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REVISTA DE MEDICINĂ MILITARĂ

- *Concise Review on Modulation Mechanism of Matrix Metalloproteinase via Oncoviruses in Aggressiveness of Malignancies*
- *Vascular Changes in Hypertension: Is There Cell Death Involved? – A Narrative Review*
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REVIEW

Concise Review on Modulation Mechanism of Matrix Metalloproteinase via Oncoviruses in Aggressiveness of Malignancies

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Abstract: *Matrix metalloproteinases (MMPs) are a member of the protein family intimately associated with zinc-containing endopeptidases, which are the main proteases implicated in extracellular matrix (ECM) degradation. The high ability of MMPs in the degradation of extracellular proteins is an important event in many normal biological as well as pathologic processes, including tumor progression, tissue repair, wound healing, developmental morphogenesis, and inflammatory diseases. This review is focused on the description of the modulation mechanism of MMPs via oncoviruses. Up to now, a large number of oncogenic viruses have been detected and recognized as etiologic factors of multiple human cancers. Actually, by expression of several viral oncoproteins of tumorigenic viruses with the simple genetic system, we are able to deregulate or modulate the pathways of cell transformation procedure in malignancy. In brief, upregulated expression of MMPs genes by direct (through DNA binding) , as well as indirect interactions (by activating PI-3K/AKT, ERK/MAPK, IFN/JAK/STAT, JNK, NF-κB signaling pathways and/or immune response) with tumorigenic viruses, have a noticeable role in the proliferation of cells, as well as angiogenesis, mobility, EMT, invasiveness, and metastatic features. In fact, the growth of tumor-related researchers offers a future perspective for the efficiency of targeted, molecular-based therapies and its anticancer effects via diverse mechanisms such as the suppression of viral oncoproteins.*

Keywords: *Matrix Metalloproteinases (MMPs), Tumor Viruses, Tumor Progression*

INTRODUCTION

Matrix metalloproteinases (MMPs), form a family of zinc-dependent extracellular matrix (ECM) remodeling endopeptidase enzymes that play a role in a number of physiological processes, like the healing of wounds, organismal development, and effectors of immune responses, as well as in pathological conditions, like pulmonary dysfunctions, autoimmune diseases, and cancer [1,2]. To date, almost 25 various vertebrate MMPs have been recognized, including 23 members in humans [3]. Based on sequence homology and specificity for substrate, the MMPs family divides into six categories of stromelysins, collagenases, matrilysins, gelatinases, membrane-type as well as other non-classified MMPs [4]. Each of these groups has its subunits that perform unique functions [5]. Chemokines, cytokines, as well as growth factors, cell-to-to-cell adhesion molecules, and binding proteins, can be mentioned as other potential targets for MMP substrates [6]. The classification of MMPs is indicated in Table 1 shown [7-11]. Matrix metalloproteinase (MMPs) activity might be regulated at three transcription levels, production of enzymes like zymogens, which require activation, as well as co-expression of tissue inhibitors of metalloproteinases (TIMPs) [12,13]. MMPs transcription is perfectly controlled, and their expression level of them is low. TIMPs are endogenous suppressors of metalloproteinases that can inhibit the MMM's catalytic activity, therefore maintaining ECM homeostasis [14]. MMPs are produced in their inactive zymogenic forms (proenzymes or pro-MMPs), which are subsequently proteolytically activated by removing propeptides by other proteases [15]. The majority of MMPs are activated in the extracellular space via plasma, tissues, and microbial proteases. The rest of the activated MMPs like MT-MMPs, MMP-11, 23, and 28 possess a furin-dependent cleavage motif RXK/RR in the propeptide sequence, which are intracellularly

activated via furin-like proteases of Golgi [16]. Any disruption in the regulation of MMPs modulatory mechanisms may associate with the progression of the disease in pathological conditions [12].

Table 1: Classification of the main members of the MMP family by chromosomal location and the group of substrates

MMP number	MMP subclass/ Family/Group	MMP name	Substrates	Chromosome
MMP-1	Collagenases	Interstitial collagenase	Collagens (I,II,III,VIII a X); gelatin; aggrecan; L-selectin; IL-1β proteoglycans, entactin; ovostatin; MMP-2; MMP-9	11q22-q23
MMP-2	Gelatinases	Gelatinase A	Collagens (I,IV,V,VII,X,XI a XIV); gelatin; elastin; fibronectin; aggrecan; MBP; osteonectin; laminin-1; MMP-1; MMP-9; MMP-13	16q13
MMP-3	Stromelysins	Stromelysin 1	Collagens (III,IV.V, a IX); gelatin; aggrecan; perlecan; decorin; laminin; elastin; casein; osteonectin; ovostatin; antactin; plasminogen; MBP; IL-1β; MMP-2/TIMP-2; MMP-7; MMP8; MMP-9; MMP-13	11q23
MMP-7	Matrilysins	Matrilysins	Collagens (IV a X); gelatin; aggrecan; decorin; fibronectin; laminin; entactin; elastin; casein; transferrin; plasminogen; MBP; β4-integrin; MMP-1; MMP-2; MMP-9; MMP-9/TIMP-1	11q21-q22
MMP-8	Collagenases	Neutrophil collagenase	Collagens (I,II,III,V,VII,VIII a X); gelatin; aggrecan; fibronectin	11q21-q22
MMP-9	Gelatinases	Gelatinase B	Collagens (IV,V,VII,X a XIV); gelatin; entactin; aggrecan; elastin; fibronectin; osteonectin; plasminogen; MBP; IL-1β	20q11.2-q13.1
MMP-10	Stromelysins	Stromelysin 2	Collagens (III-V); gelatin; casein; aggrecan; elastin; MMP-1; MMP-8	11q22.3-q23
MMP-11	Stromelysins	Stromelysin 3	Unknown (casein)	22q11.2
MMP-12	Elastase	Metalloelastase	Collagen IV; gelatin; elastin; casein; fibronectin; vitronectin; laminin; entactin; MBP; fibrinogen; fibrin; plasminogen	11q22.2-q22.3
MMP-13	Collagenases	Collagenase-3	Collagens (I,II,III,IV,IX,X,a XIV); gelatin; plasminogen; aggrecan; perlecan; fibronectin; osteonectin; MMP-9	11q22.3
MMP-14	Membrane-MMP	MT1-MMP	Collagens (I-III); gelatin; casein; fibronectin; laminin; vitronectin; entactin; proteoglycans; MMP-2; MMP-13	14q11-q12
MMP-15	Membrane-MMP	MT2-MMP	Fibronectin; entactin; laminin; perlecan; MMP-2	16q12.2-q21
MMP-16	Membrane-MMP	MT3-MMP	Collagen III; gelatin; casein; fibronectin; MMP-2	8q21
MMP-17	Membrane-MMP	MT4-MMP	Gelatin	12q24
MMP-18	Collagenases	Collagenase-4	Unknown	Unknown
MMP-19	Other	RASI-1	Gelatin; tenascin; fibronectin; collagen IV; laminin; entactin; fibrin/fibrinogen; aggrecan	12q14
MMP-20	Other	Enamelysin	Amelogrenein; aggrecan; COMP	11q22
MMP-23	Other	Unnamed	Amelogenin	11q22
MMP-24	Membrane-MMP	MT5-MMP	Pro-MMP 2	20q11.2
MMP-25	Membrane-MMP	MT6-MMP	Pro-gelatinase A; fibrin; fibronectin; collagen IV; gelatin	16p/3.3
MMP-26	Matrilysins	Endometase, matrilysin-2	Gelatin Iα; P1; fibrinogen; fibronectin; vitronectin; β-casein	Unknown

MMPs AND TUMOR PROGRESSION

Cancer is the second factor of death globally, which is estimated to account for 9.6 million deaths in 2018 [17]. Recent two decades of biomedical research have led to large amounts of information about the molecular events which occur during carcinogenesis as well as signaling pathways involved in the progression of cancer. Complex molecular mechanisms play different roles in the tumor cells and the tumor environment associated with this process [18]. Several interactions of tumor cells with the involved environment of the tumor are crucial for the pathological changes observed during the transformation of cells and carcinogenesis in the extracellular matrix (ECM), as well as cytokines, growth factors associated with ECM, along with neighboring cells (neutrophils, endothelial cells, mast cells, macrophages, fat cells, and pericytes) [19-21]. Different steps of cancer consisting of migration, attack, metastasis, and angiogenesis are in interaction with the neighboring microenvironment [22]. MMPs are the main molecules in these processes, and since they break down numerous cell adhesion molecules, indicating their roles in the regulation of cell-to-to-cell, and cell-ECM interactions [23,24]. Previous researchers have identified that MMPs play an essential role in all phases of tumor maintenance and growth, including invasiveness, migration of tumor cells, evasion from apoptosis as well as immune surveillance, angiogenesis, and metastasis, so that all these major stages may be modulated by MMPs [25].

Interferon, interleukins, growth, factors, and extracellular MMP inducers secreted from tumor cells in a paracrine, manner stimulating adjacent host cells to generate the required MMPs. MMPs produced by normal cells can be bound and used on the surface of tumor cells [26]. MMPs gene expression and its activity increase in many types of cancers in humans. And this increased expression causes an increase in tumor size, invasion, and decreased survival [27]. Thus, MMPs are used as a novel and capable therapeutic target in different types and cancer phases [28]. MMPs use their proteolytic function to remove physical barriers, developing angiogenesis and tumor metastasis [29]. Tumor development and angiogenesis depend on the facilitated accessibility of signaling molecules. For example, cytokines and growth factors by MMPs enhance by making them available for tumor cells and their microenvironment [18]. This process is performed by releasing these factors from the ECM (VEGF, IGF, and bFGF) or via secreting them from the surface of the cell (HB-EGF, EGF, TGF-a) [4]. The release of endogenous angiogenesis suppressers can regulate angiogenesis perfectly, for instance, angiogenesis inhibitors like tumstatin, endostatin, angiostatin, and endorepellin [30]. Disruptions in the regulation of cell-cell and cell-ECM interactions by MMPs during the processing of E-cadherin and integrins, respectively, affect both cell phenotype (EMT) as well as increasing cell migration [31,32]. Almost all members of the MMP family have been reported to be dysregulated in human tumors, although MMPs such as MMP-1, -2, -7, -9, -13, and 14 are more important [33]. We have demonstrated the most noticeable MMPs, which are related to the phases of tumor growth, biological effects, and their consequences in Table 2.

Table 2: Key MMPs in relation to the stages of cancer progression, biological effect, and consequences

Function	MMP	Biological effect	Consequence	Reference
Cancer cell invasion	MT1-MMP, MMP-2 and MMP-9	Proteolytic	Breakdown physical barriers	[144]
Cancer cell proliferation	MMP-1, -2, -3, -7, -9, -11, -19	Cleavage of IGF binding proteins, Shedding of membrane, anchored ligands of EGFR, including HB-EGF, TGF-a and amphiregulin, Activation of TGF-β, Shedding of HB-EGF	Proliferation	[145-147] [148] [149] [150]
Cancer cell apoptosis	MMP-7 Several MMPs	Cleaving the Fas ligand Indirect activation of the serine/serine/threonine Akt ⁄ protein kinase B through the signaling cascades of EGFR and IGFR	Anti-apoptotic	[151] [147, 152]
Tumor angiogenesis	MMP-2, -9 MMP-3, -10, -11 MMP-1, -8, -13	Degradation of COL-IV, perlecan; release of VEGF and bFGF, respectively Degradation of COL-IV, COL-XVIII, perlecan; generation of tumstatin, endostatin, angiostatin and endorepellin, respectively	Upregulation of angiogenesis Downregulation of angiogenesis	[153, 154] [4, 155]
Cell adhesion, migration, and EMT	MMP-2 MT1-MMP MMPs MMP-2, -3, -9, -13, -14 MMP-1 and -7 MMP-28	Degradation of COL-IV; generation of cryptic peptides Degradation of laminin-5; generation of cryptic peptides Integrins as substrates Overexpression this MMPs associated with EMT, a highly conserved and fundamental process of morphological transition By cleavage E-cadherincontribute to morphological transition Proteolytic activation of TGF-β	Promote migration Induction of EMT; cell migration Powerful inducer of EMT, leading to cell migration	[156, 157] [158] [159] [160] [161]
Immune surveillance	MMP-9 MMP-9, -2, -14 MMP-7, -11, -1, -8, -3 MMP-7, -8	Cleavage of IL-2R alpha Release active TGF- β Release of a1-proteinase inhibitor Cleave several members of the CC (β-chemokine) and CXC (α-chemokine) chemokine subfamilies or to regulate their mobilization	Repress T-lymphocyte proliferation Abolish T-lymphocyte reaction against cancer cells Decrease cancer cell sensitivity to NK cells Affecting leukocyte infiltration and migration	[162] [163] [164] [165, 166]

ONCOVIRUSES

Approximately 20% of all human malignancies worldwide are associated with viruses [34]. Seven viruses have been implicated as oncogenic, including HPV, EBV, HCV, HBV, Kaposi's sarcoma-associated herpesvirus (KSHV), HTLV-1, and Merkel cell polyomavirus (MCV), which have been reported to be etiologically implicated in the maintenance and progression of human cancers [35]. However, viral oncogenesis processes

are a complex process, with induction of chronic inflammation conditions, disruption of cellular DNA repair mechanisms, interrupting host genetic and epigenetic integrity, and homeostasis, viral infection may result in genome variation and cell cycle deregulation. Viral 'oncoproteins' are capable of modulating or deregulating cellular signaling pathways, making changes in the expression of genes and microRNAs in either transcription or post-transcription, and disrupting tumor suppressor factors and proteins, which regulate cell polarity, immune response, signal transduction, and cell apoptosis. Genetic and epigenetic modifications caused via oncoviruses may be resulting in the morphology and variation of cancer stem cells, which are essential for the primary initiation, development, migration, invasiveness, and chemotherapy resistance of tumors. These molecular events underlying the interactions between oncoviruses that use specific cellular genes and cellular signaling pathways are important issues to research efforts [36-38]. MMPs have several specific biological actions in every cancer phase, from the beginning to the extension of pertinent metastases. Accordingly, this review's focus is on characterizing the mechanism of modulation or deregulation of MMPs by oncoviruses.

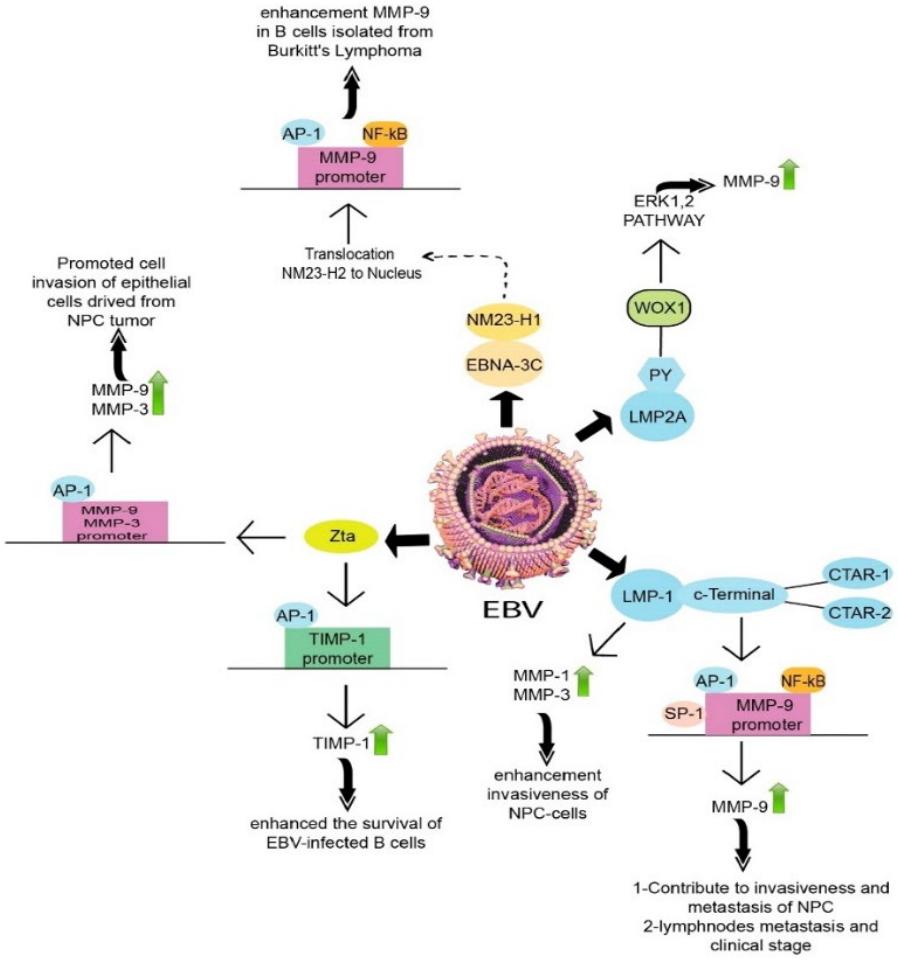
INTERACTION BETWEEN ONCOVIRUSES AND MMPs

Epstein-Barr virus

Epstein-Barr virus (EBV) is a linear dsDNA virus that belongs to the human gamma-herpesvirus family, playing a role in different B-cells lymphomas, including Hodgkin's lymphoma, Burkitt's lymphoma, post-transplant lymphoproliferative disorders, as well as other tumors like T-cell non-Hodgkin lymphomas and nasopharyngeal carcinoma [39]. In tumors associated with EBV, the virus leads to a latent infection expressing only a subset of 12 latent viral transcripts, two early RNAs (EBERs), three latent membrane proteins (LMPs), six nuclear antigens (EBNAs), and BARF transcripts. Out of these genes, EBNA1, EBNA-2, EBNA-3A, EBNA-3C, EBNA-LP, and LMP1 enable the virus to control a number of cellular signaling pathways associated with oncogenesis [40,41]. EBV LMP1 increases MMP-9 expression by recruitment of Sp-1, AP-1, and NF-kappa B transcription factors on MMP-9 promoter that is related to invasiveness and metastasis in EBV-associated NPC [42]. Previous research demonstrated that C-terminal activation regions 1 and 2 (CTAR-1 and CTAR-2), two crucial signaling domains within the carboxy terminus of LMP1, might activate the MMP-9 promoter and stimulate MMP-9 activity [43]. TANG Jian showed EBV-induced MMP-9 upregulation correlates with lymph node metastasis and clinical stage in NPC tumors [44]. Lee demonstrated that upregulation of MMP1 and MMP3 expression in LMP1-positive NPC tumors led to the enhancement of invasiveness in NPC cells [45]. BZLF1 (also known as Zta) is one of the critical inducers of EBV lytic cycle alteration and has been reported to trigger metastasis in epithelial cells [46, 47]. In vitro studies demonstrated that Zta stimulates MMP3 and MMP9 expression in epithelial cells derived from NPC tumors. Zta can activate the MMP3 promoter by AP-1 elements, and its DNA-binding domain is necessary for the process of promoter binding and MMP3 stimulation. Zta-enhanced cell migration requires MMP3 but not MMP9. Zta-elevated cell migration needs MMP3, but not MMP9. Therefore, the expression of both MMP3 and MMP9 is important for the co-expression of the two MMPs (MMP3 and MMP9) induced by Zta, which simultaneously promotes cell invasiveness of epithelial cells differentiated from NPC tumor [48]. Moreover, Zta protein via binding AP-1 site of its promoter increases TIMP-1 expression in EBV-infected B-cells as well as EBV-immortalized lymphoblastoid cell lines (LCLs). Upregulation of TIMP-1 expression through Zta enhances the maintenance of EBV-infected B-cells and promotes the progression of EBV-associated cancer [49]. EBNA3C regulates the transcription of numerous viral as well as cellular genes important for the process of immortalization by EBV in primary B-lymphocytes [50]. Nm23-H1 is a well-known metastasis inhibitor, and its low expression is correlated with metastatic tumor capability in different types of human carcinoma [51]. In vitro research showed the interaction of EBNA3C with Nm23-H1 leads to the translocation of Nm23-H1 to the nucleus and inhibits the antimotility effect. However, studies have shown that this interaction also can increase transcriptional activity on a related promoter of the target gene [52]. Interaction between EBNA3C and Nm23-H1 upregulates the expression of MMP-9 by binding the transcription factors AP-1 and NF-kB on its promoter in B-cells that were isolated from Burkitt's lymphoma [53]. LMP2A promotes cell transformation, migration, and invasion by affecting host cellular signaling pathways, mainly by its N-terminus, intracellular domain, which consists of an immunoreceptor tyrosine-based activation motif (ITAM), a YEEA motif, and two PPPPY (PY) motif [54,55]. LMP2A promotes invasion of NPC cells by the PY motif via stimulation of extracellular signal-regulated kinase (ERK)-1/2 as well as the downstream transcription factor Fra-1, therefore leading to upregulation of MMP9 expression [56]. WW domain-containing oxidoreductase WOX1, also called WWOX or FOR, is a candidate tumor inhibitor encoded by the human fragile WWOX gene, related to several signaling pathways modulating embryonic development, metabolism, cell proliferation, and apoptosis [53]. The function of WOX1 in cancer suppression is commonly used by stimulating apoptosis and sequestering some oncoproteins [57,58]. According to a previous study, the interaction of the tyrosine residue 33 of WOX1 and the PY

motif of LMP2A is needed for the LMP2A-induced ERK1/2-Fra-1-MMP9 pathway in NPC cells (Figure 1) [59]. Two SNPs (rs8113877T>G or rs17576A>G) of MMP-9 in ab_EBV positive individuals were associated with more possibility of progression to chronic lymphocytic leukemia in contrast to those individuals who were negative [60]. In summary, the upregulation of MMPs by EBV plays a significant role in EBV-triggered metastasis.

Figure 1: Schematic model of deregulation of MMPs by EBV virus

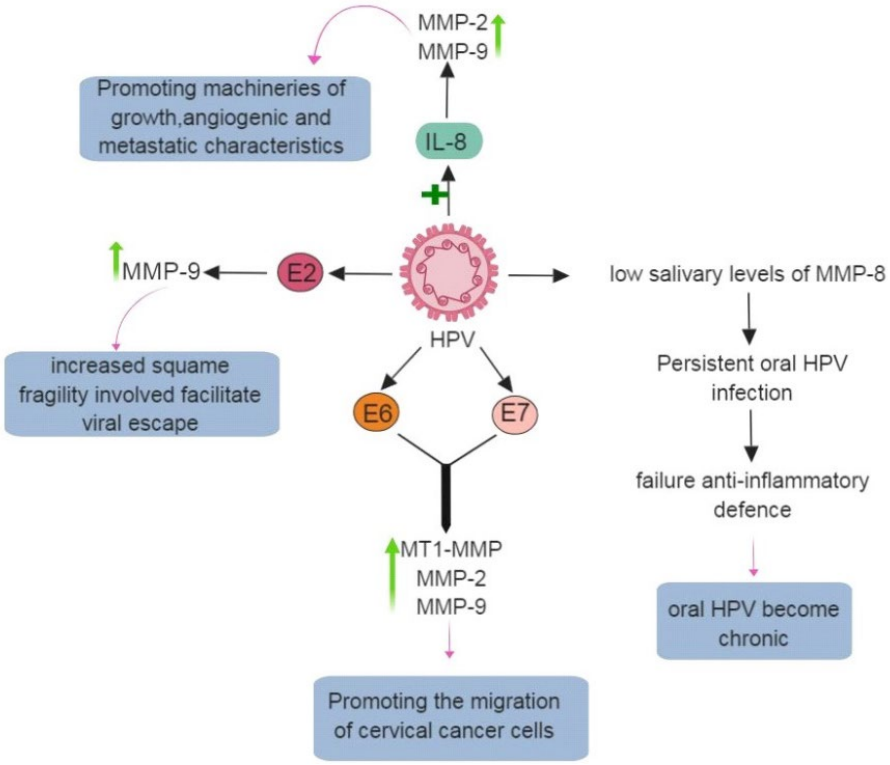


Human papillomavirus

Human papillomaviruses (HPVs), namely double-stranded DNA viruses, are recognized as one of the main agents that relate to the progression of numerous cancers such as head and neck, cervix, vagina, vulva, anal and penile carcinomas [61]. Continuing infection with high-risk human papillomavirus (HPV) types, especially HPV type 16 or 18, is essential for the development of a cancer precursor lesion and invasive cervical cancer, which is identified through deregulated and high-level expression of HPV early genes (E6 and E7) [62]. The high-risk HPV E6 and E7 oncoproteins result in transformation by a disruption in the activity of cellular tumor suppressor proteins p53 and RB, respectively, and several cellular proteins [63,64]. Induction of MT1-MMP expression in HaCaT cells, an immortalized human keratinocyte line, by the E7 protein of high-risk HPV types 16 and 8 contributes to cellular proliferation, angiogenesis, and, malignant progression, for the invasion of HPV-positive tumor cells [65]. In human keratinocytes and organotypic skin cultures, HPV8 E2 protein overexpresses the expression of MMP-9 by direct activation of its promoter, resulting in increased squamous fragility involved in facilitating viral escape [66]. A recent study demonstrated that HPV-16 E6/E7 oncoproteins cause upregulation of MT1-MMP, MMP-2, and MMP-9 to promote cervical cancer cells [67]. Previous studies reported that HPV 16 infection might lead to lung tumor cells through increasing in interleukin-17 (IL-17) expression. IL-17 along with its downstream signaling mediator, interleukin-8 (IL-8), play an efficient role in tumor angiogenesis and metastasis by modulation of various pro-angiogenic factors [68]. Induction of IL-8 by HPV upregulates the expression of MMP-2 and MMP-9 in lung adenocarcinoma that correlated with progressive

mechanisms of growth, and metastatic and angiogenic characteristics [69]. HPV-16 E6/E7 overexpressed the expression of MT1-MMP and MMP-2, which are correlated with increased cell invasion in cervical cancer [70]. According to recent research (Haukioja), low salivary levels of MMP-8 are due to continued oral HPV infection, which appears like a failure of the anti-inflammatory defense of the oral mucosa to allow an incident oral HPV infection to become a chronic infection [71]. Common skin extract immunoblots and zymographic analysis revealed increased expression of MMP-9, MMP-13, and MT1-MMP in HPV8-positive mice rather than HPV8-negative mice, which are involved in the development of HPV8-induced cutaneous tumors (Figure 2) [72]. Singh et al. have shown that SNPs -1306CC (rs243865: C>T) in the promoter of the MMP2 gene, enhanced transcription and enzymatic activity of the MMP-2 gene is associated with the incidence possibility of cervical cancer. A significant relationship between the −1306CC genotype and HPV infection in increasing the risk of cervical cancer was observed [73].

Figure 2: Schematic model of deregulation of MMPs by HPV virus

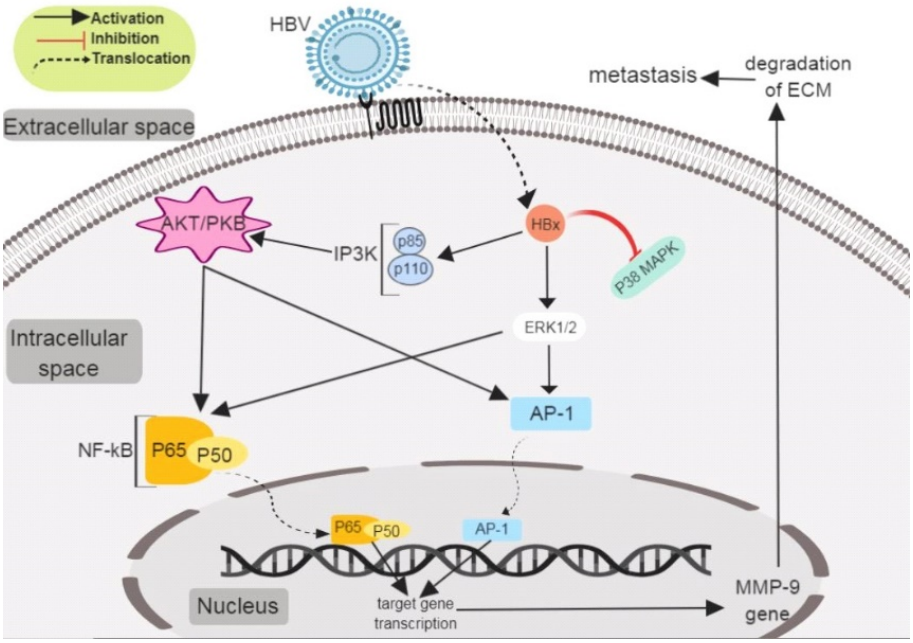


HBV

Chronic hepatitis B (CHB), an important agent responsible for liver cirrhosis and hepatocellular carcinoma, is among the primary health problems of the world [74,75]. HBV transactivator protein (HBx) enables the invasive capacity of the virus, which is mediated by increased the expression of membrane-type one matrix metalloproteinase (MT1-MMP) that subsequently activates matrix metalloproteinase-2. HBx is able to induce both MT1-MMP expression and cell invasion through dependence on cyclooxygenase-2 (COX-2) activity. Furthermore, HBx increases COX-2 expression through the activation of COX-2 gene promoter transcription in a nuclear factor of an activated T-cell-dependent (NF-AT–dependent) manner. These data indicated the capacity of HBx to augment tumor cell invasion by a mechanism consisting of MT1-MMP and COX-2 upregulation and led to new insights into HBx protein mechanism of action, its involvement in tumor metastasis and hepatocellular carcinoma resulting from intrahepatic metastasis to HBx protein expression following establishment of tumor [76]. HBx protein plays a crucial role in the progression of hepatocellular carcinoma (HCC). HBx is often the only HBV protein that would be detected in hepatic tumor cells, which enhances tumor cell invasion and metastasis. HBx, by inducing the PI-3K/AKT and ERK pathways except for the P38 MAPK pathway, leads to the activation of NF-kB and AP-1 transcription factors. HBx triggers the translocation of NF-kB and AP-1 into the nucleus, leading to increasing MMP-9 expression. Thus, HBx modulates the transcriptional function of MMP-9 that is essential to the incursion and metastasis of tumors through PI-3 K/AKT and

ERK pathways. These findings suggest that HBx could induce MMP-9 and, in turn, in the invasion and metastasis of cancer, by activating ERK and PI-3K signaling pathways (Figure 3) [77].

Figure 3: Molecular mechanism of HBX-activated MMP-9 expression. HBx induces PI-3K/AKT and ERK pathways but not the p38 MAPK pathway. This process leads to the stimulation of NF-kB and AP-1 transcription factors. Translocation of NF-kB and AP-1 activated by HBx into the nucleus leads to an increase of MMP-9 expression. Therefore, HBx via regulation of transcriptional activation of MMP-9, plays an important role in the invasion and metastasis of tumors via PI-3K/AKT and ERK pathways

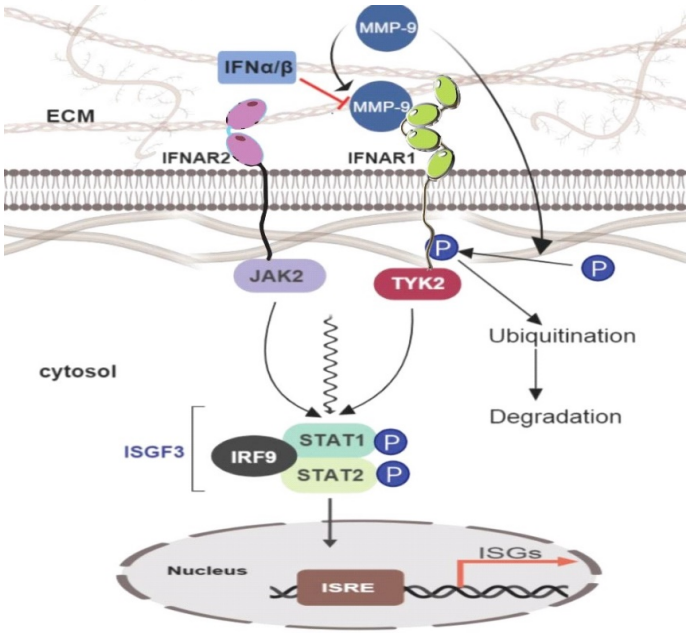


Genes that were defined to be modulated in the presence of HBx include those genes involved in signal transduction, cell cycle, cytokine production, metabolism, apoptosis, cell adhesion, and oncogenesis. The functions of other less-defined genes also were affected. Transactivation by HBx upregulates the translation, transcription, and release of matrix metalloproteinase-3 (MMP-3), which manifests as a cell migratory phenotype. This effect of HBx was suppressed in the presence of an MMP-3-specific peptide inhibitor. HBx applies selective control of transcription in hepatoma cells as well as inducing cell migration via activation of MMP-3. Therefore, by upregulation of MMPs, HBx increases the invasion of tumor cells and the destruction of the extracellular matrix that is reported to increased cell migration [78]. The progression of tumors tightly depends on a permissive microenvironment that is highly correlated with the destiny of tumor cells. The normal structure of ECM is of high importance in the modulation of cell growth. For invasiveness, cancer cells must overwhelm the mechanical barriers caused by the ECM. It has been demonstrated which conformational changes in the ECM by MMPs have a noticeable role in the mobility and spread of tumor cells, resulting in invasion and metastasis. The activation of MMP induced by HBx signifies a role of HBx protein in the development of HCC metastasis. However, HBx is demonstrated to relieve transcriptional stimulation activity, it is not defined in what way HBx induces the expression of MT1-MMP [79,80]. HBV replication is assisted by MMP-9 through repression of IFN/JAK/STAT signaling as well as IFNAR1 function. Thus, these outcomes have discovered a novel role for MMP-9 in viral replication, revealing a new mechanism by which HBV escapes host immunity to maintain persistent infection. Indeed, there is a positive feedback loop between the injection of HBV and the production of MMP-9. Firstly, HBV activates MMP-9 production in PBMCs and macrophages. Subsequently, HBV replication would be facilitated by MMP-9 through repressing IFN/JAK/STAT signaling, interacting with IFNAR1 to prevent its binding to IFN-α and promoting the ubiquitination phosphorylation, degradation of IFNAR1 and ISRE, subcellular distribution, and interferon-stimulated response element. Thus, MMP-9 would benefit HBV by blocking innate immunity to launch or maintain chronic infection (Figure 4) [81].

In hepatitis B infection, apoptosis of liver cells that is induced by varying degrees of inflammation plays an essential role in the outcome of CHB [82]. The MMP-9 expression level is directly related to TNF-α and IL-6. Because IL-6 and TNF-α can

regulate MMP-9 activity in a concentration- and time-dependent mode, overexpression of MMP-9 can degrade ECM to destroy the basement membrane and lead to disorganized and loose tissue structures, leading to further penetration of TNF-α and IL-6. These factors can interact with each other and create a vicious cycle, resulting in pathological changes in the liver. In conclusion, the MMP-9 level is increased remarkably in patients with varying degrees of CHB, it would be important in the pathological progress of the liver, and it has a close correlation with inflammation, which can provide a theoretical basis for clinical treatment [83].

Figure 4: MMP-9 assists HBV replication by suppressing IFN/JAK/STAT signaling and IFNAR1. Indeed, HBV induces MMP-9 in infected patients and in HBV-stimulated leukocytes. Then, MMP-9 comforts HBV replication through repressing IFN/JAK/STAT signaling, interacting with IFNAR1 to block its binding to IFN-α, and promoting the phosphorylation, ubiquitination, subcellular distribution, and degradation of IFNAR1

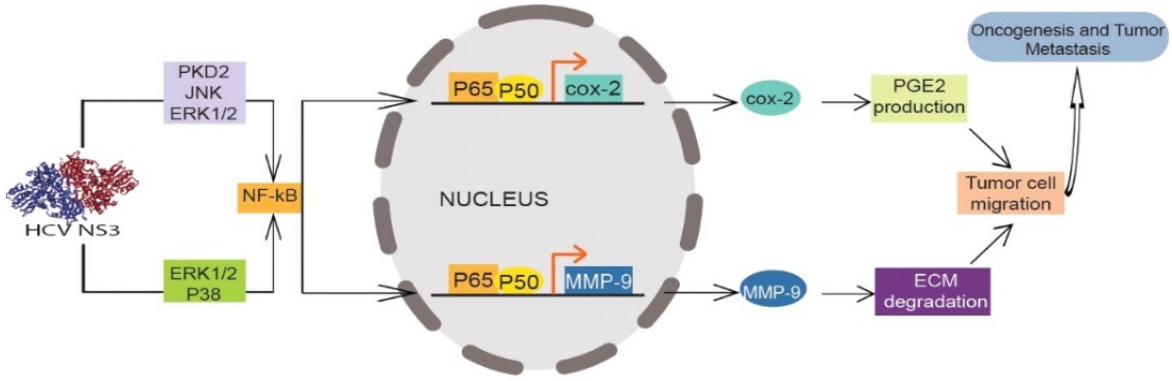


HCV

Hepatic inflammation, cirrhosis, developing liver fibrosis, and hepatocellular carcinoma (HCC) are common problems in HCV patients [84,85]. In the liver, the main reason for fibrogenesis is pathological repletion of the extracellular matrix (ECM), which leads to an imbalance in the rate of enhanced matrix synthesis to reduce the degradation of connective tissue proteins that can be controlled by matrix metalloproteinases (MMPs). Recent studies showed that increasing levels of hepatic gelatinases, including MMP-9, and MMP-2, are related to the progression of HCC and the fibrotic index of chronic HCV patients [86,87]. The disparity between matrix metalloproteinases (MMPs) and tissue suppressors of matrix metalloproteinases (TIMPs) is remarked to be a significant index of degradation of the extracellular matrix. MMPs are members of gene families that can degrade collagen and play an essential role in the natural mechanism of tissue repair in pathological states, including liver fibrosis. Thus, an increase of the MMP-1/TIMP-1 ratio by IFN treatment can relieve liver fibrosis of chronic hepatitis C that demonstrates the anti-fibro genetic effect of IFN on the factors modulating hepatic fibrosis [88]. During chronic liver harm, activated hepatic stellate cells (HSC) turned backward into myofibroblast-like cells and proliferated. This stage is an important factor in the creation of fibrosis and subsequent cirrhosis [89]. HSCs are considered important sources of fibrillar as well as nonfibrillar collagens of the liver, matrix-degrading proteases (MMPs), and their specific blockers (TIMPs). Briefly, recent studies on patients with chronic hepatitis C reported important variations in hepatic TIMP and MMP expression. It is likely that TIMP expression was affected via the inflammatory conditions of the disease, which can reflect the effects of the disease. Promoted fibrosis enhances the expression of MMP-7 and MMP-2 and provides a condition for the destruction assessment of connective tissue [86]. The primary activity in the biosynthesis of different prostaglandins (PG) and thromboxanes catalyzes via cyclooxygenases [90,91]. There are two cyclooxygenases (COX) isoforms: COX-1 is basically expressed in several cell types and is the main factor in the homeostatic activity of PG, while COX-2 is triggered by different proinflammatory stimuli, including lipopolysaccharide (LPS) and cytokines [92-94]. COX-2 is involved in fibrogenesis, inflammation, and carcinogenesis [95-97]. Therefore, different researchers reported that

increased COX-2 expression and PG production might be associated with liver dysfunction and tumorigenesis in various animal models [98-100], in addition to fibrosis and hepatocellular carcinoma (HCC) among humans [101,102]. HCV core and NS5A proteins, both alone and in association with the impact of endotoxin and proinflammatory cytokines, are capable of regulating the expression of COX-2 and MMP-9 genes through chronic HCV infection. Therefore, this mechanism may be considered evidence for this process that chronic HCV infection may result in liver fibrosis. Thus, it seems reasonable that much PGs generated in hepatocytes via the correlated effect of HCV proteins in addition to inflammatory factors may affect neighboring liver cells by autocrine and/or paracrine pathways, therefore participating in hepatocarcino-genesis [103]. The E2 envelope glycoprotein of the hepatitis C virus (HCV) is an important modulator of HCV interaction with cell surface receptors. E2 interacts with an extracellular loop of human CD81; it's a receptor expressed on various cell types such as hepatocytes and B-cells. CD81 on the cell surface is expressed in human hepatic stellate cells (HSC) at a high level, Matrix metalloproteinase-2 (MMP-2; gelatinase A), an important enzyme responsible for the degradation of the normal hepatic extracellular matrix, upregulates subsequently after binding E2 toCD81. Furthermore, E2/CD81 interaction in human HSC causes an increasing amount of MMP-2 via increasing the binding activity of activator protein-2/DNA via ERK/MAPK phosphorylation. Increased degradation of the hepatic extracellular matrix in HCV-concentrated regions may be associated with inflammatory as well as parenchymal damage [104]. HCV replication may be regulated by signal cascades, cellular factors, and viral proteins [105-108]. The HCV nonstructural protein 3 (NS3) has a serine protease required for the synthesis of viral protein and helicase activity needed for viral replication [109,110]. NS3 also is a major protein in modulating cell proliferation, indicating its role in carcinogenesis [111,112]. HCV NS3 protein facilitates tumor cell invasion in two ways. Firstly, NS3 induces several signaling pathways like protein kinase D2 (PKD2), JNK, and ERK1/2 to modulate the transcription factor NF-κB, and subsequently, it regulates the expression of COX-2 [113], and COX-2, in turn, activates MMP-9 expression. Secondly, NS3 induces the ERK1/2–p38 signal pathway to modulate the activity of NF-κB, which triggers the translocation of NF-κB to the nucleus and binding of NF-κB to MMP-9 promoter, which consequently upregulates MMP-9 expression. MMP-9 (an important enzyme in degrading the extracellular matrix), and COX-2 (the main enzyme in prostaglandin E2 production) exert key roles in tumor invasion and tumorigenesis. Therefore, NS3 enhanced tumor cell metastasis by MMP-9 and COX-2, leading to tumorigenesis (Figure 5) [114]. The MMP2, MMP9, and TNF-α levels have an effective increase in chronic HCV patients. However, no considerable correlation has been reported in the levels of MMPs and TNF-α between fibrotic and cirrhotic cases. In consequence, MMP2, MMP9, and TNF-α revealed high reproduction capacity in distinct patients afflicted with liver fibrosis (F1–F4) compared to the control group. In contrast, MMP9, MMP2, and TNF-α were not distinguishable factors for liver fibrosis in patients with chronic HCV. Therefore it is likely that they would be used as prognostic markers for liver fibrosis [115].

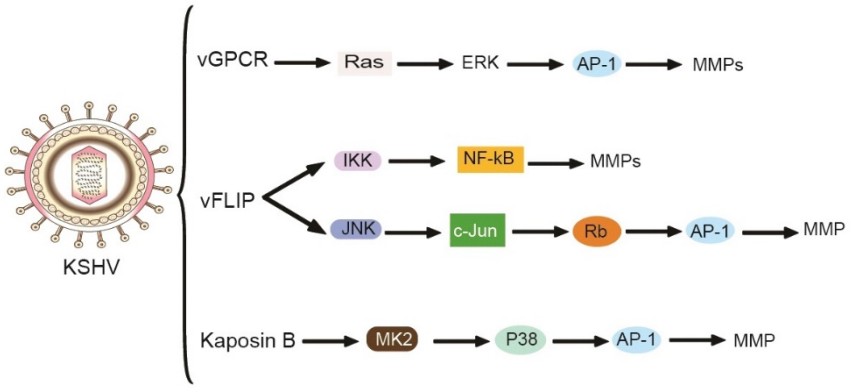
Figure 5: The mechanism of hepatoma cell invasion by HCV NS3 protein. First, NS3 activates multiple signaling components such as protein kinase D2 (PKD2), JNK, and ERK1/2 to regulate the activity of transcription factor NF-κB, which in turn regulates the expression of COX-2, and then COX-2 activates MMP-9 expression. Second, NS3 activates ERK1/2–p38 signaling pathway to regulate NF-κB activity, stimulates the translocation of NF-κB from the cytosol to the nucleus and the binding of NF-κB to the MMP-9 promoter, and thus activate MMP-9 expression.Thus, NS3 enhances tumor cell migration through COX-2 and MMP-9, resulting in oncogenesis or tumorigenesis



KSHV

Human herpesvirus-8 (HHV-8) or Kaposi sarcoma-associated herpesvirus (HHV-8 /KSHV) is a ds DNA virus with 165 kb in length. The HHV-8 life cycle consists of two states, lytic replication, and a latent phase. After primary infection, HHV-8 uses the cellular machinery of the host for replication and is subsequently observed in a latent phase as an episome. Like all herpesviruses, the B lymphocyte is the primary source of HHV-8 latency. The second sites for viral latency are endothelial cells, but these cells are the first infected cells in Kaposi sarcoma (KS). During latency, HHV-8 can regulate host cellular signaling pathways by different cellular mechanisms. Latent proteins, which include viral Cyclin a (v-Cyclin), LANA, kaposin, viral FLICE inhibitory protein (v-FLIP), and several miRNAs contribute to the pathogenesis of malignancies associated with HHV-8 [116]. HHV-8 has been identified in a Kaposi sarcoma lesion in an AIDS patient in 1994. This virus was reported to be associated with many B cell lymphoproliferative disorders (LPDs) such as posttransplant and germinotropic LPDs, multicentric Castleman disease (MCD), as well as primary effusion lymphoma (PEL) [117]. MMPs possibly associate with the mechanism of invasive KS growth and can be a proper target for effective therapeutic measures [118]. Developing KS lesional cells express gelatinases (MMP-2, MMP-9), stromelysin-1 (MMP-3), matrilysin (MMP-7), and collagenase (MMP-1, MMP-13) [119]. Cheng F. et al. demonstrated KSHV induces endothelial-to-mesenchymal transition (EMT). EMT was triggered by the viral proteins vGPCR and vFLIP via activation of the Notch pathway, which leads to a gain of membrane-type-1 matrix metalloproteinase (MT1-MMP) (Figure 6) [120]. The transcription factor PROX1 expression was recently investigated in KS. PROX1 binds the MMP14 promoter to repress its transcription. PROX1 negatively regulates MMP14 in normal tissues. Silvia Gramolelli et al. revealed that infection of lymphatic endothelial cells (LECs) with KSHV decreases PROX1 expression [121].

Figure 6: Schematic model of deregulation of MMPs by KSHV virus

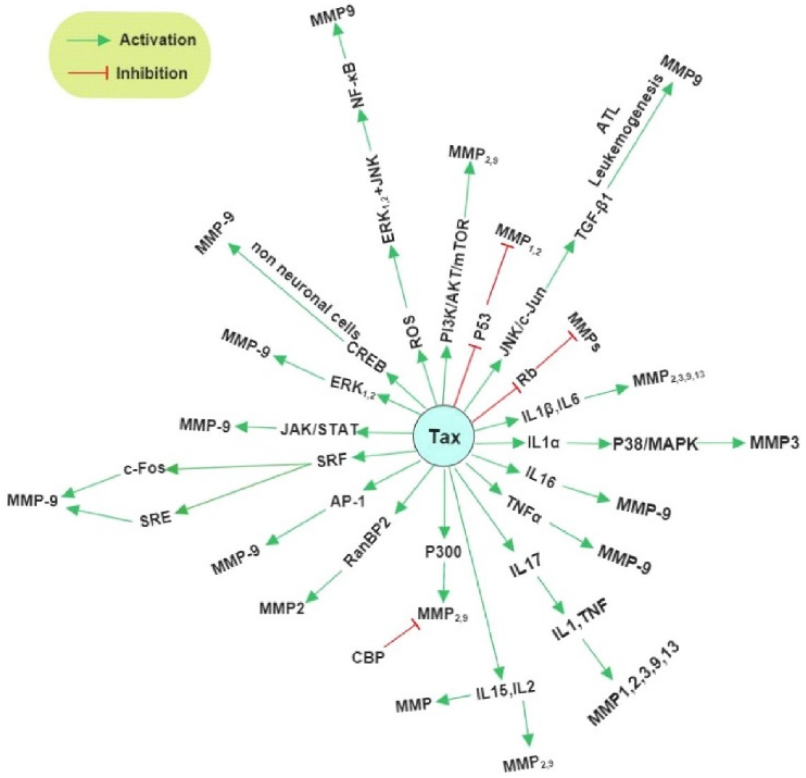


Human T-cell lymphotropic virus type I (HTLV-I)

HTLV1 is a member of the retroviridae family, which is an etiologic factor of Adult T-cell Leukemia (ATL).ATL is a malignancy responsible for the extremely aggressive proliferation of CD4+ cells with no known cure [122]. ATL commonly originates from an infection that is acquired in early life [123]. Also, besides ATL, other manifestations of infiltrated T-cells, matrix metalloproteinases (MMPs), and cytokines of viral infection are Sjogren syndrome with an inflammatory appearance and HAM/TSP associated with neurological symptoms and incomplete paralysis of limbs [124-126]. The 3' end of the genome (pX region) encodes some specific genes, including Rex, Tax, p12, p13, p30, and HTLV-1 bZIP factor (HBZ) [127,128]. HTLV-1 encodes two important genes, basic leucine zipper factor (HBZ), and tax, that are recognized to exert key roles in the infectivity of HTLV-1, survival, and growth of leukemic cells. Unlike HTLV-1 tax, HBZ escapes the antiviral response and is not detectable via the immune system. HTLV-1 provirus pX region encodes HBZ protein and in contrast to Tax, expressed in 100% of ATLL cells [123,129,130]. Although the proliferation of T cells is increased by HBZ, its function is interestingly antagonist to many tumorigenic activities of tax, such as activation of classical signaling pathways such as NFAT, AP-1, CREB, and NF-κB. Tax Repression through HBZ provides a condition for HTLV-1 to develop ATL by evading the immune system [123,131]. Tax may interact with cellular factors including AP-1, CBP/p300, CREB/ATF, and NF-κB. Both HTLV-1-replication and T-cell proliferation are generally enhanced by activating of IκB kinase (IKK)-NF-κB that is stimulated by viral Tax oncoproteins. Viral transactivator Tax is a presumed E3 ubiquitin ligase that is unitedly related with UbcH7, UhcH5c, and UbcH2 and is responsible for direct activation of IKK via binding to the NEMO subunit [132,133]. Bazarbachi et al. proved the interaction of HTLV-I-transformed cells in relation

to endothelial cells triggers the gelatinase activity of MMP-9 and MMP-2 in endothelial cells and decreases the tissue blocker of MMP. In a study by Szymocha et al. on infected astrocytes (T cells) with HTLV-1, contact with T cells is the factor that the astrocyte obtains a typical phenotype of gliosis and causes the release of proinflammatory cytokines (i.e. IL-1 α, IL-6, TNF-α) and matrix metalloproteinases (MMP-3, MMP-9). The high expression of MMPs and their endogenous suppressors (TIMP-3 and TIMP-1) cause MMP/TIMP imbalance. It changes the extracellular matrix [134]. Giraudon et al. showed cytokines secreted by infected glial cells with HTLV-1 are responsible for increasing the expression of TIMP-3, and MMP-3, MMP-9, while MMP-2, TIMP-1, and TIMP-2 remained stable (Figure 7) [135].

Figure 7: The demographic characteristics of the nurses (average score and standard deviation of nurses' clinical competence and moral reasoning)



MATRIX METALLOPROTEINASE INHIBITORS

Nowadays, MMPS targeting suppression of the activity of MMPs in the extracellular matrix as a novel anticancer strategy could be reconsidered. TIMPs, which are natural inhibitors of MMPs, at first, were the compounds to be developed for medical research. Hypothetically, TIMPs with potent inhibitory activity specifically can suppress the function of some MMPs which could lead to a useful therapeutic effect. However, their clinical application is still hampered by the absence of an efficient gene delivery style that has restricted the clinical use of this method [136]. Nevertheless, the progress of several types of natural synthetic suppressors of MMPs has been completely adopted and broadly investigated in clinical trials and researched in various cancers during the past decade (Table 3). Generally, synthetic suppressors of MMPs divide into three distinct pharmacologic groups including collagen non-peptidomimetics and peptidomimetics, tetracycline derivatives, and bisphosphonates [137].

THE RELATION BETWEEN INHIBITING OF MAJOR SIGNALING PATHWAYS BY MMP INHIBITORS AND ONCOVIRUSES

As previously mentioned, in HBV infection, HBx controls the transcription induction of MMP-9 which plays a key role in migration as well as metastasis of the tumor via PI-3 K/AKT and ERK pathways. Therefore, these data show that HBx plays a major role in inducing MMP-9 and, in turn, in the invasion and metastasis of cancer via activating ERK and PI-3K signaling pathways [77]. On the other hand, all the findings of one study already implied that 3-azidoWA suppresses 3-azidoWA-mediated MMP-2 expression through the attenuation of internal phospho-ERK as well as phospho-Akt expression in a dose-dependent condition that may have a significant role in the prevention of mouse angiogenesis as a natural inhibitor [138].

Table 3: An overview of the mechanisms deregulation MMPs by oncoviruses

Virus	Viral Factor	Number MMP	Mechanism/Effect	References
EBV	LMP1	MMP-9	Enhanced MMP-9 expression through induction of the MMP9 promoter by recruitment transcription factors AP-1, Sp-1, and NF-kappa B on MMP-9 promoter	[42]
	LMP1	MMP-1, MMP3	Upregulated MMP-1 and MMP-3 expression in NPC tumors	[45]
		MMP-9	Upregulated MMP-9 expression correlation with lymph nodes metastasis and clinical stage	[44]
	Zta	MMP-9, MMP-3	Overexpressed MMP-9 and MMP-3 expression in epithelial cells derived from NPC tumor	[48]
	EBNA3C	MMP-9	The interaction between EBNA3C and Nm23-H1 upregulated expression of MMP-9	[53]
	LMP2A	MMP-9	Upregulated MMP-9 expression by activation of extracellular signal-regulated kinase (ERK)-1/2 and a downstream transcription factor Fra-1	[56]
	Zta		Zta protein also up-regulated TIMP-1 gene expression in EBV-infected B cells and EBV-immortalized LCLs	[49]
HPV	E7	MT-1 MMP	E7 protein of the high-risk HPV types 16 and 8 lead to the induction of MT-1 MMP in in HaCaT cells	[65]
	E2	MMP-9	Increases the expression of MMP-9 in human keratinocytes and organotypic skin cultures	[66]
	E6/E7	MT1-MMP, MMP-2 and MMP-9	Upregulated expression of MT1-MMP, MMP-2 and MMP-9 in cervical cancer	[67]
		MMP-2, MMP-9	Up-Regulates MMP-2 and MMP-9 Expression and Activity by Inducing Interleukin-8 in Lung Adenocarcinomas	[69]
		MMP-8	Persistent oral HPV infection was associated with a low salivary MMP-8 concentration	[71]
		MMP-2, MMP-9, MMP-13 and MT1-MMP	Increased expression of MMP-9, MMP-13 and MT1-MMP in HPV8-positive mice than HPV8-negative animals	[72]
		MMP-2	A significant relationship was observed between the −1306C>T functional polymorphism in MMP-2 gene and HPV infection in increasing the cervical cancer risk	[73]
HBV	HBX	MT1-MMP, MMP-2	Up-regulated MT1-MMP expression by cyclooxygenase-2 (COX-2) activity and activates MMP-2	[76]
	HBX	MMP-9	Increased expression and regulate the transcriptional activaton of MMP-9 by activation of ERK and PI-3K signal pathways	[77]
	HBX	MMP-3	Up-regulated thetranscription,translationandsecretionofMMP-3	[78]
HCV	CORE AND NS5A	MMP-9	Up-regulated expression of MMP-9 with the synergistic effect of endotoxin and proinflammatory cytokines during chronic HCV infection	[103]
	E2	MMP-2	Up-regulation of MMP-2 by interaction between E2 and CD81and E2/CD81 interaction induced the up-regulation of MMP-2 by increasing activator protein-2/DNA binding activity via ERK/MAPK phosphorylation	[104]
	NS3	MMP-9	Activates MMP-9 expression by activates PKD2, JNK, and ERK1/2 to regulate NF-κB which regulates COX-2 expression , finally cox-2 activates mmp-9 expression Activates ERK1/2–p38 signal pathway to regulate NF-κB activity, stimulates the translocation of NF-κB from cytosol to the nucleus and the binding of NF-κB to the MMP-9 promoter	[114]
KSHV	vGPCR and vFLIP	MT1-MMP	Increases of MT-MMP by induced EMT through activation of Notch pathway, leading to gain of MT1-MMP	[120]
		MMP-14	KSHV reduced prox-1 expression(Prox-1 binds to MMP14 promoter and represses its transcription),therefore prevents the repression of MMP-14 by prox-1 and induces mmp-14 expression	[121]
HTLV-1	TAX	MMP-2 MMP-9	Induces the gelatinase activity of MMP-9 and MMP-2 in endothelial cells and downregulates the tissue inhibitor of MMP	[134]
		MMP-9 MMP-3	Increases expression of MMP-9 and MMP-3 bysecreted proinflammatory cytokines (IL-1alpha, IL-6, TNF-alpha) by infected glial cells with HTLV-1	[134, 135]

Moreover, the results of a study by LU et al. provide molecules for the molecular signaling basis of the effects of newly synthesized quinazolinone MJ-29 on the inhibition of metastasis and invasion that could be beneficial as a therapeutic strategy to treat human oral cancer [139]. Accordingly, it can be concluded that 3-azidoWA and MJ-29 as natural MMP-9 inhibitors may have a more effective inhibitory ability on HBV, and HCV-related cancers by attenuating the internal phospho-ERK and phospho-Akt protein expression, which led to the repression of ERK and PI-3K signal pathways.

Other natural compounds such as curcumol via activation of JNK1/2, Akt, and nuclear translocation of NF-κB p65 [140], as well as magnolol through the inhibition of TNF-α by suppressing NF-κB binding activity [141], both caused the reduction of MMP-9 expression in MDA-MB-231 cells, breast cancer cell metastasis, and human urinary bladder cancer 5637 cells, respectively. Meanwhile, there is a possibility that these natural inhibitors can also modulate MMP-9 protein expression through multiple signaling pathways such as JNK, Akt, and TNF-α in HCV-related cancers. Antcin-H, a pure compound that is isolated from A.cinnamomea, can downregulate MMPs through inhibition of the FAK-ERK-C/EBP-/β/c-Fos-MMP-7 pathway, especially MMP-7 expression in renal cancer cell [142] (Table 4).

Table 4: Natural and synthetic inhibitors of MMPs in inhibiting targets of major signaling pathways in various types of cancer

Inhibitor	Class	Selectivity	Type of cancer studied	Inhibiting targets of major signaling pathways	Reference
Batimastat	Peptidomimetic	Broad spectrum	malignant ascites and malignant pleural effusion		[33]
Marimastat	Peptidomimetic	Broad spectrum	Breast cancer, small cell lung cancer, on-small cell lung cancer		[33]
BB-2516	Peptidomimetic	Broad spectrum	Head and neck squamous cell carcinoma (HNSCC)	through interference with autocrine loops in EGFR/c-erbB signaling pathways	[143]
BAY 12-9566	Nonpeptidomimetic inhibitor	MMP-2, MMP -3	advanced ovarian cancer, Breast cancer		[167]
AG3340	Nonpeptidomimetic inhibitor	MMP-2, MMP -3	non-small cell lung cancer		[168]
BMS-275291	Nonpeptidomimetic inhibitor	MMP-2, MMP -9	Non-small-cell lung cancer, prostate cancer, colorectal cancer, Breast cancer		[169, 170]
CGS 27023A	Small molecule	Broad spectrum	Advanced solid cancer		[33]
Col-3 (metastat)	Chemically modified tetracycline	MMP-2, MMP -9	Soft-tissue sarcoma, AIDS-related Kaposi's sarcoma, high-grade gliomas		[171, 172]
Prinomastat	Small molecule	Broad spectrum	Non-small cell lung cancer, , prostate cancer		[136]
Tanomastat	Small molecule	MMP-2, MMP-3, MMP-9	Ovarian cancer, adenocarcinoma of the pancreas, non-small cell lung cancer		[136]
Doxycycline	Tetracycline derivative	Broad spectrum	polycystic ovarian cancer		[136]
SB-3CT	Small molecule	MMP-2, MMP-9	T-cell lymphoma and prostate cancer models		[173]
ND-322	Small molecule	MMP-2/MT1-MMP	melanoma		[174]
curcumol	Major active components in the essential oil of RhizomaCurcumae	MMP-9	Breast cancer cell metastasis	via JNK1/2 and Akt(Ser473)-dependent NFκB signaling pathways	[140]
3 -azidoWA	derivative of Withaferin A	MMP-2	human cervical HeLa and prostate PC-3 cells	concomitant marked reduction in pAkt and pERK signaling by modulating extracellular par-4	[138]
MJ-29	2-phenyl6-pyrrolidinyl-4-quinazolinone derivatives	MMP-2/9	Oral squamous cell carcinoma	Down-regulation of MAPK and AKT Signaling	[139]
antcin-H	pure compound isolated from A. cinnamomea	MMP-7	Renal Cancer Cell	Partly through Inactivation of FAK-ERK-C/EBP-/β/c-Fos-MMP-7 Pathways	[142]
magnolol	the main constituent of Magnoliaceae	MMP-9	human urinary bladder cancer 5637 cells	Inhibition TNF-α-induced MMP-9 expression by suppressing NF-κB binding activities	[141]

The presence of MMP-inhibitory effects in virus-related cancers is still unknown. It has been determined that BB-2516, among all synthetic inhibitors, decreases the protein expression of a broad spectrum of MMPs by interfering with autocrine loops of EGFR/c-erbB signaling pathway in head and neck squamous cell carcinoma (HNSCC) [143]. However, more studies are necessary to understand the connections between MMP inhibitors and these signaling pathways in oncovirus-related cancers, making it especially important to examine how the relationships are.

CONCLUSIONS

In summary, matrix metalloproteinases as cellular proteases play the main role in biological processes as well as pathological conditions, including tissue repair, wound healing, developmental morphogenesis, inflammatory diseases, and tumor progression. Oncoviruses, in many cases, promote tumor progression through multiple mechanisms that affect the expression of these cell proteases. As oncoviruses upregulate the expression of MMPs genes through direct (via DNA binding) as well as indirect interactions (via activating PI-3K/AKT, ERK/MAPK, IFN/JAK/STAT, JNK, NF-κB signaling pathways or immune response), causing escape from the immune system and play a key role in cell proliferation, migration, angiogenesis, EMT, invasion, metastatic features, and subsequent tumor progression. Today, several compounds have been studied as matrix metalloproteinase inhibitors. Various studies have shown that these inhibitors activate the immune system and suppress tumor progression by inhibiting signaling pathways activated by viruses. With these interpretations, these cell proteases may be used as biomarkers to detect different stages of the development of oncovirus-related tumors, soon followed by inhibitors of these proteases in the treatment of viral tumors.

Conflict of interest

The authors report no conflicts of interest.

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Ethics approval and consent to participate

The study protocol was reviewed and approved by the ethics committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1399.520).

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REVIEW

Vascular Changes in Hypertension: Is There Cell Death Involved? – A Narrative Review

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Abstract: *Background: Cardiovascular disease can be triggered and accelerated by hypertension and the underlying changes in the structure and function of arteries. The purpose of this review is to explore some vascular changes that occur in hypertension as a consequence of the imbalance between cell proliferation and cell death, processes that play an important role in stabilizing the thickness of the arterial wall during vascular remodeling. Methods: The authors conducted research through PubMed and Google Scholar databases using the following search formula: (hypertensive*) AND ((vascular modifications)) OR (vascular changes)) AND (cell death). Results: From 40 articles, only 17 publications were included in this study, taking into account four processes that can be preceded or followed by inflammation and depend on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli: Cell proliferation and growth; Cell migration; Cell death; Degradation or reorganization of extracellular matrix. Conclusions: To summarize, maladaptive vascular changes in hypertension can represent a major argument for prompt and maximal therapeutic intervention in patients with high cardiovascular risk. Moreover, they can represent an important step in discovering new markers of cardiovascular risk and in the development of new targeted therapies for different pathways of cellular signaling through which the reversibility of abnormal vascular changes could be obtained.*

Keywords: *cell death; inflammation; cellular metabolism alteration; vascular remodeling; hypertension; pathophysiology*

INTRODUCTION

Cardiovascular disease, one of the leading causes of death worldwide, can be triggered and accelerated by hypertension and the underlying changes in the structure and function of arteries. Knowing the pathophysiological mechanisms that are responsible for the maladaptive changes occurring at the vascular level in the context of hypertension, can represent a first step in the development of new targeted therapies for different pathways through which the reversibility of the abnormal vascular changes can be obtained.

Some studies have shown that hypertension linked to the imbalance between cell death and cell proliferation could explain vascular remodeling: accelerates endothelial apoptosis, promotes cell migration, and accelerates the proliferation of vascular smooth muscle cells [1] resulting in the thickening of the middle tunic of the arteries [1,2]. The role of cell death in blood pressure charge is still unknown [3]. The prevalence of hypertension increases with age and the proposed pathophysiological mechanisms are represented by hemodynamic changes, arterial stiffness (due to elastic lamina disruption and intimal hyperplasia), and neurohormonal and renal dysfunction [4]. In the elderly, vascular injuries can heal through the formation of vascular scars due to the increase in the proliferation of smooth muscle cells and the reduction of their apoptosis and decrease in sensitivity to apoptotic stimuli in fibroblasts and myofibroblasts, with secondary fibrosis [5,6].

In terms of the cell death process, some major pathways have been described (apoptosis, necrosis, and autophagy), depending on the injury and the type of cell [7]. Programmed cell death and necrotic cell death sometimes have mechanisms that can be overlapped [5]. The initiation of apoptosis is the result of death signaling pathways, intrinsic (mechanisms triggered by cellular stress through hypoxia, oxidative stress, and ADN damage) or extrinsic (mediated by transmembrane receptors of the TNF receptors family). In apoptosis, an

essential process in maintaining tissue homeostasis, as a result of caspase cascade activation and imbalance between pro and antiapoptotic factors, the cell is broken for-ming membrane-bound vesicles and apoptotic bodies (after the shrinkage of the cell, fragmentation of nucleus and chromatin and plasma membrane blebbing [7]) responsible for clearance of dead cells and also for intercellular communication [8]. Necrosis is an accidental form of cell death but in certain instances is initiated or modulated under programmed control mechanisms. Unlike apoptosis, which requires energy consumption, necrosis is a process in which cells die without energy consumption; in the necrotic cell death process, as a result of the breaking of the cell membrane and the elimination of the cell content in the extra-cellular space, inflammation occurs. It has been described as a form of programmed necrosis (necroptosis), caspase-independent, with mitochondrial changes involvement [5]. Another type of cell death, autophagy, is the process through a staving or deprivation of growth factors cell generate energy and metabolites by digesting its organelles or macromolecules. There were also described multiple sub-types of cell death, depending on caspase involvement or the presence of some of the necrosis criteria: aponecrosis, paraptosis, anoikis, cornification, etc [5].

Recent studies examine how certain factors affect endothelial apoptosis in patients with hypertension and other comorbidities [1,4,9]. Endothelial cell apoptosis was found in patients with hypertension and peripheral arterial disease by studying endothelial cell cultures [9]. Vascular cell re-modeling, an active process, involves changes in at least 4 cellular processes: cell growth and cell migration, inflammation, cell death, and degradation or reorganization of the extracellular matrix. It is dependent on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli [10].

Although the in vitro studies [11] demonstrated a relationship between the level of some plasma membrane receptors involved in apoptosis induction (named death receptors) [12] and the phenomenon of apoptosis, the causal link between cell death and vascular changes was not proved yet [11].

Considering that the phenomenon of cell death and cardio-vascular outcomes are linked through metabolic risk factors intervention and exogenous causes of cellular stress that lead to cell destruction via different pathways and demonstrating to what extent the alteration of cellular signaling, the alteration of the extracellular environment and the triggering of cell death mechanisms are responsible for the abnormal remodeling of the arterial wall in the hypertensive patients, the research could be directed to discover new markers of cardiovascular risk and new therapies that can be targeted in patients with cardiovascular risk factors. The benefit could be major, the measurement of death receptors levels related to cell death phenomena that can predict cardiovascular outcomes and death [11], in patients with high cardiovascular risk, became a major argument for prompt and maximal therapeutic intervention in this specific populational group.

The purpose of this review is to explore some vascular changes that occur in hypertension as a consequence of an imbalance between cell proliferation and cell death, processes that play an important role in stabilizing the thickness of the arterial wall during vascular remodeling.

MATERIALS AND METHODS

A comprehensive search through PubMed and Google Scholar using the following search formula: (hypertension*) AND ((vascular modifications)) OR (vascular changes)) AND (cell death) was performed. Only full-text freely available articles published in English between 2000 and 2021 were used. Articles that were most relevant to the subject of this review were included after thorough consideration. Inclusion criteria were published in English, publication within the mentioned period, and inclusion of hypertensive models or animal models, or cell cultures studies. From 40 articles, only 17 publications were included in this study. All the selected papers were reviewed by an independent reviewer to check that the interpretations were correct.

Hypertension has a great impact on the cardiovascular system; the thickness of the arterial wall during the remodeling process could be the result of cell death mechanisms triggered by factors potentially involved in the etiology of arterial hypertension. Considering that the connection between cell proliferation and cell death is responsible by a vascular re-modeling process in hypertension, we take into account for our study four processes that can be preceded or followed by inflammation and depend on the interaction between local growth factors, vasoactive substances, and hemodynamic stimuli:

- Cell proliferation and growth
- Cell migration
- Cell death
- Degradation or reorganization of extracellular matrix

RESULTS

The search strategy identified 40 articles of which 17 were deemed to be eligible for inclusion in this systematic review. Of these, 12 studies addressed markers of cell proliferation/growth, 7 studies addressed cell migration, 12 studies addressed cell death processes/markers, 7 studies focused on

the degradation of extracellular matrix, and 13 studies addressed inflammation markers (shown in Table 1).

Table 1: Type of processes involved in vascular remodeling

Reference	First author, year of publishing	Study type	Pathophysiological mechanisms, Type of process					
			Arterial remodelling	Inflammation	Cell proliferation/ cell growth	Cell migration	Cell death	Reorganization/ degradation of extracellular matrix
1	Brown, I.A.M *et al,* 2018	Review	X	X	VSMC hypertrophy and hyperplasia	X	X	
2	Xu, C. *et al,* 2001	Experimental study	X		X		X	
16	Martinez-Quinones, P. *et al,* 2018	Review	X	X	VSMC hypertrophy and hyperplasia	X		X
10	Renna, N.F. *et al,* 2013	Review	X	X	X	X	X	X
21	Schiffrin, E.L. 2012	Review	X	X	VSMC hypertrophy and hyperplasia		X (apoptosis; anoikis – in endotelial cells)	X
19	Virdis, A. *et al*, 2014	Review	X	X	X	X		
15	Sun, H.J *et al,* 2020	Review	X			X		X
18	Munoz-Durango, N. *et al,* 2016	Review	X	X	X	X	X	X
17	Bruno, R.M. *et al*, 2017	Research article	X	X	X		X (apoptosis)	
3	Abdelbary, M. *et al*, 2019	Comparative study		X	X		X (necrosis, apoptosis)	
22	Intengan, H.D.; Schiffrin, E.L. , 2001	Review	X	X	X		X (apoptosis)	X
9	Gardner, A.W. *et al*, 2019	Randomized Controlled Trial		X			X (apoptosis)	
26	Krishna, S. M. *et al*, 2021	Randomized Controlled Trial	X	X			X (apoptosis)	X
27	Fekete, A. A. *et al*, 2016	Randomized Controlled Trial	X	X				
28	Li, X. *et al*, 2022	Review	X	X	X	X	X (apoptosis)	X
32	Xu, W.;Erzurum, S.C., 2011	Review	X		X		X (apoptosis)	
33	Levy, M. *et al*, 2007	Clinical research	X	X	X		X (apoptosis)	

In hypertension, the connection between cell proliferation and cell death could be critical in determining the thickness of the arterial wall during the remodeling process. There are 2 types of criteria for classifying cell remodeling:

- according to the size of the middle tunic: hypertrophic, eutrophic, and hypotrophic
- according to the size of the lumen: internal and external remodeling.

The consequences of cell remodeling are:

- high pulse pressure,
- stiffening of the arterial wall
- changes in the extracellular matrix.

All consequences of cell remodeling are determined by the

release of growth factors and cytokines. The immune system, namely T lymphocytes, plays a key role in remodeling, more precisely based on a T-reg/T-effector imbalance.

Because of its ability to modify the vascular tone, vascular endothelium plays a significant role in hypertension. Endothelial dysfunction is caused by a reduction in nitric oxide (NO) availability, which causes endothelial dysfunction. The main cause of the decline in NO is the excessive production of reactive oxygen species (ROS)[13].

Blood pressure is affected by the phenomenon of cell death that can occur at different levels of the arterial wall, however, in hypertension the role of different types of vascular cell death is unclear. Researchers discovered that vascular smooth muscle cell growth is increased by cell death [1]. The cell death process is thought to play a key part in remodeling, but it is unclear whether it is the main process or a growth-related compensatory mechanism, being influenced by reactive oxygen species, NO, angiotensin type 2 (AT-II), and endothelins.

Due to vascular damage, elevated blood pressure values may alter the integrity of the endothelium and may also represent a risk factor for the onset and progression of vascular atherosclerosis changes leading to the destabilization of atheroma plaques [14]. Atherosclerosis favors the processes of inflammation and necrosis of the blood vessel and plaque can become unstable without proper treatment and cause even more complications.

DISCUSSION

In our study, we collected a lot of information about vascular modifications in hypertension. From the 17 selected articles, in 15 we found information about vascular remodeling (88.23%), in 14 articles we found arguments for the presence of the inflammatory phenomenon (82.35%), 13 articles (77.47%) argued for the presence of cell death and also 13 described cell proliferation, 7 articles (41.17%) described the phenomenon of cell migration, and 8 articles (47.05%) described reorganization of the extracellular matrix.

Starting from the information provided by the articles included in our study, our hypothesis related to the involvement of cell death at the vascular level in arterial hypertension seems to be valid. Maintaining a balance between the processes of cell proliferation, cell migration, cell death, and degradation of the extracellular matrix could ensure adequate vascular elasticity and delay the installation of specific hypertensive vascular changes. It seems that cells from all layers of the vascular wall are involved and can be affected by different types of death, the damage to each cell type leading to cellular metabolic changes and changes in the extracellular environment.

Vascular tone and vascular remodeling

The endothelial cells which form the tunica interna of the arterial wall, are essential for regulating hemostasis, vascular tone, and host defense [15]. Secretion, protection as a barrier, and local metabolism are the endothelium's three primary activities, each of which is altered by hypertension. The endothelial cells produce vasoactive substances such as nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H2S)[15], prostacyclin (PGI2), endothelin 1 (ET-1), AT-II, prostaglandins. Normally, there is an equilibrium between these substances to maintain proper blood flow. In hypertension, at the vascular level is an increased production of vasoconstrictor factors (ET-1, AT-II, prostaglandins) which determine endothelium dysfunction (increased permeability, induce inflammation, and edema). It was discovered that the substances and structures that are released from a cell when it dies can affect the endothelial function in an autocrine, paracrine or endocrine model [16].

The endothelium is important for both short-term vascular tone modulation and long-term vascular remodeling. Nitric oxide is the most significant endothelium-derived vasodilator molecule which, under healthy conditions, is capable of inhibiting the primary fundamental pathways driving the formation of atherosclerosis and thereby improving vascular health. Reduced NO availability and endothelial dysfunction are caused by NO breakdown by reactive oxygen species (ROS) in physiological aging and many pathological diseases, including arterial hypertension.

Essential hypertension patients (HT) had a lower endothelial response to acetylcholine (Ach) than normotensive people (NT) in each age group, but it was proved that the vascular response to Ach in both HT and NT patients decreases as they become older [17]. In the pathogenesis of endothelial dysfunction and the cardiovascular consequences related to endothelial inflammation [18], apart from the effects of nitric oxide and carbon monoxide, the role of hydrogen sulfide has been also described. H2S formation was detected in smooth muscle cells, vascular endothelial cells, and cardiomyocytes and it was shown that H2S could protect endothelial cells against apoptosis by attenuating the overproduction of ROS and inhibiting mitochondrial apoptosis [15].

The vascular smooth muscle cells (VSMCs) that form tunica media are responsible for vasoconstriction, vasodilatation, and the process of remodeling [19]. The hyperplasia and hypertrophy of VSMCs are the processes that underlie vascular remodeling. The most frequent type of remodeling in hypertension is the inward type causing the narrowing of the arterial lumen. The outward type of remodeling occurs during anti-hypertensive treatment and is also caused by increased

blood flow[16]. Some studies have revealed that a marked cell death influences and accelerates the proliferation of vascular smooth muscle cells, resulting in the thickening of the middle tunic of the arteries [2]. As a result of cell death through necrosis and the breakdown of plasma membrane integrity, the contents of necrotic cells are released, triggering a proinflammatory response [3].

Fibroblasts that form the tunica externa secrete chemokines, cytokines, and growth factors that contribute to normal vascular function and participate in the degradation of the extracellular matrix components. They play an important role in remodeling as a response to injury. However, fibroblasts suffer structural changes that are accompanied by proliferation and migration into the tunica media during hypertension [16].

Cell remodeling

Cell remodeling involves cell growth and cell migration, cell death, and degradation of the extracellular matrix and is dependent on multiple factors [10]. The media-to-lumen ratio (M/L), modifying the vessel wall width for increasing muscle mass, the reorganization of cellular and noncellular elements and the lumen dimensions are the most prominent manifestations of these adaptations [20]. As a result, remodeling can be classified based on the degree of change of the medium arterial tunic in hypertrophic, which is the most common, hypotrophic, or eutrophic remodeling, as well as the size of the lumen in internal or exterior remodeling [21]. With age, large arteries undergo outward hypertrophic remodeling and stiffening [4], and in hypertension, this process may be accelerated, resulting in higher pulse pressure [22-24]. The elastic laminae, on the other hand, develop multiplication and fragmentation in severe hypertension, with increased deposition of collagen and fetal fibronectin, leading to greater stiffness [23]. Small artery remodeling has typically been associated with increased media thickness in hypertension, but new studies have shown that there are two forms of remodeling, inward eutrophic and inward hypertrophic, depending on whether the media cross-sectional area is enlarged [23]. The discovery that inward eutrophic remodeling may be prominent in some kinds of hypertension has raised the prospect that countervailing mechanisms in hypertension, including apoptosis, may compensate for or modify growth [23,25]. The outer and lumen diameters are decreased, the media cross-sectional area is unchanged and the M/L is increased without stiffening in inward eutrophic remodeling [25]. Inward eutrophic remodeling reduces the outer diameter of the vessel through a mix of growth and apoptosis, with apoptosis concentrated to the periphery, whilst inward growth decreases lumen diameter, which may explain the preservation of media volume. It is uncertain whether apoptosis is a growth-related compensatory mechanism or a primary process[25]. Mechanical forces exerted on the arterial wall also play a significant role in remodeling. Changes in the extracellular matrix, the cellular release of growth factors and cytokines, and vascular sensitivity to circulating humoral factors are all caused by them. Force duration, load, and force properties all have an impact on all of these factors [1].

Low-grade inflammation

In hypertension and cardiovascular disease, low-grade inflammation plays a critical pathogenic function [26-28]. Innate and adaptive immune systems, specifically T cells, are implicated, according to a significant body of research. T-lymphocytes play a key role in the development of hypertension-related vascular remodeling. The balance between T-effector and Treg lymphocytes is a critical regulatory system that, when disrupted, favors blood pressure increase and the development of organ damage [21]. Treg lymphocytes have potent anti-inflammatory characteristics, ensuring vascular homeostasis and protecting the arterial wall against the onset of atherosclerosis [21,29]. In humans, the majority of data establishing essential hypertension as a chronic low-grade inflammatory state found a clear and independent link between c-reactive protein (CRP), tumor necrosis factor (TNF), Interleukin 1 beta (IL-1 beta)[30], Interleukin-6 (IL-6), and adhesion molecules and vascular alterations in essential hypertensive patients [21].

An important role in the process of vascular remodeling is played by the renin-angiotensin-aldosterone system (RAAS)[29,31] because of its role in VSMC migration and inflammation [24,32]. This system acts through 2 types of angiotensin II receptors AT1-R and AT2-R, but the AT1-R is thought to be the primary receptor that causes Ang II's proliferative and profibrotic actions [20,33]. The AT2-Rs are thought to cause opposite effects, preventing vascular deterioration. But there are times when AT2-R acts similarly to AT1-R (for example, in renovascular hypertension it causes tonic renal medullary vasoconstriction) [32,34]. Recent studies showed that there exists an AT1-R autoantibody (AT1-AA) which is an agonist of AT1-R. AT1-AA raises AT2-R expression and encourages cell migration through cytoskeletal remodeling [34]. The RAAS is implicated in physiological and pathological actions when it comes to vascular changes. It reduces ROS generation and modifies vascular tone via vasoconstriction and vasodilatation. During hypertension, this RAA system induces fibroblast proliferation, collagen deposition, and vascular hypertrophy [20].

Cell death

Apoptosis is a type of gene-controlled cell death that was first discovered in development as a mechanism for fine-tuning

growth. The vascular system has a large number of apoptosis modulators. Reactive oxygen species, NO, angiotensin type 2 receptors, and the endothelin system are all possible candidates. Reactive oxygen species play a key role in the pathogenesis of hypertension and vascular cell apoptosis [35], with O_2^- inducing proliferation and H_2O_2 inducing apoptosis through a protein kinase C–dependent pathway, respectively. Angiotensin II is also a potential apoptotic trigger [25,31,36].

Recent studies examine how certain factors affect endothelial apoptosis in patients with hypertension and other comorbidities. Endothelial cell apoptosis was found in patients with hypertension and peripheral arterial disease (PAD) by studying endothelial cell cultures. They demonstrated that physical activity can lower this type of cell death[9]. PAD patients present increased cardiovascular stress, inflammation, and oxidative stress. Inflammation and oxidative stress speed up the progression of myopathy and increase the risk of apoptosis and sarcopenia. In older persons, exercise decreases oxidative stress and improves reactivity to oxidative processes [9].

Inflammation, VSMC apoptosis, and oxidative stress in patients with hypertension [1,18,19] can also play an important role in the pathology of abdominal aortic aneurysm AAA [37,38]. According to some studies [39] a member of the seric proteinase inhibitor superfamily, Human Kallistatin, acts as a protective factor by blocking the pathway that stimulates AAA generation [39], and even whey-protein supplementation has a beneficial effect, improving vascular function by inhibiting the serum angiotensin-converting enzyme [40].

Lesions that appear on the arterial wall and the dysfunction of endothelial cells determined by high blood pressure values are factors that contribute to atherosclerosis. In the injured artery intima, extra low-density lipoprotein (LDL) accumulated suffer processes of oxidation and can attract monocytes and macrophages, forming the foam cells. The local inflammatory reaction is severe [41] and it triggers a cascade of events [38,42]. Foam cell secretion, deposition, and apoptosis [43] are crucial steps in the occurrence of atheroma plaque [44]. In advanced stages, the macrophage function of phagocytosis is destabilized, the atheromatous plaque continues to grow and, in the end, it will become unstable, promoting local inflammation and necrosis without the proper treatment [41]. Studies have revealed that at the cellular level microRNA plays a role in the control of atherosclerosis [41,45], acting on the TGF-β signaling pathway, inducing proliferation, migration, and apoptosis of endothelial cells, VSMCs, and macrophages [37,43,46].

Sometimes, associated with long-term uncontrolled hypertension and left ventricular dysfunction (or other conditions such as left heart disease, thromboembolic pulmonary condition, lung disease, liver disease, hematologic disease, metabolic disease, connective tissue disease, human immunodeficiency virus infection, thyroid diseases, etc.), pulmonary hypertension may occur [47,48]. In this situation, through the lungs arteries that have a reduced lumen and thickened walls, blood will flow slowly. Pulmonary arterial hypertension (PAH) is defined by mean pulmonary arterial pressure (mPAP)>20 mmHg at rest assessed by right heart catheterization [47-49]. Histopathological changes at the pulmonary level are neointima formation in pulmonary arteries, wall thickness, and plexiform lesions formed by the proliferation of endothelial cells. There is an increased level of ROS that contributes to oxidative stress and vascular remodeling [50], similar to the changes found in the arteries of hypertensive patients. It has been discovered that there is a deficient activity of NO-synthase, the level of NO is low and so are the vasodilatory effects. The role of proapoptotic and antiapoptotic proteins has been incriminated in the initiation of the pathogenesis of PAH and the proliferation of apoptosis-resistant vascular cells [51,52]. Both reversible and irreversible PAH express the apoptotic markers (caspase-3 and p-53) [51,53], while irreversible PAH expresses BCL-2 only in severely wounded endothelial cells [51]. Apoptosis plays an important role in pulmonary hypertension among the proliferation of VCMCs and plexiform lesions [51,52].

In our study we highlighted some evidence in favor of the hypothesis that cell death is involved and has consequences on vascular changes in people with arterial hypertension; the vascular changes occurring in the hypertensive patient may be due to the alteration of the cellular metabolism, which may represent a triggering factor of cell death processes by activating abnormal cellular signaling pathways. However, there are insufficient data to show the cell death types involved and the types of cells in the vascular walls involved in the process of vascular remodeling, therefore we consider that further studies are necessary to elucidate these aspects

CONCLUSIONS

Cell death and cardiovascular outcomes seem to be linked through metabolic risk factors intervention and exogenous causes of cellular stress. Demonstrating that alteration of cellular signaling, the alteration of the extracellular environment, and abnormal cell death mechanisms are responsible for the abnormal remodeling of the arterial wall in hypertensive patients could direct future research toward the identification of new molecules as plasma membrane receptors involved in apoptosis and new therapies that can be targeted in patients with cardiovascular risk factors. Once the vascular impact of imbalances between cell proliferation and

cell death will be demonstrated, its early detection could become an argument for maximum therapeutic intervention in this population group.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

Authors' contribution
EB conceived of the presented idea. AMD, EB contributed to documentation on the topic of interest, work conception and revised the manuscript. EB, ŞSB, OA performed the final revision of the manuscript. All authors discussed the results and contributed to the final manuscript. ŞSB and OA gave final approval. All authors have read and agreed to the published version of the manuscript. EB, ŞSB, OA had equal contributions.

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ORIGINAL ARTICLE

Is Still Neurology Desirable as a Specialty for Aspiring Physicians?

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Abstract: *This article aims to highlight the burnout burden among neurologists and provide information on some of its causes and consequences. We report an original article that reviews our current understanding of burnout matter in the neurology field. The main search engine through which we performed extensive literature research was PubMed, Scopus, and Google Scholar. The following information was collected: triggers of burnout and factors that perpetuate it, major complaints, values, and goals that dictate how coaching with it. Physician burnout is an umbrella term characterized by symptoms determined by exposure to chronic workplace stressors. Simply put, it encompasses many dimensions: emotional exhaustion, feelings of cynism, detachment (depersonalization), and a sense of ineffectiveness at work (low personal accomplishment). Burnout is common among all medical specialties, but neurology ranks at the top of the list. Several identified factors continue to fuel this problem. Properly addressing them, rethinking practice, and implementing wise, modern strategies can make significant contributions to reducing it. Aside from the chronic harm to the physician, burnout puts at risk the healthcare community at many levels including patients, other professionals, finances, etc. This paper wants to emphasize that burnout in neurology should be regarded as a global crisis. Thus, some strategies are proposed.*

Keywords: *neurology, burnout, work-life, emotional exhaustion, stress, fatigue, depersonalization, second-victim phenomenon, complaints, functional neurology*

INTRODUCTION

Physician burnout is an umbrella term characterized by symptoms in response to prolonged exposure to chronic stressors related to the workplace. This topic attracted interest from researchers since it exhibits a concerning prevalence. In the field of medicine, we can consider burnout a pandemic that has a negative impact both on doctors and their patients, but most of the time this reaches far beyond that, to families and the overall healthcare system. All medical specialties are affected, but some of them carry a higher percentage of burnout. Over 40% of general physicians experienced at least one symptom of burnout, neurology being ranked the second specialty affected, with more than 60% of neurologists signaling symptoms [1,2]. Medscape 2022 report on Physician Burnout and Depression showed that 47% of neurology specialists are experiencing mental health challenges, which represents a substantial increase in its prevalence compared with the previous year's reports when it was 42% [3]. The purpose of this article is to highlight the burnout burden among neurologists and provide information on some of its causes and consequences.

BURNOUT AND NEUROLOGY

To date, many research studies have contributed to a comprehensive overview and understanding of the root causes that fuel physician burnout.

The prevalence of neurological disorders increased in the last two centuries and the increased burden that comes along can be attributed to explosive population growth, people reaching older ages, unhealthy lifestyles, and unawareness of the importance of prophylactic measures [4].

In opposition to this growth, the global shortage of neurologists led to inequalities in the availability of patient care with those already existing clinicians being overwhelmed. The Association of American Medical Colleges biannually

investigates the number of patients per active physician. Reports published two years ago indicated that the number was higher in neurology (23.429) than in ophthalmology (17.162), cardiovascular diseases (14.717), radiology (11.896), psychiatry (8.544), emergency medicine (7.332), internal medicine (2.758), and is approaching specialties with the highest burnout rates such us urology (32.490) [5]. Thus, the low number of practicing neurologists per patient increases the amount of work, compromises patient care, and exacerbates burnout.

Switching to professional working hours per week, an interesting study concluded that neurologists worked 57.1 hours per week, with 42.3 hours being spent performing inpatient activities. In our neurology department, the average number of professional working hours per week is 59, with a peak of 100 on rare occasions (absenteeism of other colleagues because of medical conditions/vacations or lack of staff) [6].

From a holistic point of view, burnout can be regarded as an indicator of quality and duration of life, given that it has a direct damaging effect on health: overnight shifts, exposure to all kinds of infective agents, irregular and exaggerated effort, lack or inadequate physical exercise, emotional detachment, interrupted meals or maladaptive coping strategies such as excessive caffeine intake or other power drinks, alcohol, and drug abuse. Johann Ludwig Casper, a prominent name in forensic medicine stated that there is no profession with a comparable physical and mental force demand as the medical one, an observation comparable with recent studies [7]. It is also worth mentioning that being on call requires working very fast or accomplishing more tasks being short on time. Very similar ideas are presented in a meta-analysis of 17 studies that in people with high demands, the risk of mortality is 20% higher than in the reference group [8]. Similar opinions are found in a study involving 1.671 American neurologists, where 60.1% of the responders had at least one symptom of burnout [2].

According to the World Health Organisation and International Classification of Diseases, burnout has become 2019 a legitimate medical disorder and has been given its ICD-10 code (Z73.0 - Burn-out state of vital exhaustion). Burnout is a state of chronic stress, that occurs after the body system is repeatedly triggered. It can exacerbate mental conditions like anxiety disorders, depression, and sleep problems. Some authors focused on the cortisol secretion parameters and concluded burnout is a mental syndrome with unique pathophysiology [9]. Moreover, as it often happens, the presence of a disease leads to a higher chance for another one to develop. Burnout influences not only subjective well-being but other biological processes as well. In a large study of nearly 9000 employed adults, was found that burnout is a significant risk factor for developing coronary heart disease and finally, heart attacks [10]. These findings support the idea that physicians with high demands as neurologists are exposed to challenges regarding their health and are more vulnerable to stress and burnout.

There is an excessive workload and clerical duties, including finishing notes, phone calls, ordering tests and reviewing results, responding to phone calls and patient requests, and prescribing medication. Assessing indicators related to burnout in a national survey on 9561 neurology residents and fellows after studying 354 responses to the survey the following was revealed: regarding the amount of time spent on clerical tasks directly related to patient care" only 26.2% of residents and 29.7% of fellows agreed that it is reasonable [2]. Another particular challenge is finding meaning in work and this often translates to helping others. Some neurological disorders do not come with treatments or cures or are associated with a significant burden of disability. In the same study, in the matter of, satisfaction with the job" only 68.9% of residents and 63.1% of fellows were more likely to be satisfied with their career choice and in line with, work being meaningful", 78.8 % of residents and 85.6% of fellows had positive feelings towards their job [11]. In comparison to attending physicians, an important number of neurology residents experience burnout due to extra workload and the challenges that come with a new environment, 73% of residents mentioned at least one symptom from the constellation of burnout. Furthermore, both residents and practicing physicians are affected by the second-victim phenomenon, which refers to feelings of guilt caused by medical errors which result in poor patient outcomes [12,13].

Some authors focused instead on sources and outcomes of burnout, on potential interventions to treat it or to prevent the onset. It must be mentioned that burnout is a consequence of systemic deficiencies rather than a result of poor uncoping skills. Increasing trainee resilience and cutting clerical tasks through hiring administrative workers or through using user-friendly technology [14], positive reinforcement and support during residency, and implementing regular burnout evaluations such as Maslach Burnout Inventory. The last one mentioned is the most widely accepted evaluation of burnout and outlines three key aspects of this response: the state of feeling emotionally worn out, feelings of cynicism toward work, and a personal sense of inefficiency in the workplace [15].

The supply-and-demand issue that perpetuates burnout and challenges patient care in neurology could be overcome by recruiting other medical staff. For example, nurse practitioners could get in charge of some medical tasks, without actually

exceeding their abilities, as the healthcare oath foremost promotes abstaining from all intentional wrong-doing and harm. This topic with potential usefulness has already been addressed in an article published in the JAMA journal, but it aroused a lot of controversies [16]. Most complaints came from those in need of help, like physicians, stating that to figure out the pathology, prove competency, and be able to give medical assistance one has to go for over a decade in training. This amount of time is spent in school only by doctors, to fully become licensed, and no other alternative shorter programs can replace this training. But maybe they can approach it. An answer to this problem could be by enhancing nonphysician practitioners in medical student courses or by attending residents, giving them templates and free access to educational programs. In conjunction with this, in the UK for example, specialist nurses are known for their crucial role. They can choose and qualify for various neurological conditions (multiple sclerosis, Parkinson's disease, and epilepsy). Sometimes they can order tests, administer basic treatments, and in some states even prescribe medications.

Another indicator of burnout and shortage of neurologists is also projected by the difficulty in making an appointment for new patients and by lengthy waiting lists. Data from 626.000 first appointments scheduled in 2016 with 1.044 providers concluded that wait times for new patients are longer for high-demand specialties, with neurology patients waiting approximately 32.3 days for first appointments [17]. This is particularly relevant since waiting for more than a month often determines patients to cancel their appointments, and never return to the clinic with consequences resulting from delayed diagnosis and treatment. Moreover, significant information was revealed by 2021 research on NHS data conducted by Neurological Alliance – a coalition with over 80 organizations including the MS Society, Parkinson's UK, and MND Association. Thus, they showed that by the end of March 2021, over 150.000 people were waiting for an assessment by a specialist and over 10.000 of these have been waiting for over a year [18]. These numbers indicate how overwhelmed are the neurology services, putting a lot of pressure on existing physicians, and prolonging a state of excessive stress and fatigue that is burnout.

As a consequence of different workplace stressors and poor coping mechanisms, it was estimated that 10-15% of doctors will develop a problem with substance addiction at some point in their careers [19]. Regarding the neurology field, in a published survey among all specialties, answers from 470 chronic pain/neurology subjects as well, it was shown that in this category of physicians there is a prevalence of 3.7% marijuana use. The issue is not limited to illegal drugs, pain medicine, and alcohol count as well. The anonymous questionnaire indicated a prevalence of 88.6% of alcohol use in physicians from the category of chronic pain/neurology, also being the group with the highest prevalence for benzodiazepine of 15.5%. In addition to this, they found 16.3% prevalence for minor opiates and 1.9% for major opiates, hence being ranked the third specialty. Neurologists frequently come across chronic pain patients in need of benzodiazepines and opiates and somehow this exposes them to these drugs. They ranked second only to psychiatrists in the matter of the risk of using benzodiazepines [20].

Many neurologic diseases require interdisciplinary care and this sometimes creates the setting for conflict between providers offering healthcare services [21]. To reduce the burnout burden, neurologists should try to understand and develop the concept of teamwork. Literature describing the issue of individual vs. teamwork in neurology is scarce, but limited evidence suggests there are more benefits from the adoption of a teamwork approach, where feasible. By avoiding professional isolation, rethinking professional roles, and abandoning territorial behavior.

Receiving complaints is another issue that lies at the heart of burnout in neurology as well. Many of these complaints arise from the limits that go along with disabling or cureless illnesses, giving a false feeling that the healthcare workers did not do anything possible to resolve the case. This situation can be overcome by improving the informative part of the medical act with better communication skills with the patient and through offering honest expectations for patients and their families. Moreover, it is not uncommon that patients to have a low level of medical education and not understand the limits of medicine, priorities, or medical procedures, so this is another important factor that promotes complaints [22,23].

We propose some strategies for the prevention of burnout on neurology specialists (Table 1). There are three categories of factors: related to patient education, related to the workplace, and the healthcare system, and factors related to patient education. An important contributor that fuels burnout is the offering of patients by medical professionals who come across neurological diseases in their practice and whose residency programs do not provide minimum knowledge. Many of these patients have functional neurological disorders which do not always require neurologist interventions. The same problem is with incidental findings on brain imaging such as minimal white matter changes with the improbability of multiple sclerosis or other significant CNS diseases. Poor medical knowledge, not understanding the limits of medicine, medical priorities, or investigations are factors of complaints between relatives. Time-consuming work, the complexity of neurological diseases, communicating a difficult diagnosis, or spending more time doing bureaucratic tasks than caring for

patients, all of these are frustrating. Adequate working conditions, generous salaries, creation of functional interdisciplinary teams, filling vacancies, and introduction of telemedicine and artificial intelligence in the field of neurology, would be some solutions for attracting young doctors to this specialty.

Table 1: Significant factors contributing to burnout among neurologists and strategies for prevention

Main factors related to burnout	Proposed solutions
Factors related to the patient education	
Many referrals to neurology that do not need neurology intervention Eg: Functional neurology (often, patients with functional conditions tend to show more dissatisfaction) [22].	Educational initiatives/ online courses targeting other medical professionals that send patients and have minimal to no knowledge of common neurological diseases. Early neuroscience curricula in medical school.
The pandemic of cardiovascular risk factors: high blood pressure and cholesterol, obesity, smoking, and poor diet - put a person at high risk for neurological diseases as well.	Preventive medicine. Screening programs and campaigns to raise awareness.
Factors related to the workplace and healthcare system	
Complaints from patients/families arise from: - the poor medical knowledge - not understand the limits of medicine, medical priorities, or investigations (23)	Legal assistance for the doctors involved.
Shortage of neurologists because of not choosing the specialty [24].	Programs in medical school promote and value neurology. Adequate salary.
Neurologists choose urban over rural settings [25].	Improving rural health settings. Providing medical tools/gadgets/making technology available so the rural residents have equal opportunities to receive medical treatment.
The complexity of neurological diseases creates a job mismatch.	Development of team-based neurologic care Increased need for subspecialty medical care Eg: In Romania, there are no specialists in movement disorders or neuro-ophthalmology
Responsibility fatigue at the workplace.	The schedule of work must be dependent on the complexity of the tasks.
The neutral attitude of the hospital management board [26].	Providing support, understanding human needs, expand vacation policies.
Many rescheduling of appointments decreases physician and healthcare system efficiency overall.	Telemedicine and e-consults increase the capacity to respond to the demand for care and help patients with difficulties in coming to their clinic visits [27].
Mounds of paperwork - spending more time doing bureaucratic tasks than caring for patients. Lack of access to medical records - especially in older patients with many investigations.	Digitalization and artificial intelligence are the future of healthcare. Enabling tracking system for ordered tests/ follow-ups.
Time-consuming tasks. Eg: documenting a history of falls in Parkinson	Artificial intelligence Eg: smartwatches or other wearables that quantify falls or periods of inactivity and already provide a pattern or a conclusion.
Factors related to the physician	
Communicating a difficult diagnosis (often the first interaction occurs already at the time of critical illness).	Programs/Training courses that will help doctors improve their medical communication with patients.
The emotional burden of the specialty itself.	Providing necessary psychological assistance to guide neurologists facing mental health problems like depression or anxiety.

CONCLUSIONS

Overall, burnout can be regarded as a matter of specialty, and it has built up slowly over time, creating a vicious cycle, perpetuated by workforce dropout or choosing other medical specialties with a better lifestyle. Identification of burnout effects in neurology is policy relevant for some reasons: it affects the overall healthcare system with all its components, work-life integration (family), emotional and physical well-being, and social engagement. To conclude, it must be regarded as a worldwide issue.

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REVIEW

Prognostic Value of Serum Biomarkers in Secondary Peritonitis - A Literature Review

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Abstract: *Background: Infection and sepsis represent major complications after abdominal surgery for secondary peritonitis. Finding new tools that can help identify patients at risk for developing postoperative complications is of the utmost importance for clinicians. We tried to evaluate the use of different biomarkers in evaluating the postoperative prognosis of patients with secondary peritonitis. Methods: We searched the available literature on the usefulness of serum biomarkers in evaluating prognosis in patients with secondary peritonitis. Results: Elevated postoperative lactate levels and high procalcitonin (PCT) levels were associated with negative outcomes. C-reactive protein values increase rapidly in response to the surgical insult but have no value in assessing the overall prognosis. Conclusion: Procalcitonin (PCT) is superior to other biomarkers in predicting severe septic complications and overall mortality in patients with secondary peritonitis.*

Keywords: *biomarkers, peritonitis, C-reactive protein, lactate, procalcitonin, presepsin*

INTRODUCTION

Secondary peritonitis is a condition caused by the spread of bacteria and contents of intraabdominal organs into the peritoneal cavity [1] and is the second leading cause of sepsis in intensive care units worldwide [2]. Overall mortality is 6%, but mortality rises to 35% in patients who develop severe sepsis [2]. Secondary peritonitis requires immediate surgical intervention but after the initial procedure, persisting or new-onset abdominal sepsis continues to be a major problem in the postoperative course [3].

Using clinical symptoms such as fever, tachycardia, tachypnea, non-specific laboratory findings, and scoring systems are valuable tools to assess overall severity and prognosis but will not allow a differentiation between an infectious and non-infectious etiology of a systemic inflammatory response. The clinical assessment of sepsis biomarkers has gained considerable interest in identifying patients at risk for severe complications following surgical intervention for secondary peritonitis [1].

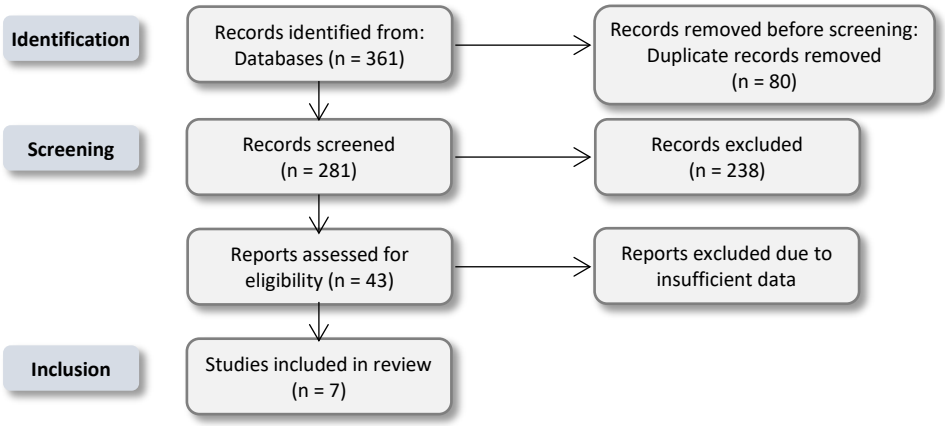
This review aimed to identify the most important biomarkers evaluated in patients with secondary peritonitis and their association with prognosis.

MATERIALS AND METHODS

We searched in three databases using the keywords „peritonitis“ and „prognosis“ in the title or abstract AND specific biomarkers by full name and abbreviated form in full text: lactate, C-reactive protein (CRP), procalcitonin (PCT), presepsin.

Due to the scarcity of the results, a broader search was conducted using the keywords „peritonitis“ AND „biomarker“ to ensure that all relevant literature is covered. The search was last updated on 24/06/2022. A total of 361 publications were primarily retrieved in PubMed, Embase, and Web of Science. No articles were added after manually searching major scientific journals and other references. After excluding 80 duplicates, 281 titles were screened for eligibility and 43 articles were assessed for full-text evaluation. Only English-written human studies were accepted and most articles were excluded due to irrelevancy. We focused only on circulating biomarkers for prognosis in secondary peritonitis. No cut-off point was set for the date of publishing due to the paucity of articles. At the end of the process, seven articles were included in the review. The search strategy is detailed in Figure 1.

Figure 1: Search strategy – identification of studies via databases and registers



OVERVIEW OF THE SELECTED ARTICLES

Seven articles were included in the current review. Four studies were prospective and three were retrospective, and one study included a control group. Biomarkers of interest were lactate, procalcitonin, and C-reactive protein. No study evaluating presepsin was identified. All studies focused on mortality and severity of septic shock induced by secondary peritonitis.

Further details on the selected studies are summarized in Table 1.

SERUM BIOMARKERS CORRELATED WITH PROGNOSIS IN SECONDARY PERITONITIS

Lactate

Lactate is the metabolic end product of anaerobic glycolysis. Approximately 1500 mmol of lactate is produced daily in all tissues, but mostly in skeletal muscle, brain, intestine, and red blood cells [6,9]. The concentration of arterial lactate is a reflection of the delicate balance between net production and clearance. Disturbances in lactate production, clearance, or both lead to hyperlactatemia and it is seen in a variety of conditions such as sepsis, shock, cardiac arrest, tissue hypoxia, burns, or after some pharmacological agents [6].

The prognostic value of lactate in patients with secondary peritonitis was evaluated in four studies. Negi et al. conducted a prospective study to evaluate the utility of arterial lactate measured on admission (AL1) and 24h after surgery (AL24) as well as lactate clearance as prognostic indicators for mortality in patients with secondary peritonitis. The study concluded that mean values of initial lactate, postoperative lactate, and percentage lactate clearance correlated significantly with higher morbidity and mortality [6]. Of these three lactate indices, postoperative lactate (AL24) was the most accurate in predicting morbidity and mortality [6]. Elevated lactate levels persisting postoperatively probably indicate irreversible pathophysiological changes and global tissue hypoperfusion, indicative of poor lactate clearance and higher morbidity and mortality. Andreevich et al., after conducting a retrospective study, concluded that increased lactate levels both before and 72h post-operatively were associated with a negative prognostic[8]. In the study conducted by Suarez-de-la-Rica et al., 24h postoperative lactate concentrations predicted mortality in the Surgical Critical Care Units, but overall mortality was predicted better by concurrent high procalcitonin and lactate peak concentrations [7].

Jung et al. conducted a retrospective study that aimed to compare the performance of the qSOFA score alone with a combined score derived from qSOFA and serum lactate levels for predicting in-hospital mortality for patients with secondary peritonitis. The study results showed that the qSOFA score alone has poor sensitivity and a modest predictive performance in patients with secondary peritonitis [5]. The addition of one point for hyperlactatemia (plasma lactate level > 2.0 mmol/L) resulted in improved sensitivity (5). The use of the qSOFA + lactate score can be useful to assist clinicians in immediately assessing the risk of mortality in patients with secondary peritonitis [5].

Most studies agreed that elevated post-operative lactate levels correlate better with negative outcomes than preoperative lactate levels.

Procalcitonin

Procalcitonin (PCT) is the 116-amino acid pro-peptide of the biologically active hormone calcitonin [10]. The clinical value of PCT assessment in infection and sepsis has been investigated in almost every medical discipline so far. Most studies have shown that, in general, only in the presence of systemic host response to bacterial or fungal stimuli, does PCT concentration rise above the reference range of 0.5 ng/mL. However, new data demonstrated an early production of PCT related to tissue damage after trauma and the magnitude of

the hypovolemic challenge, yet unrelated to infection (4, 10). We analyzed four studies on the usefulness of PCT measurements in predicting negative outcomes in patients with secondary peritonitis. In 246 patients with intraoperatively proven secondary peritonitis of various origins, Reith et al. showed that PCT is a sensitive parameter for the prediction of severe septic complications. Moreover, the study demonstrated that PCT reflects the effectiveness of the surgical procedure in eliminating the septic intra-abdominal focus in the postoperative course (4, 10). PCT was of excellent prognostic value and achieved a sensitivity of 84% and a specificity of 91% in discriminating survivors from non-survivors within the first four postoperative days [4]. Similar observations have been reported in a study conducted by Rau et al. on 82 patients with intraoperatively proven secondary peritonitis. Pro-calcitonin values were elevated in patients who developed septic multiple organ dysfunction syndromes (MODS) with peak levels on the third and fourth days after the onset of symptoms. Comparing the preoperative and postoperative courses, PCT concentrations were significantly elevated in patients with septic MODS during the immediate postoperative course. A persistent procalcitonin elevation beyond the first week after the onset of symptoms was evident in non-survivors, whereas normal concentrations were measured in survivors [3].

Table 1: Studies focused on mortality and severity of septic shock induced by secondary peritonitis

Author	Study design	Biomarker	No. of patients	Correlation with prognosis
Akcay et al (2014)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	84	Serum PCT levels follow a falling trend after surgery as long as the postoperative course remains uneventful. PCT was a better marker than CRP in following postoperative complications and overall prognosis.
Reith et al (2000)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	246	A reduction in the PCT concentration correlated with improvement in the infection and sepsis and was found in all surviving patients. Compared with the values for CRP, PCT showed significant differences between survivors and patients who died, while CRP did not [4].
Rau et al (2007)	prospective	procalcitonin (PCT), C-reactive protein (CRP)	82	The cumulative analysis of all PCT and CRP values measured in the first, second, and third to fourth weeks identified persisting PCT levels greater than 1 ng/mL as an excellent means to assess the overall outcome. Irrespective of the interval, PCT was significantly better than CRP in assessing overall prognosis [3].
Jung et al (2018)	retrospective	lactate	457	The qSOFA score alone has poor sensitivity and a modest predictive performance in patients with secondary peritonitis. The addition of one point for hyperlactatemia (plasma lactate level > 2.0 mmol/L) resulted in improved sensitivity. The use of the qSOFA + lactate score will assist clinicians in immediately assessing the risk of mortality in patients with secondary peritonitis [5].
Negi et al (2020)	prospective	lactate	224	Mean values of initial lactate, postoperative lactate, and percentage lactate clearance were found to correlate significantly with higher morbidity and mortality in patients with secondary peritonitis. Of these three lactate indices, postoperative lactate was the most accurate in predicting morbidity and mortality [6].
Suarez-de-la-Rica et al (2014)	retrospective	lactate, C-reactive protein (CRP), procalcitonin (PCT)	221	Both PCT and lactate provided early prognostic information, more accurate than clinical scores, for overall mortality [7].
Andreevich et al (2021)	retrospective	lactate, C-reactive protein (CRP)	136	Increased lactate and CRP values both before and 72 h after surgery were associated with negative prognostic. PCT value before surgery was insignificant and only an increase in PCT 72h after surgery was associated with a negative outcome [8].

In a prospective study conducted on 84 patients, Akcay et al. were able to demonstrate that serum PCT levels follow a falling trend after surgery as long as the postoperative course remains uneventful. In addition, they showed that after the surgical intervention PCT was a better marker than CRP in following postoperative complications and overall prognosis in patients with secondary peritonitis [1].

Suarez-de-la-Rica et al. compared the prognostic value of lactate, procalcitonin, and c-reactive protein. Peak lactate (>1,8 mmol/L) and PCT (>100 ng/mL) concentrations were associated with mortality, but peak PCT level was the best biomarker for overall mortality. In this study, concomitant high peak lactate and peak PCT concentrations considered as a single dummy variable were highly predictive of overall mortality [7].

C-Reactive Protein

C-reactive protein (CRP), named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae, was the first acute-phase protein to be described [11]. CRP is an evolutionarily conserved protein. From arthropods to humans, CRP has been found in every organism where its presence has been sought [12].

C-reactive protein is a useful marker of systemic inflammation without discriminating infection from other inflammatory processes [7]. When it comes to the value of CRP in evaluating prognosis in patients with secondary peritonitis, after searching the available literature, we found five studies that included this biomarker. All studies agreed that measured CRP values, which increase rapidly in response to the surgical insult, had no value in assessing overall prognosis. C-reactive protein did not reveal any differences between patients with or without septic MODS or in non-survivors and survivors in the perioperative course [3].

CONCLUSIONS

Although a multitude of biomarkers is elevated in patients with secondary peritonitis in the perioperative course, only a few are reliable parameters for assessing the course and prognosis in this category of patients. Many studies have clearly shown that procalcitonin (PCT) is superior to other biomarkers in predicting severe septic complications and overall mortality. In combination with careful clinical patient assessment, PCT offers distinct advantages for the evaluation of response to treatment and prediction of outcome in patients with secondary peritonitis. In regards to the fact that procalcitonin is a well-known „sepsis“ parameter, this may not embrace the real properties of PCT, which remain to be defined.

Further studies must be conducted to obtain more knowledge about the importance of serum biomarkers in patients with secondary peritonitis and their correlation with prognosis.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Etiology of Chronic Kidney Disease and Comorbidities in Chronic Hemodialysis Patients

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Abstract: *Introduction: As general population tends to have increasing life expectancy, the risk associated with developing chronic kidney disease (CKD) with multiple incapacitating concequences, also increases. Method: For the present study, we registered the data from the observation files of 37 patients diagnosed with CKD undergoing treatment by chronic hemodialysis and noted the CKD associated diagnoses included in the notion of comorbidities. We monitored their statistical incidence both in the whole group and separately, in women and men using TTEST and CORREL. Results: The median age of the subjects was 55.86 (± 12.00) years. The study population mean weight was 74.90 (± 14.44) kg, with a mean weight of 69.33 kg for female subjects, and 77.92 kg for males, respectively. Diabetes was identified in 35.13% of patients, whilst heart failure was present in 16.21% of patients. Conclusions: Following the analysis of the information about the patients with CKD in the dialysis program, which we included in the study group, we observed the existence of variations that occur with age, significant correlations between age and weight and between albuminemia and weight. The most common comorbidity is high blood pressure followed by anemia.*

Keywords: *chronic kidney disease; chronic hemodialysis patients; comorbidities; plasma albumin*

INTRODUCTION

Chronic kidney disease (CKD) can develop due to a multitude of pathologies that all have in common the evolution to kidney failure, with gradual or rapid loss of the main function of the kidneys, that is to remove waste products and excess fluid from the body [1]. The last stage of the development of CKD, especially in patients who are treated by peritoneal dialysis or hemodialysis, is characterized by multiple organs and systems damage [2].

Not only CKD, but also the treatment of its various manifestations, mainly affect the body tissues and systems, at all levels: nervous, cardiovascular, respiratory, endocrine, hematopoietic, digestive, renourinary [2].

The tendency to increase life expectancy of the population is also associated with an increase of the incidence and prevalence of CKD, considering the already known relationship between aging and decreased kidney function, which is seen as a consequence of the physiological aging of the kidneys [2,3]. An even steeper increase in the number of patients with CKD is expected in the coming years. In this context, the medical goal becomes to slow down the progression to terminal stages, achievable primarily by diagnosing the condition as early as possible [1].

Compared to conservative treatment, extrarenal treatment methods provided clear advantages for patients with chronic end-stage renal disease, the duration and quality of life being

considerably superior. There are several methods of extrarenal purrification: peritoneal dialysis, hemodialysis, hemodiafiltration. The decision for each type of treatment is based primarily on medical considerations, depending on the underlying disease and the comorbidities present [4]. Diabetic patients are usually at higher risk of developing more severe forms of chronic diseases (e.g. cancer) [5,6,7]. Peritoneal dialysis is preferred for diabetic patients with CKD as this method of treatment ensures the maintenance of a residual diuresis for a longer time, the prevention of complications due to the present vascular damage, the possibility of intraperitoneal administration of insulin and a better hemodynamic stability than chronic hemodialysis. In cases where the patient has a poor state of hygiene or if peritoneal dialysis does not allow a satisfactory volume control to be maintained, chronic hemodialysis is preferred [4].

MATERIALS AND METHODS

For the study we performed, we registered the data from the observation files of 37 patients diagnosed with CKD undergoing treatment by chronic hemodialysis, in the Nephrology Department of the Bucharest University Emergency Hospital.

We noted their general data and the presence of associated comorbidities, as well as the determined values of plasma albumin concentration.

The statistical data processing regarding the incidence of comorbidities revealed important information for understanding the clinical and biochemical context of this particular type of patient in the hemodialysis department.

Among the tests performed on the patients that had presented for chronic dialysis, we noted the value of plasma albumin concentration, because it is a parameter that provides information about the loss of protein in the urine and the nutritional status of renal patients. We determined the statistical differences between the values from women and men, as well as the correlations between this parameter and age, respectively weight.

We performed the statistical processing using the Student test (t-test) to determine the statistical significance of the differences in the values of the parameters determined in women and men and the Correl test to determine whether there are correlations with statistical significance between the parameters we tracked.

We noted the diagnoses associated with CKD, included in the notion of comorbidities and we monitored their statistical incidence both in the whole group and separately, in women and men.

RESULTS

In terms of gender distribution, of the 37 patients, 13 (35.13%) were women and 24 (64.86%) were men.

The median age of the subjects was 55.86 (± 12.00) years. For the women, the median age was 56.46 (± 10.86) years, whereas for the men it 55.54 (± 12.78) years. Although there is a slight gender difference regarding age, this failed to be statistically significant (p> 0.05).

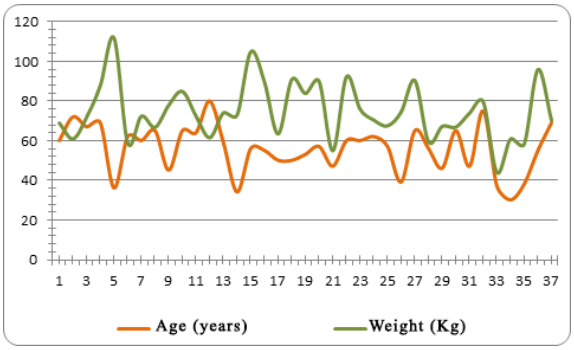
Regarding weight, as presented in Table 1, the study population mean weight was 74.90 (± 14.44) kg, with a mean weight of 69.33 kg for female subjects, and 77.92 kg for males, respectively. As presented in Table 1, this difference was found to be statistically significant, p = 0.01.

Table 1: Average weight and the statistical difference between men and women

	Average weight in women (kg)	Average weight in men (kg)
Average value	56.46	52.27
Standard Deviation	10.87	17.16
p (t-test) 0.43		

Applying the Correl test, we observed that age and weight are two parameters that correlate statistically significantly (p = 0.04) (Figure 1).

Figure 1: Significant correlation between the values of weight and age



For the 37 patients, we noted the value of the plasma albumin concentration and determined the difference in values according to the gender of the patients and the statistical significance of this difference, using the Student test (t-test) (Table 2).

It is observed that the albuminemia values that were determined in these patients are not outside the normal laboratory limits of 3.5-5.2 g/dl (Table 2). By applying the

Correl test, we calculated the statistical significance of the correlations of albuminemia with age and weight (Table 3).

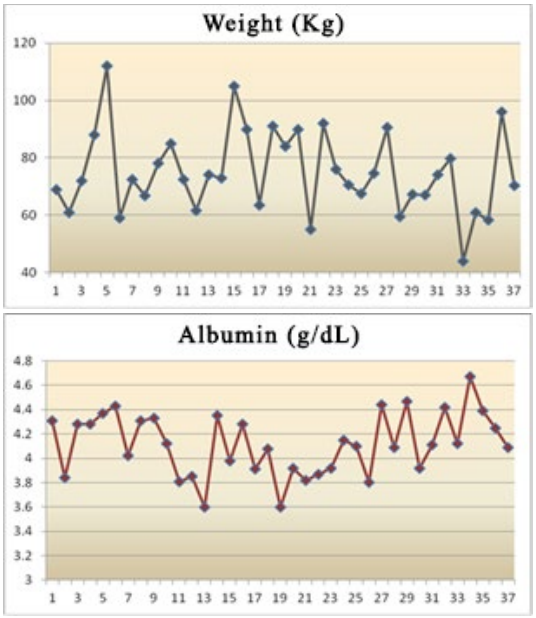
Table 2: Statistical significance of differences in plasma albumin concentrations in patients in the group by sex

Albuminemia(g/dl)		
	Women	Men
Average value	4.10	4.25
Standard deviation	0.24	0.27
t-test	p = 0.41	

Table 3: Statistical significance of correlations between plasma albumin concentrations with age and weight in patients in the group and by sex

Significance of the correlation between plasma albumin concentration and		
	Age	**Weight**
Women	p = 0.1	p = -0.2
Men	p = -0.37	p = 0.1
Total	p = -0.24	p = 0.02

Figure 2: Correlation weight-albuminemia



We observed that separately, in women and men, the plasma concentrations of albumin do not correlate significantly with age or weight, but, after using the Correl test for the whole study group, a significant correlation between albuminemia and weight has been identified.

We made this determination, knowing that the albumin dosage is a test to assess the general state of nutrition, especially in elderly patients with various conditions, in this category falling most of the patients with CKD in the lot we studied.

COMORBIDITIES DIAGNOSED IN THE STUDIED GROUP

Diabetes mellitus, dyslipidemia, atherosclerosis, hypertension, arteriopathy obliterans of the lower limbs

Diabetes is present in 35.13% of patients, 3 of women and 10 of men, which means 23.07% and 41.66%, respectively. Dyslipidemia occurs in a number of 3 male patients, which means a percentage of 8.1% of the total group. Atherosclerosis is noted in a female patient with kidney disease and in 9 male patients, the incidence rate being 27.02% in the whole group. High blood pressure is a more common comorbidity in patients in the study group: 11 out of 13 women are hypertensive and 23 out of 24 men with CKD are hypertensive. Lower limb arteriopathy is present in only 2 of the male patients, which means 5.4% of the group. Both patients also have diabetes and high blood pressure.

Heart failure, cardiomyopathy, arrithmia, valvulopathy, chronic coronary heart disease

Heart failure is present in 5 of the women and only 1 of the men, this comorbidity is therefore found in 16.21% of patients. Cardiomyopathy is rarer, 1 female patient and 2 of the men in the group, ie 8.1% also have this diagnosis. Arrhythmias are more common in women, 5 out of 14, ie 35.7%, presenting this manifestation in the form of atrial fibrillation with medium or large ventricular allure or in the form of supraventricular tachycardia. Among men, only 2.33% have atrial fibrillation with a medium ventricular allure. Thus, 18.91% of the total group have a form of arrhythmia. Valvular damage is identified in 4 patients out of a total of 37, ie 10.81%, more frequently in women 3 out of 14 and only in 1 male patient. Coronary ischemic disease is diagnosed in 3 women and 6 men, the incidence being 24.32%.

Diabetic/hypertensive/mixed nephropathy, chronic tubule-interstitial nephropathy, autosomal dominant polycystic kidney disease, single kidney, chronic pyelonephritis, chronic glomerulonephritis

Chronic tubulointerstitial nephropathy is diagnosed in a single male patient. The incidence at group level is 2.7%. Out of 13 women in the group, hypertensive nephropathy is present in 2 and diabetic nephropathy in 3 of them, a total of 38.46% with nephropathy. Of the 24 men in the group, 6 have diabetic nephropathy, 4 have hypertensive nephropathy and 2 have mixed nephropathy, a total of 50% of patients with nephropathy. A number of 4 of the 13 women, 30.76%, are also diagnosed with autosomal dominant polycystic kidney disease. Of the 24 men, 4 also have this diagnosis, representing

16.66%. At the level of the whole group, the percentage is 21.62%. Only one male patient has a single kidney, the cause being congenital. The incidence at group level is 2.7%. And pyelonephritis rarely occurs, only one patient, a woman, has this diagnosis. The incidence at group level is 2.7%. More common is chronic glomerulonephritis, which is present in 6 of the men, respectively 25%, and in 2 of the women, respectively 15.38%, with an incidence in the whole group of 21.62%.

Kidney stones, parathyroidectomy, secondary hyperparathyroidism, anemia, chronic viral hepatitis

Kidney stones are present in 2 women and 3 men in the group, which means an incidence of 13.51%. Secondary hyperparathyroidism is diagnosed in only 2 patients, a man and a woman, the incidence is 5.4% in the whole group. Surgical removal of the parathyroid glands was performed in 3 women and 1 man in the group, the frequency being 10.81%. Anemia is more common, 7 of the 13 women, ie 53.8% and 15 of the 24 men, ie 62.5%, have pathologically low hemoglobin. Out of the whole lot 59.45% of patients have anemia. Chronic hepatitis B infection is uncommon as viral hepatitis B is present in 2 women, 1 man in the group is infected with hepatitis B virus and 1 is coinfected with hepatitis B + C viruses. The frequency of chronic viral hepatitis in the group is 10.81%.

DISCUSSIONS

Gender and age are factors that influence some characteristics related to CKD in patients with type 1 or type 2 diabetes, and certain particular aspects of pathogenesis, diagnosis and treatment management have been identified, but the mechanisms are incompletely known and more studies are needed to clarify these connections which would be very useful in order to reduce the number of cases of renal patients and to prevent or control the complications of this condition. Considering the data published by Eriksen O. et al. [5], old age and the male gender are predictors of worsening kidney disease and the rapid progression to severe kidney failure. In the study of Giandalia A et al. [8] the authors concluded that men are more affected, because in their studies, the number of men enrolled is higher than the number of women, results similar to our study where the percentage of men was 64.86%.

Weight is an important parameter in assessing the nutritional status of the patient with CKD. In obese people with CKD, the progression of the disease to renal failure is faster compared to cases of normal weight. Also kidney transplantation or hemodialysis, which may become necessary in advanced stages of the disease, may be more difficult and may have more frequent complications in obese people [9]. After initiating treatment with hemodialysis or peritoneal dialysis, in patients with a glomerular filtration rate below 35 ml/min/1.73 m^2, in stage 3 CKD, weight loss is a factor that increases the risk of mortality by 54% compared to with patients whose weight remains constant. Even in patients with moderate renal impairment, weight loss should be closely monitored and the patient's nutritional status monitored before dialysis is required [10].

In the final stages of the kidney disease, hypoalbuminemia is common, caused by factors that act simultaneously: reduced liver synthesis, increased degradation and renal loss and the glomerular filter membrane is damaged and becomes more permeable allowing the passage of negative albumin molecules from plasma. In our study we observed that separately, in women and men, the plasma concentrations of albumin do not correlate significantly with age or weight. However, applying the Correl test to the whole group, resulted in a significant correlation between albuminemia and weight. We made this determination, knowing that the albumin dosage is a test to assess the general state of nutrition, especially in elderly patients with various conditions, in this category falling most of the patients with CKD in the lot we studied. According to Haller C. et al. [11] the inflammatory phenomenon in kidney disease is also involved in the pathogenesis of hypoalbuminemia. Inflammation correlates with the risk of mortality, hypoalbuminemia is not in itself a pathogenic factor of renal impairment, but is a major predictor of unfavorable prognosis.

Diabetes mellitus is one of the main negative prognostic factors for chronic diseases [12]. According to Anders HJ. et al. [13] the prevalence of type 2 diabetes and CKD increases the incidence of diabetic kidney disease, also known as diabetic nephropathy. The combination of the two diseases can be in the form of diabetic kidney disease if the kidney damage is the consequence of diabetes, it can be a kidney disease caused by individual mechanism in the diabetic patient or it can be a coincidence of simultaneous occurrence of the two diseases. The involvement of the membrane-glucose cotransporter membrane and hyperglycemia are associated with the activation of the renin-angiotensin-aldosterone system, glomerular hyperfiltration and tubular hyperabsorption occur, which creates a particular situation that requires appropriate treatment. Whatever the mechanism, the patient's evolution is more unfavorable compared to those who have only one of the conditions [13,14]. Our study results for 35,13% of the patients, that were diagnosed with diabetus mellitus type II, found that 69.23% of them have further developed diabetic nefropathy.

In the study of Tsimihodimos V et al. [15] it was shown that lipid metabolism is disrupted in patients with CKD even in the early stages and can be very severe in some cases, further impairing kidney function. The pathogenic mechanisms are not

fully understood, but studies show that dyslipidemia is a pathogenic factor for both kidney disease and the associated cardiovascular disease [16]. In our study dyslipidemia was identified in only 8,1% of the patients.

Atherosclerosis sets in early and progresses rapidly in patients with CKD. It usually increases the risk of cardiovascular disease and, as the severity of kidney disease increases, so does the mortality rate, according to Olechnowicz-Tietz S. et al. [17]. In our study population 27.02% were found to suffer from atherosclerosis.

Considering the cardiovascular risk, compared to the general population, patients with CKD, according to the study of Matsushita K et al. [16] have a constant increased cardiovascular events risk. Studies identified that 70% of CKD patients, and up to 80-90% of those on dialysis, are diagnosed with high blood pressure [18,19]. Considering all these data, our studies results that 91.9% of CKD subjects were suffering of/diagnosed with hypertensive disease are consistent with the data in the literature.

As shown in the study of Maric-Bilkan C. et al. [20], in the absence of diabetes, women have a considerably lower risk than men of vascular, microvascular or macrovascular diseases. The opposite trend is observed in diabetic patients: women are at higher risk for these complications. Microvascular lesions that occur in nephropathy, neuropathy, and retinopathy are more common in men. Macrovascular lesions consisting of chronic coronary heart disease, myocardial infarction, peripheral artery damage, and stroke are more common in women. To explain this, the focus has been on sex steroids, which play an important role in regulating heart function. Estrogens are generally considered hormones with cardioprotective effects, and androgens with adverse cardiac effects. But the effects of these hormones on the target organs are complex and different, and the presence of diabetes interferes with the pathophysiological mechanisms that cause vascular damage [14,18,19].

Renal comorbidities are, in part, primary kidney disease that has progressed unfavorably, with the patient reaching the stage of CKD that requires dialysis in order to survive [20]. Another part of these comorbidities is secondary, the pre-existing disease being another. This is the case with nephropathy, which develops during the course of hypertensive disease or diabetes [14]. The percentage of incidence of nephropathy of any etiology in the study population was 45.94%. It is a high percentage, which is explained by the fact that, as we have shown above, diabetes and hypertension are precursors or caused by CKD.

Our results are consistent with literature data showing that 11.2% of patients in a large group of with CKD present with kidney stones. The same study by Chuang TF. et al [21] shows that patients with kidney stones are 1.82 times more likely to develop CKD.

Secondary hyperparathyroidism is found in chronic dialysis patients, the incidence of this comorbidity being reduced by treatments with phosphorus chelators, calcium supplements and calcitriol. As shown in the study of Llach F. et al [22] there are situations when parathyroidectomy is necessary, namely when clinical signs such as persistent hypercalcemia, pruritus, metastatic calcifications appear [23]. In our study hyperparathyroidism was found in only 5,4% of the patients but out of the whole lot, 10.81% of the patients had already been treated with parathyroidectomy. The latter can cause insufficiency of many organs.

Blood characteristics such as blood group or different blood components (eg. lymphocytes) have been linked to chronic diseases or their treatments toxicity [24-27]. According to the study of Ueda N. et al. [28] hemoglobin levels below 12 g/dl for women and below 13 g/dl for men are considered anemia. Iron and erythropoietin deficiency causes anemia in patients with CKD. Hepcidin is a hormone produced by the liver in inflammation or when the body's iron deposits in the form of ferritin or hemosiderin are saturated. Hepcidin binds to and inhibits ferroportin, thereby decreasing the release of iron from the enterocyte and reticuloendothelial cells and decreases plasma iron. Inflammatory status in CKD increases hepcidin regardless of the availability of iron to the body, so it becomes necessary to administer iron, but also to reduce inflammatory phenomena [29]. It was a frequent occurence in our study as 62.5% of our patients were diagnosed with anemia.

Hepatitis B or C virus infections can cause chronic kidney damage but do not have a high incidence according to Chacko EC et al. [30]. If the infection occurs in patients with CKD, anti-infective treatment has many side effects and may worsens the prognosis. Similar results were shown in our study as only 10.81% of the patients presented with concurrent viral hepatitis and CKD.

CONCLUSIONS

Following the analysis of the information about the patients with CKD in the dialysis program from our study weight has been found to be significantly higher in men, compared to women. Albuminemia was within normal limits in all patients, significantly correlating with the body weight. Patients' comorbidities identified in our study were similar to the ones found in the current literature to be related to CKD. The most common comorbidity is high blood pressure, identified in over 90% of the subjects. Anemia is the second most common

comorbidity, occurring in almost 60% of patients. With high frequency, in over 20% of patients, we observed the presence of diabetes, atherosclerosis, ischemic coronary heart disease, nephropathy of various etiologies, autosomal dominant polycystic disease and chronic glomerulonephritis.

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Review

The Role of Coronary CT Angiography in the Management of Patients with Coronary Atherosclerotic Disease

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Abstract: *Coronary CT angiography is a non-invasive method of analyzing the coronary lumen through which the atheromatous bur-den can be evaluated, with the analysis of the type of plaque (soft, calcified, mixed) and the impact on the arterial lumen (stenosis, occlusion). The anatomical evaluation by coronary CT is indicated in symptomatic patients with low and medium risk factors for coronary atherosclerotic disease, in those with inconclusive laboratory and EKG results, patients with un-certain stress test results, in the evaluation of coronary grafts and intrastent stenoses. Depending on the result of the angiography, the severity of the stenosis and the management of the patient are established. In the case of mild and medium stenoses, risk factors management and drug treatment are recommended. For severe stenoses, patients are referred for interventional coronary angiography or functional evaluation. Coronary CT angiography increases the certainty of coronary atherosclerotic disease diagnosis. It has a superior discriminative capacity of plaques with low attenuation, the main predictor of myocardial infarction, with almost five-fold higher risk. Coronary CT angiography increases the adaptation of medication, and the inclusion of statin therapy decreases the risk of fatal and nonfatal myocardial infarction at 5 years and increases the quality of life.*

Keywords: *coronary CT angiography, coronary atherosclerotic disease, calcium score, CAD-RADS*

INTRODUCTION

Atherosclerotic disease is the main cause of death in Europe [1]. Lifestyle factors (smoking, diet, level of physical activity) but also biological risk factors (hypertension, hypercholesterolemia, hyperglycemia) contribute to this [2].

The management of patients with coronary atherosclerotic disease is complex, including numerous investigative methods. Of these, coronary CT angiography has been analyzed more and more, over the last decade, due to technological progress. Coronary CT angiography is a non-invasive method of evaluating the heart and coronary arteries. Coronary CT angiography is performed using a computer tomograph that involves high-speed scans for moving organs and contrast material containing iodine, administered in injectable intravenous form to obtain the best images of the coronary arteries and the heart. Thus, the atheroma plaques in the coronary arteries can be identified, the type of plaques analyzed (soft, calcified, mixed), and the consequences on the arterial lumen (stenoses, occlusions) evaluated. Coronary anomalies can be detected, valve morphology can be analyzed, as well as the degree of myocardial perfusion.

MATERIALS AND METHODS

The main reference studies used in evaluating and managing patients with coronary atherosclerotic disease are extensive studies that have contributed to the current state of knowledge in this field, demonstrating the usefulness of the investigation in diagnosis, monitoring, and recommendations for the treatment of the disease.

The PROMISE study (Prospective Multicentre Imaging Study for Evaluation of Chest Pain) – from the year 2017 – showed that coronary CT angiography is a superior means of evaluation for identifying patients at risk with nonobstructive

coronary atherosclerotic disease, compared to classical functional exploration means, in patients with stable angina pectoris and with an average probability of coronary atherosclerotic disease. The patients were randomly divided into two groups, 4500 patients were investigated by coronary CT angiography and 4602 patients were evaluated by functional means (exercise electrocardiogram, exercise ultrasound, and exercise myocardial scintigraphy). The patients were subsequently monitored for 26 months. The significantly lower incidence of coronary events in patients investigated by coronary CT angiography compared to those evaluated by functional means (33.4% versus 78.0%) demonstrates that coronary CT angiography had a significantly higher discriminative capacity for predicting coronary events than functional explorations (c -index, 0.72; 95% CI, 0.68-0.76 versus 0.64; 95% CI, 0.59-0.69; P=0.04) [3].

The SCOT HEART study (Scottish Computer Tomography of the Heart) is large, started in 2010, and is still ongoing, which will continue until 2027, with some of the results already published. It is a randomized parallel study, which included, between 2010-2014, 9848 participants with angina pectoris suggestive of coronary atherosclerotic disease and with mild and medium risk factors. The patients were divided into two groups that were evaluated differently: 4146 patients by coronary CT angiography and the rest were managed by standard anatomical and functional means. The main diagnostic results showed that coronary CT angiography increased the certainty of the diagnosis of coronary atherosclerotic disease (relative risk [RR] = 1.79, 95% confidence interval [CI] 1.62-1.96, p < 0.001) and reduced the frequency of the diagnosis of angina due coronary atherosclerotic disease (RR = 0.93, 95% CI 0.85-1.02, p = 0.13) compared to standard care [4]. The main clinical result was a decrease in the risk of fatal and nonfatal myocardial infarction at 5 years, which was 2.3% in the coronary CT angiography group, compared to 3.9% in the group managed by standard means. The secondary results consisted of an increase in the quality of life, the adaptation of medication, and the inclusion of statin therapy. In those cases where the lesions were severe, patients were referred for angiography and stenting [5].

Other secondary, very important results emerged in 1769 patients, followed for 4-5.7 years, which proved that the presence of plaques with low attenuation is the main predictor of myocardial infarction, independent of the presence of cardiovascular disease risk factors, of the score of calcium or area of stenosis. Patients with low-attenuation plaques had an almost 5-fold higher risk of myocardial infarction (95% CI, 2.06-10.5; P<0.001). In 41 patients who suffered an acute myocardial infarction, both low-attenuation plaque load and calcium score were increased (336 [62-1064] versus 19 [0-217] Agatston units; P<0.001), and the presence of obstructive coronary artery disease (54% versus 25%; P<0.001) [6].

The UK's National Institute for Health and Care Excellence's 2016 NICE chest pain guideline recommended coronary CT angiography as the first line of evaluation in patients with new stable chest pain. Emphasis is placed on anatomic explorations over functional ones, a choice to which cost-effectiveness also contributes [7,8].

However, this approach raised concerns that, after the first evaluation with coronary CT angiography, there would be an increased referral to further investigations, such as invasive coronary angiography. To test this, a retrospective study was conducted on 652 patients who underwent coronary CT angiography from 2017 to 2018. The results showed that 92 patients had moderate (50-70%) or severe stenosis (>70 %). Of these, 69 were directly referred for invasive coronary angiography, of which 63 underwent the investigation, and severe atherosclerotic coronary disease was confirmed in 40 patients (63%). Among the patients with moderate stenosis, 18 were referred to cardiac stress echography, which was positive in one case. The remaining patients had mild stenosis in 62 cases, 36 studies were inconclusive, and the rest of them (462 patients) were normal or had minimal stenosis [9]. The study's conclusions showed that coronary CT angiography is an effective first-line method in the evaluation of patients with newly appeared stable angina pectoris, most of them having normal results or minimal coronary atherosclerotic disease. In patients with severe stenosis, it had an increased detective capacity, the stenosis being confirmed by invasive coronary angiography in 63% of cases.

The positive predictive value for coronary stenosis of coronary CT angiography, compared with stress tests, was evaluated and published in a study from 2019. A total of 800 patients with stable angina pectoris from two centers were included, one in the UK where 400 patients were investigated with coronary CT angiography and a center in Portugal where 400 patients were investigated with exercise tests (treadmill exercise stress test EST or stress imaging test). The patients in the two groups were equally distributed in terms of gender and BMI. Of the 400 coronary CT angiographic examinations, 387 (96%) were diagnostic. Of these, 92 patients (23%) had CAD-RADS 3-5 coronary obstruction, of which 67 (72%) underwent coronary angiography and 61 patients (91%) had atherosclerotic coronary obstructive disease confirmed. In the Portuguese group, 400 patients with stable angina evaluated by stress test with the positive result, recommended for interventional angiography, were included. On interventional angiography, the obstructive coronary disease was diagnosed in 51% of patients. In conclusion, the positive predictive value

of coronary CT angiography is 91%, statistically significantly higher ($p<0.0001$) than stress tests (51%) [10]. The study concludes that coronary CT angiography, due to its significantly higher predictive value than exercise tests, has a good indication as a first test for evaluating coronary stenosis, and could mean the avoiding of unnecessary interventional angiographic tests.

The AGATSTON coronary artery calcium score is a semi-automatic measurement of the degree of calcification of the coronary arteries. It is an investigation performed with a low dose of radiation at the beginning of the coronary CT angiographic examination, before the administration of contrast substance. The main role is an early stratification of the degree of coronary atherosclerotic infiltration.

An automatic calculation of coronary calcifications is made according to the density in Hounsfield units and the area occupied by the plaque.

Depending on the value of the total calcium score, coronary artery disease is divided into five degrees: no evidence of disease (calcium score 0), minimal damage (1-10), mild (11-100), moderate (101-400), severe (>400), the risk of major cardiac events increases in patients with an Agatston score greater than 160 (Table1) [11].

Table 1: Calcium score according to the Agatston scale

Agatston score	Coronary artery disease
0	no evidence of calcium
1-10	minimal
11-99	mild
100-399	moderate
400-999	severe
1000+	extensive calcium deposits increased risk of coronary events in the next year

The calcium score showed a good correlation with coronary CT angiography in the diagnosis of coronary plaques, with the limitation of not being able to evaluate the soft component of the plaque.

Special emphasis is given to the Agatston score of zero, there is the concept of "the power of zero", which means that patients are considered low risk and if there are no other risk factors, that leads to the avoidance of certain preventive drug therapies, such as statins. Another important role of the calcium score is personalizing the prescription of statins and aspirin among the general population but also the population with special conditions such as severe hypercholesterolemia or diabetes [12].

Coronary calcium score (CAC) has become a popular investigation due to its accessibility, speed, tolerability, low cost, reproducibility, and financial benefits due to the exclusion of complex coronary pathologies that involve more expensive investigations [13].

There are practice guidelines that include structured recommendations for the treatment and prevention of coronary atherosclerotic disease but there are some variations between countries in using coronary calcium score as a reference for inpatient treatment. However, there is a common agreement that coronary calcium scoring assessment is to begin in patients over 40 years. In asymptomatic patients with an intermediate risk of atherosclerotic coronary disease and a calcium score of over 100, the initiation of statin treatment is recommended. In patients with a calcium score of 0, the risk is decreased and the administration of statins is retained, with a repeat of the calcium score after 10 years. These conclusions were reached following the MESA study (Multi-Ethnic Study of Atherosclerosis) in which the 10-year risk of cardiac events was between 1.3-5.6% for a calcium score of 0 and 13-26.6% for a calcium score over 300. The MESA study concluded that in conditions of other cardiovascular disease risk factors maintained constant, the relative risk of cardiac events increases proportionally with the increment of the calcium score [14].

For the good management of patients with coronary atherosclerotic disease, the Guide of the European Society of Cardiology (ESC) was updated in 2019. It established coronary CT angiography as the first line of anatomical evaluation of patients with coronary atherosclerotic disease. The previous recommendations (ESC guide from 2013) proposed a combined approach between anatomical and functional means.

The current management of patients with angina pectoris and suspected obstructive coronary disease consists of a 6-step approach.

1. In the first stage, patients are evaluated through a clinical examination to identify patients with unstable angina or acute coronary syndrome, with the determination of symptoms.

2. The second step involves the evaluation of the general condition and the quality of life - patients with unstable angina or acute coronary syndrome are not included.

3. The third step includes performing some basic tests (blood tests, resting electrocardiogram, resting cardiac ultrasound) and evaluating the function of the left ventricle.

4. Step four consists of assessing the risk of clinical probability of obstructive coronary disease.

5. The fifth step involves diagnostic tests, which are chosen depending on the clinical probability of obstructive coronary disease but also multiple clinical factors, comorbidities, expertise, and access to examinations.

Thus, the anatomical evaluation by coronary CT is recommended as an initial diagnostic test in symptomatic patients in whom the coronary atherosclerotic disease cannot be excluded by clinical evaluation, in those with a low and medium probability of coronary atherosclerotic disease.

Functional tests are recommended in situations where coronary CT angiography is non-diagnostic or has shown stenoses whose functional significance is uncertain.

Invasive angiography is recommended for those with very high clinical probability, symptoms that do not respond to drug treatment, and typical angina at a low level of effort, in patients with an uncertain diagnosis on functional tests.

Coronary CT angiography is not recommended in extensive calcifications, irregular heart rhythm, significant obesity, the impossibility of performing respiratory commands, and any other situations in which qualitative images cannot be obtained.

6. The sixth step, depending on the result of the above tests, the management of the patient and the drug treatment are decided.

Severe calcifications and poor clinical images can lead to an overestimation of the severity of stenoses. In patients that present revascularization through stents and bypass grafts, the accuracy of the coronary CT angiographic examination is impaired in some cases due to the presence of blooming artifacts and the incomplete evaluation of the native vessels. Coronary CT angiography is not recommended in any situations in which qualitative images cannot be obtained: extensive calcifications, significant obesity, and the impossibility of performing respiratory commands [15].

CAD-RADS Coronary Artery Disease Reporting and Data System is a method of evaluating coronary atheromatous plaques developed by the American Cardiology Society with the main purpose of standardizing the reporting of coronary atherosclerotic disease.

Also, the method includes recommendations for patient management depending on the result of coronary CT angiography (Table 2).

Table 2: CAD Rads score for the quantification of stenoses and patient management

CAD-Rads	Stenosis	Additional test
0	0%	-
1	1-24% minimum	-
2	25-49% mild	-
3	50-70% moderate	functional evaluation
4A	70-90% in 1 or 2 vessels	functional evaluation or interventional coronary angiography
4B	>50% in the left main trunk >70% in 3 vessels	interventional coronary angiography
5	100% total occlusion	interventional coronary angiography and viability assessment
N	Non-diagnostic study	additional evaluations

Thus, stenoses are divided into 5 categories, as follows:

• CAD-RADS 0 means the absence of plaques or stenoses and implies the search for other causes of angina pectoris;

• CAD-RADS 1 are plaques without stenosis or minimal stenosis (1%-24%) and imply preventive therapy and risk factor management;

• CAD-RADS 2 includes mild stenoses (25-49%) and includes preventive therapy and risk factor management, especially in patients with non-obstructive plaques in several segments;

• CAD-RADS 3 are moderate stenoses (50-69%). Patient management consists of anti-ischemic pharmacological treatment, preventive with the treatment of risk factors;

• CAD-RADS 4 are severe stenoses, which include two sub-categories: A = severe stenoses (70-99%) and B = tri coronary stenoses (≥70%) or left common coronary trunk (>50%). For category A, interventional coronary angiography or functional evaluation is recommended and for category B, interventional coronary angiography is recommended, to which anti-ischemic drug treatment and treatment of risk factors are added;

• CAD-RADS 5 is total occlusion (100%), and interventional coronary angiography and/or viability assessment is recommended, to which anti-ischemic drug treatment and treatment of risk factors are added;

• CAD-RADS N includes non-diagnostic studies that cannot exclude obstructive coronary disease, and require additional/alternative investigation.

All vessels larger than 1.5 mm in diameter must be included in the classification.

CAD-RADS 1 category also includes plates with positive remodeling and no evidence of stenosis.

There are four modifiers of the CAD-RADS score that are mentioned in the result with the "/" symbol, these are 1 N (non-diagnostic), 2 S (stent), 3 G (graft), and 4 V (vulnerability)[16].

RESULTS

The selected studies show that coronary CT angiography increases the certainty of the diagnosis of coronary atherosclerotic disease. It can predict coronary events, decreases the risk of fatal and nonfatal myocardial infarction at 5 years, and increases the quality of life. Coronary CT angiography has a good indication for being the first test for evaluating coronary stenosis due to its higher predictive value than exercise tests, thus avoiding several unnecessary interventional angiographic tests. The management of patients with coronary atherosclerotic disease can be conducted depending on the result of coronary CT angiography. The detection of severe lesions by coronary CT angiography makes intervention possible in the next stage by angiography and angioplasty.

DISCUSSION

The indications for coronary CT angiography are numerous - from retrosternal pain, clarification of inconclusive non-invasive studies, and assessment of coronary atherosclerosis and by-passes or stents.

The management of the patient with coronary atherosclerotic disease involves a clinical examination and basic tests (blood tests, resting electrocardiogram, resting cardiac ultrasound). Anatomic evaluation by coronary CT is recommended as an initial diagnostic test in symptomatic patients with low and intermediate probability of atherosclerotic coronary artery disease. Functional testing is recommended in situations where coronary CT angiography is nondiagnostic or has revealed stenoses of uncertain functional significance. Depending on the severity of the stenosis, the management of the patient is established. For minimal and small stenoses, preventive therapy and the management of risk factors are chosen. For moderate stenoses, anti-ischemic pharmacological treatment is added, and for severe stenoses, interventional coronary angiography or functional evaluation is recommended.

Also, with the help of coronary CT angiography, other structures such as bones, soft tissues, lungs, or blood vessels can be visualized simultaneously during the scan, being useful for a possible differential diagnosis.

There are limitations of coronary CT angiography such as pregnancy or chronic renal failure, and other contraindications: cardiac arrhythmias, hyperthyroidism, and iodine allergy. In patients with revascularization through stents and bypass grafts, the accuracy of the coronary CT angiographic examination can be impaired due to blooming artifacts and incomplete evaluation of the native vessels. Coronary CT angiography is not recommended in any situations in which qualitative images cannot be obtained: extensive calcifications, significant obesity, and the impossibility of performing respiratory commands.

CONCLUSION

Numerous studies have proven coronary CT angiography as the main investigation for coronary anatomical evaluation. Coronary CT angiography provides important information about the presence and extent of coronary atheroma plaques. A major advantage is the early diagnosis of coronary atherosclerosis, based on which preventive treatment can be administered. Coronary CT angiography decreases the risk of myocardial infarction, allows drug treatment adaptation, and increases the quality of life.

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All authors have read and agreed to the published version of the manuscript.
The respective roles of each author are: ASC first/lead author performed the research, wrote the paper, and is the corresponding author, secondary authors DC and SMS supervised the paper, implemented feedback, and conceptualized some ideas.

Ethics approval and consent to participate
The manuscripts contain a study that hasn't been performed on humans, ethical review and approval were not applicable.

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Not applicable.

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Original Article

Does Intravenous Immunoglobulin Therapy Have a Role in the Treatment of Severe Coronavirus-19 Patients?

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Abstract: *We wanted to evaluate the efficacy of intravenous immunoglobulin (IVIG) treatment in Covid-19 patients in the intensive care unit (ICU). Sixteen (66.7%) male and 8 (33.3%) female adult severe Covid-19 patients who were treated with IVIG in the tertiary ICU between April 2020 and October 2021 at Ankara City Hospital were retrospectively reviewed. The median age of the patients was 63 (interquartile range (IQR):19). The patients were evaluated in terms of laboratory values and clinical results before and after IVIG treatment. There was no statistically significant difference between survived and deceased Covid-19 patients treated with IVIG in terms of age, gender, the onset of symptoms, presence of concomitant diseases, APACHE-II scores, duration of mechanical ventilator support, and length of stay in the ICU. When the laboratory values before and after IVIG treatment were compared, a significant decrease was identified only in alanine transaminase levels (p=0.01). However, there was a statistically significant difference in mortality in 5 patients with Guillain-Barré syndrome who all survived (p=0.001). IVIG treatment was effective in Covid-19 patients with Guillain-Barré syndrome. However, IVIG treatment did not have a statistically significant effect on pre and post-treatment laboratory values, morbidity, and mortality of severe Covid-19 patients in the ICU.*

Keywords: *Covid-19, critical care, Guillain-Barré syndrome, Intravenous immunoglobulin*

INTRODUCTION

Coronavirus disease 2019 (Covid-19) is caused by a RNA virus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1,2]. Covid-19 can lead to asymptomatic infection, mild symptoms, or severe pulmonary symptoms with hyperinflammation [3]. In severe Covid-19 patients, management of hyperinflammation and cytokine storm with appropriate antiinflammatory agents is recommended [4]. Different anti-inflammatory agents have been tried on critically ill patients during the Covid-19 pandemic.

IVIG is a biological agent which contains pooled polyclonal immunoglobulin G from the plasma of approximately one thousand healthy blood donors [1]. IVIG is used for the treatment of immunodeficiency, autoimmune and inflammatory diseases [5]. IVIG has not only anti-inflammatory effects but also the ability to neutralize bacterial toxins [6]. IVIG treatment has also been tried in epidemic diseases related to viruses such as influenza A H1N1, Middle East Respiratory Syndrome (MERS-CoV-2), and Severe Acute Respiratory Syndrome (SARS), and it has been reported that IVIG treatment decreased viral load, caused clinical improvement and decreased mortality in patients with a severe course [7,8]. In light of experience, IVIG treatment has been recommended to be used for the management of patients with Covid-19 [1,9-13]. However, the role of IVIG treatment in patients with severe Covid-19 remains unclear. Thus, this study aimed to evaluate the efficacy of IVIG treatment administered to severe Covid-19 patients in the ICU.

MATERIALS AND METHODS

Local ethics committee approval for this study was obtained from Ankara City Hospital (E2-21-835). The electronic records of 640 Covid-19 patients who were followed up between April 2020 and October 2021 in tertiary ICU were retrospectively reviewed. Thereafter, sixteen (66.7%) male and 8 (33.3%) female adult Covid-19 patients who were treated with IVIG in

the ICU between April 2020 and October 2021 at Ankara City Hospital were included in this study for further analysis. The definitive Covid-19 diagnosis was based on both real-time reverse transcription polymerase chain reaction (RT-PCR) test results and ground glass appearance with pneumonia on thorax computed tomography. These severe Covid-19 patients had also respiratory distress with peripheral capillary oxygen saturation (SPO2) ≤ 93% in room air before admission to the ICU.

IVIG treatment was administered due to sepsis in 6 (25%) patients, Guillain-Barré syndrome in 5 (20.8%) patients, hematological disorders such as hemophagocytic syndrome, chronic lymphocytic leukemia and autoimmune hemolytic anemia in 9 (37.5%) patients, rheumatologic and immunological disorders such as immunodeficiency, systemic lupus erythematosus, myositis and vasculitis in 4 (16.7%) patients. In all patients, IVIG was administered at 0.4 g/kg/day for 3 consecutive days. These 24 patients were evaluated in terms of laboratory values before the first IVIG treatment session and after the third IVIG treatment session. Thus, neutrophil, lymphocyte and platelet count, neutrophil/lymphocyte ratio, lactate dehydrogenase, d-dimer, fibrinogen, ferritin, procalcitonin, c-reactive protein (CRP), interleukin-6, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were noted. Demographic data, the onset of symptoms, concomitant diseases, APACHE II scores, duration of mechanical ventilator support, length of ICU stay, and mortality rates were also recorded.

For all statistical analysis, Statistical Packages for the Social Sciences (v17.0 SPSS 24 Inc. Chicago, IL) software was used. Categorical data were presented as numbers and percentages, while quantitative data were presented as mean standard deviation or median and interquartile range (IQR), as appropriate. The differences between the two groups were evaluated with the Mann-Whitney U test and the chi-square test. The statistical significance level was accepted as $p<0.05$.

RESULTS

The median age of the patients included in this study was 63 (IQR:19), and the median time to symptom onset was 6.5 days (IQR:6). The median APACHE II score of these patients was 16 (IQR:9). The median length of stay in the ICU was 22.5 (IQR:34) days, whereas the median mechanical ventilator time was 19 days (IQR:29) (Table 1). Eighteen (75%) patients had concomitant diseases such as diabetes mellitus type 2, hypertension, and coronary artery disease. The most common disease accompanying Covid-19 was hypertension in 13 (54.2%) patients. The most common symptom was dyspnea in 17 (70.8%) patients. Fever was present in 7 (29.1%) and cough in 5 (20.8%) patients. Other less frequent symptoms were headache, sore throat, nausea, vomiting, impaired taste and smell, myalgia, and weakness. Complications, which were not related to IVIG treatment, such as gastrointestinal bleeding in 2 patients, cerebrovascular accident in 1 patient, myocardial infarction in 1 patient, pneumothorax in 6 patients, and seizures in 2 patients were observed in total in 12 (50%) patients. Antibiotics were empirically administered in 15 (62.5%) of the patients. Unfortunately, 16 (66.7%) patients passed away due to complications related to Covid-19 (Table 1).

Table 1: Characteristics of the Intravenous Immunoglobulin Treated Covid-19 Patients

Characteristics of Covid-19 patients	Survived	Deceased	Total	*P* value
Gender (n, %) Male Female	 6 (75) 2 (25)	 10 (62.5) 6 (37.5)	 16 (66.7) 8 (33.3)	 0.67*
Age (years) (median, IQR)	61.5 (29)	63.5 (19)	63 (19)	0.85**
The onset of symptoms (days) (median, IQR)	6.5 (3)	6.5 (6)	6.5 (6)	0.60**
Length of ICU stay (days) (median, IQR)	29.5 (42)	22.0 (23)	22.5 (34)	0.42**
Mechanical ventilator duration (days) (median, IQR)	26 (59)	16 (27)	19 (29)	0.32**
Presence of concomitant disease (n, %) Diabetes mellitus type 2 Hypertension Coronary artery disease	7 (87.5) 4 (50) 6 (75) -	11 (68.8) 4 (25) 7 (43.8) 5 (100)	18 (75) 8 (33.3) 13 (54.2) 5 (20.8)	0.62* 0.36* 0.21* 0.13*
Complications (n, %)	5 (62.5)	7 (43.8)	12 (50)	0.67**
Antibiotic use (n, %)	4 (50)	11 (68.8)	15 (62.5)	0.41**
APACHE II score (median, IQR)	15 (9)	18.5 (12)	16 (9)	0.13**

*†IQR: interquartile range. ‡ICU: intensive care unit; *Chi-square test was used. **Mann-Whitney U test was used*

There was no statistically significant difference between survived and deceased patients in terms of gender, age, the onset of symptoms, length of stay in the ICU, duration of mechanical ventilator support, accompanying symptoms, concomitant diseases such as diabetes mellitus type 2, hypertension, coronary artery disease, complication development, antibiotic use, and APACHE II scores. However, a significant difference was identified in mortality when the effect of IVIG treatment administered for Guillain-Barré syndrome in 5 patients and other disorders were compared (p=0.001). All patients with Guillain-Barré syndrome survived after IVIG treatment. All 6 patients, who were treated with IVIG for sepsis, died. Three patients died and 1 patient survived with rheumatological and immunological diseases. Seven of 9 patients with hematological diseases passed away, whereas 2 patients survived (Table 1). When the laboratory values before and after IVIG treatment were compared, a significant decrease was found only in ALT levels (p=0.01) (Table 2).

Table 2: Laboratory Parameters of Covid-19 Patients Before and After IVIG Treatment

Laboratory parameters	Before IVIG treatment	After IVIG treatment	*P* value*
Neutrophil count x10^9/L	8.4 (6.5)	8.0 (7.8)	0.84
Lymphocyte count x10^9/L	0.59 (0.49)	0.59 (0.45)	0.26
Platelet count x10^9/L	206.5 (152)	210 (142)	0.48
Neutrophil/lymphocyte ratio	13.0 (10.4)	11.8 (7.0)	0.39
Lactate dehydrogenase (U/L)	456.5 (349)	459.5 (193)	0.18
D-dimer (mg/L)	2.79 (4.91)	2.69 (6.05)	0.74
Fibrinogen (g/L)	5.4 (2.9)	4.4 (2.8)	0.22
Ferritin (µg/L)	1011 (1417)	715 (1198)	0.38
Procalcitonin (µg/L)	0.30 (0.35)	0.51 (1.17)	0.10
C-reactive protein (g/L)	66 (136)	95 (151)	0.76
Interleukin-6 (pg/mL)	31.1 (34.3)	38.2 (61.4)	0.40
Aspartate aminotransferase (U/L)	44.5 (50)	40.5 (26)	0.21
Alanine aminotransferase (U/L)	52.5 (48)	34.5 (46)	0.01

*In all patients, IVIG was administered 0.4 g/kg/day for 3 consecutive days. Numerical data are given as a median and interquartile range. IVIG: intravenous immunoglobulin. *Wilcoxon test was used.*

DISCUSSION

The severity of the cases, the IVIG dose, and the time of administration should be considered when evaluating the effect of IVIG on Covid-19. In our study, the patients who underwent IVIG therapy were severe Covid-19 patients and all of the patients received IVIG treatment at the same dose and duration (0.4 g/kg/day for 3 consecutive days).

Huang et al. reported that 45 non-severe Covid-19 patients who were treated with IVIG had no benefit beyond standard therapy in terms of duration of fever, virus clearance time, length of hospital stay, and use of antibiotics [14]. However, after the initiation of IVIG therapy in Covid-19 patients, there have been several studies published reporting that IVIG treatment reduced mortality in Covid-19 patients. Gharebaghi et al. reported that 30 Covid-19 patients were treated with IVIG four vials daily for three days and the in-hospital mortality rate was significantly lower in the IVIG group compared to the control group (6 (20.0%) vs.14 (48.3%), p=0.022) [15]. Sakoulas et al. administered IVIG 0.5 g/kg/d for 3 days to 16 Covid-19 patients, reporting that patients treated with IVIG had shorter median ICU stay (2.5 vs 12.5 days, p=0.006) in addition to improvement of hypoxia compared to the control group [16]. Raman et al. reported that 50 Covid-19 patients were treated with IVIG, who had a significantly shorter hospital stay, and faster recovery with an improvement in oxygen saturation. Moreover, Raman et. al claimed that positive RT-PCR tests of Covid-19 patients treated with IVIG became negative in a shorter period compared to the control group (7 vs 18 days) [17]. Ali et al. administered each group with 10 patients with different doses of IVIG 0.15, 0.20, 0.25, and 0.30 g/kg. Even 10 of 40 participants (25%) passed away, Ali et al. reported that IVIG increased survival chances and reduced the risk of Covid-19 progression [18]. Esen et al. reported that 51 Covid-19 patients who received IVIG had prolonged median survival time and reduced CRP levels [19]. Shao et al. stated that early administration (admission ≤ 7 days) of high-dose IVIG (> 15 g per day) not only improved the prognosis but also reduced 60-day mortality in severe Covid-19 patients [20].

The latest Surviving Sepsis Campaign Guidelines did not recommend IVIG therapy for the treatment of sepsis due to the low quality of evidence from previous studies [21]. All of our 6 patients treated with IVIG for sepsis died, however, all patients with Guillain-Barré syndrome survived after IVIG treatment in this study. In addition, there are several case reports of Covid-19 patients who had neurological disorders such as acute disseminated encephalomyelitis and Guillain-Barré syndrome which were reported to be successfully treated with IVIG [22-27]. Moreover, IVIG treatment has been reported to be effective in Covid-19 related immunological diseases such as multisystem inflammatory disease, myocarditis, and Kawasaki disease [28].

However, there are several studies claiming that IVIG treatment did not affect the need for mechanical ventilation, improvement in chest tomography, and reduction in mortality in severe Covid-19 patients [29]. Tabarsi et al. administered 400 mg/kg IVIG daily for three days to 52 patients, in addition to hydroxychloroquine and lopinavir/ritonavir treatment. IVIG treatment was initiated after 3.84 ± 3.35 days of admission and Tabarsi et al. did not identify any significant difference between the IVIG and control group in terms of the need for mechanical ventilation and mortality [29]. Hou et al. reported that 113 Covid-19 patients were treated with IVIG. Patients who received IVIG had a longer hospital stay of 23.0 days (19.0–31.0) with a higher mortality rate than the control group (9 (19.1%) vs 4 (6.1%), p= 0.032) [30]. Hou et al. stated that IVIG improved neither in-hospital mortality nor the need for mechanical ventilation in severe Covid-19 patients. Liu et al. stated that they treated 421 severe Covid-19 patients with IVIG and they observed no significant differences in 28-day mortality, acute respiratory distress syndrome, acute hepatic injury, acute kidney injury, diffuse intravascular coagulation, myocardial injury, shock and invasive mechanical ventilation between the IVIG and control group [31]. Focosi et al. evaluated 2401 Covid-19 patients from 10 studies and concluded that IVIG was not associated with reduced death risk, thus the current evidence from the medical literature did not support IVIG therapy in Covid-19 patients [32]. In a recently published retrospective, multi-center cohort study of critically ill Covid-19 patients, 109 patients treated with standard therapy and 74 patients who received IVIG therapy were compared [33]. Salehi et al. reported that there was no difference in length of stay in the ICU, duration of mechanical ventilation, and mortality between standard therapy and IVIG therapy groups. Thus, Salehi et al. concluded that IVIG therapy was not beneficial in critically ill Covid-19 patients [33].

Within our study, there was no statistically significant difference in mechanical ventilation duration and length of stay in the ICU between critically ill Covid-19 patients who survived or died (p=0.32 and p=0.42, respectively). However, we did not have the opportunity to compare critically ill Covid-19 patients who received IVIG therapy plus standard treatment and only standard treatment.

Moreover, the effect of IVIG on mild Covid-19 cases requires further research. Huang et al. reported that 45 non-severe Covid-19 patients who were treated with IVIG had no benefit beyond standard therapy in terms of duration of fever, virus clearance time, length of hospital stay, and use of antibiotics [14].

In this study, laboratory parameters before and after IVIG treatment were evaluated. There was neither a statistically significant increase nor decrease in acute phase reactants such as CRP, procalcitonin, ferritin, and fibrinogen before and after IVIG therapy (p=0.76, p=0.10, p=0.38, and p=0.22, respectively.) The only laboratory parameter which had a statistically significant decrease after IVIG therapy was ALT in critically ill Covid-19 patients (p=0.01) (Table 2).

IVIG may slightly increase liver function tests. However, Chen et al. reported that IVIG was effective in alleviating hepatic ischemia-reperfusion injury in a rat model by reducing ALT, AST, IL-6, and TNF-alpha [34]. Moreover, Omma et al. reported improvement in liver function tests including ALT after IVIG treatment [3].

IVIG treatment is an expensive therapy option in developing countries. Therefore, we had to administer IVIG as a salvage therapy only to severe Covid-19 patients. Unfortunately, 16 (66.7%) of 24 severe Covid-19 patients passed away despite IVIG treatment.

IVIG therapy has the risk of several side effects such as thromboembolism, renal failure, meningoencephalitis, and anaphylaxis [35]. We have encountered no side effects during IVIG therapy in our ICU.

The main limitation of this retrospective study was the small sample size. A larger sample size will help to learn more about the positive and negative effects of IVIG therapy in Covid-19 patients. As our intensive care unit was reserved for only severe Covid-19 patients during the pandemic, this study lacked a control group. The effect of IVIG on severe Covid-19 cases requires further well-designed prospective randomized controlled studies.

CONCLUSION

IVIG treatment was effective in Covid-19 patients with Guillain-Barré syndrome. However, IVIG treatment did not have a statistically significant effect on pre and post-treatment laboratory values, morbidity, and mortality of severe Covid-19 patients in the ICU.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.
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Authors' contribution
AConceptualization, S.G.I., C.M.A., M.E.Y., S.I.; methodology, M.E.Y., S.I.; software, M.E.Y.; validation, S.G.I., C.M.A., M.E.Y., S.I.; formal analysis, M.E.Y.; investigation, M.E.Y.; resources, S.G.I., C.M.A., M.E.Y., S.I.; data curation, S.G.I, C.M.A, M.E.Y., S.I.; writing-original draft preparation, M.E.Y.; writing-review and editing, S.G.I., C.M.A., M.E.Y., S.I.; visualization, M.E.Y.; supervision, S.I.; project administration, S.I. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate
The study was conducted under the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Ankara City Hospital (E2-21-835).

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Original Article

Deregulation of the Hippo Signaling Pathway in Virus-Associated Cancer

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Abstract: *Hippo signaling has been recognized as a newly identified tumor suppressor signaling pathway that can regulate cellular processes including regeneration, cell death, differentiation, and development. Its dysregulation through overexpression of YAP (Yes-associated protein) and TAZ (PDZ-binding motif) as two main oncogenic factors of the Hippo pathway has a crucial role in several cancers. Because of the limited prognosis and therapeutic targets, further understanding of molecular pathways involved in tumorigenesis is one of the interesting issues in studies. Here, we demonstrated that some viruses, through dysregulation of the Hippo signaling pathway can be implicated in transformation, metastasis, and chemotherapeutic drug resistance in virus-related cancer and also help processes involved in the pathogenesis of viral infection such as persistence.*

Keywords: *Hippo signaling pathway, virus, cancer*

INTRODUCTION

The Hippo signaling pathway was discovered to be involved in controlling organ size and cell differentiation in Drosophila melanogaster for the first time, and it has recently been recognized as a critical tumor-suppressor network [1]. Consequently, the correlation of deregulated Hippo pathway signaling in several cancer types such as breast, liver, lung, prostate, gastric and colorectal tumors has been reported. In addition to this role, the Hippo pathway is involved in the conservation of tissue-specific stem cells, tissue renewal, and damage recovery [2,3].

The Hippo pathway is a kinase cascade that includes kinases MST1 and MST2 (also known as STK4 and STK3), large tumor suppressors 1 and 2 (LATS1 and LATS2) and adaptor proteins SAV1 (Salvador homolog 1), and MOB kinase activator 1A and 1B (MOB1A and MOB1B). MST1 and MST2 induce LATS1 and LATS2 phosphorylation, which in turn facilitates LATS-dependent phosphorylation of the homologous oncoproteins YAP (Yes-associated protein; encoded by YAP1) and TAZ (PDZ-binding motif, also known as WWTR1) that inhibit the activation of transcriptional co-activators Yorkie (Yki), YAP, and TAZ [4-6].

Hippo pathway main effectors YAP and TAZ, phosphorylation by the Hippo core complex can retain them in the cytoplasm where they expose to proteasomal degradation [7]. Since TAZ/YAP mutations rarely happen other mechanisms are more likely responsible for Hippo's relevance in cancer. Growing evidence suggests that a complex network of interactions with critical pathways can modulate YAP/TAZ activity such as TGFB, GPCR, Notch, EGFR, and angiogenesis [8].

In the case of modulation of Hippo signaling, stimulation of G protein-coupled receptors (GPCRs) associated with Gas might lead to phosphorylation of LATS and consequently down-regulation of YAP. GPCRs as integral membrane receptors activated by external ligands their binding leads to the activation of the alpha-subunits of subclasses of G proteins. In

contrast, stimulation of Ga12/13-coupled GPCRs is associated with suppression of LATS and up-regulation of YAP [9,10].

Other instances of membrane receptors that reported to modulate YAP expression are the receptor tyrosine kinases EGFR and ERBB4 [11]. The EGFR ligand amphiregulin (AREG) is an important YAP target that provides a positive cycle that mediates YAP and EGFR signaling and can stimulate intensifying proliferative signals from both pathways. Also, ERBB4 as a member of the EGFR family, via interaction of YAP and possibly TEAD1 with its intracellular domain in the nucleus, demonstrated either induction of YAP activation or increase expression of YAP target genes [12].

Oncoviruses are associated with about 12% of all human cancers. People carry at least one of these oncoviruses in their life, but these infections only a small proportion of these individuals go on to develop cancer [13]. The interaction between viral factors and the host is a complex process to provide a desirable microenvironment for oncogenesis [14]. The aim of this review is the improvement of our understanding of mechanisms of oncogenesis modulation by different viruses and a better understanding of tumorigenesis that may result in more effective targeted therapy in the future. By the recent rises in viral-related carcinomas and the link between Hippo and viruses, we assessed to determine if Hippo signaling is the main target in such cancers.

HEPATITIS B Virus

In recent decades human hepatocellular carcinoma (HCC) with approximately 746,000 deaths per year, identified as the sixth most common cancer and the third leading reason for cancer-related deaths in human populations [15].

Hepatocarcinogenesis, due to several genetic and epigenetic changes which occur through different stages of the disease is known as a complex process [16]. As regards the fact that hepatitis B virus (HBV) infection has a key role in the establishment of HCC, it is important to know its proteins' roles in Hepatocarcinogenesis. HBV encoded four proteins and among these proteins, the HBV X protein (HBx) is a multifunctional protein and has a critical role in hepatocellular carcinogenesis. Multiple signaling pathways are participating in the HCC progression [17,18].

Many studies are designed to assess the molecular and cellular mechanism of HCC because finding a suitable treatment for HCC is an immediate task for healing patients suffering from HCC. Recently, numerous researches have shown the role of the Hippo pathway in organ size control, cell proliferation, cell death, and tumor progression [19,20]. Although the role of YAP, which is a downstream factor of the Hippo signaling pathway in HCC, remains unclear, this review provides a vision for the regulation of YAP upstream and downstream effectors involved in hepatic cancer [21].

Interestingly, the hyperexpression of YAP can result in epithelial-to-mesenchymal transition (EMT) and may inhibit apoptosis, growth factor-independent proliferation, and anchorage-independent growth [22]. The liver has an amazing characterization that can lead to having unique features such as Growth control which in the liver has some unusual features. The liver has been known for its noticeable capacity to renew consequently to a two-third partial hepatectomy (PHx) or injury. The natural liver in an adult is composed of two parenchymal cell types — hepatocytes and cholangiocytes [23].

Dedifferentiated hepatocytes as multipotent stem cells are capable to be the source of tissue renovation in the damaged liver [24]. Factors of the Hippo signaling pathway including MST1 and MST2 have been demonstrated to have key roles in adjusting or restricting liver progenitor cells. Thus, it is predicted that the Hippo can be involved in the suppression of stem/progenitor cell proliferation in the mature liver [25]. Furthermore, some studies also showed that knocking out YAP resulted in hepatocyte and cholangiocyte injury and loss [26].

In synergy with the functional roles of Hippo signaling, any change of upstream effectors of the hippo pathway like Mst1/2 can result in over-proliferation and sometimes Hepatocarcinogenesis. Since HBV is commonly the major reason for HCC; YAP possibly has an important role in its carcinogenesis. Interestingly, YAP expression is frequently shown to be increased in HBV infections resulting in HCC, infected cell lines, and HBx transgenic mice. Through the CRE-dependent HBx attaching to the YAP promoter, HBx can increase YAP and follow stimulate YAP target genes which leads to the proliferation of hepatoma cells. Findings revealed the importance of the Hippo signaling pathway in cancer and suggested new treatments for HBV-infected patients to inhibit progression to HCC [27].

Studies have revealed that the loss of Mst1/2 may result in the promotion of several tumors in the liver of mice. The loss of both Mst1/2 is required to affect the liver since the loss of Mst1 or Mst2 is not effective on liver size or the number of liver tumors [28]. These findings firmly revealed the effect of Mst1/2 on YAP activity. Interestingly, lack of YAP phosphorylation and impairment of Mst1/2 activity that occurs in carcinogenesis have been reported in 30% of HCCs [29].

The liver-specific NF2/merlin inactivation may lead to an increase in hepatic stem cell number with a lack of any changes in differentiated hepatocytes [30]. The studies on Sav or Mst1/2 positive livers for assessment of the effectiveness of

YAP/TAZ and Lats1/2 phosphorylation in the hippo pathway displayed complex results regarding the need for Mst1/2 or Sav in Lats1/2 phosphorylation [29,31] and since in mouse fibroblasts, for YAP phosphorylation, presence of Mst1/2 was not crucial, it is considered that the pathway may be specific in hepatitis [29].

In a study on transgenic mice, it was shown that YAP induction can lead to abnormal hepatocyte expansion after 2 months and consequently several nodes have been reported in hepatitis [32]. The nodes were reported to be a collection of hepatocytes in a generative condition that condensed the near parenchyma and revealed severe HCC. Also, this liver-specific loss of either Mst1/2 in hepatocytes leads to noticeable liver growth because of its deregulated cell proliferation [28].

On the other hand, when transgenic mice models with YAP hyperactivity crossed with mice lost its DNA-binding domain TEAD2 do not promote HCC. Lacking YAP overexpression may lead to having mice with livers deficiency in Nf2/merlin after crossing with mice that have the defective TEAD2 gene, confirming the therapeutic concept of using the TEAD with YAP in the complex. Verteporfin injection has been demonstrated to reduce liver mass in YAP transgenic animal models and also has no side effect on the wild mice tissue homeostasis, defining the potency of compounds for therapeutic administration in patients with HCC. Verteporfin displayed numerous important features that make its clinical execution for HCC promising [32].

In HCC cell lines induction of YAP results in further resistance to doxorubicin-induced apoptosis; while, inhibition of YAP expression by RNA interference has been reported to reverse YAP-increased chemoresistance. These processes are mediated by the MAP kinase signaling pathway induction rather than an AKT-dependent mechanism of chemoresistance [33]. This process of chemoresistance occurs after treatment by the MEK1/2 suppressor U0126 before doxorubicin injection, which noticeably reduces cell vitality in YAP overexpressing HCC lines [34].

Survivin is accepted as an antiapoptotic protein and in HCC patients identified to be associated with a weak prognosis. Recent studies have shown YAP overexpression is in associate with survivin induction in patients with HCC. It seems that YAP has this oncogenic capacity on the cell with a survivin increase leading to carcinogenesis [35].

HUMAN PAPILLOMAVIRUS

Cervical cancer considers the most important cancer in women because it is the fourth leading reason of cancer-related death in women. Due to the detection of human papillomavirus (HPV) DNA in the majority of cervical cancer patients, high-risk HPV types recognize as the cause of cervical cancer [2].

In a study by Chunbo He et al results implicated that YAP1, one of the main oncogenic effectors of the Hippo pathway, was (11%) amplified several times, whereas LATS1/2, MST1, and FATs, that identified as upstream tumor inhibitors of the Hippo pathway, were not detectable or mutated in cervical cancer patients. YAP1 induction in cervical cancer cells noticeably triggers the expression of EGFR and its related ligands AREG and TGFa. On the other hand, TGFa and AREG, through EGFR signaling, can block the Hippo pathway and induce YAP protein (dephosphorylated LATS1, MOB1, and YAP1) to promote cervical cancer cell growth. Recognition of this circumstance between Hippo/YAP and EGFR in human cervical cancer provides an opportunity to combine targeting of the Hippo/YAP and EGFR pathways to have an efficient way to treat cervical cancer [36].

In addition, hyperactivation of YAP1 can be an important mediator to the persistence of HPV infection. First, studies report that hyperactivated YAP1 increases the expression of HPV receptor molecules such as ITGRA6, SDC1, and EGFR, which through the early stage of HPV infection can help the HPV entry process. Second, is that hyperactivation of YAP1 in cervical epithelial cells may reduce viral pattern recognition by the innate immune system. Some studies report that TLR2 and TLR4 and adaptor proteins MYD88 and TRIF were suppressed, which could facilitate HPV infection. Third, there is evidence that ectopic expression of YAP may suppress the activation of transcriptional factors that are essential for the production of type I IFNs, including IRF1, IRF3, and IRF7, leading to the reduction of IFNA1, IFNB1, and IFNE in hers. IFNs can activate antiviral factors of the immune system and play their role in the progress of innate and adaptive immune responses. Also, some studies showed that the expression and activation of main components of the type I IFN pathway, like IFNAR2, STAT1, JAK1, and IRF9, are suppressed by the expression of YAPS127A in the epithelium of the cervix. Suppression of the IFNAR2/JAK1/STAT1 signaling pathway leads to decreasing of antiviral ISGs, such as MX1, CH25H, and IFITMs (blockers of virus entry), and APOBEC3G, OAS1, ISG15, and IFI44L (suppressors of virus translation and replication) [37].

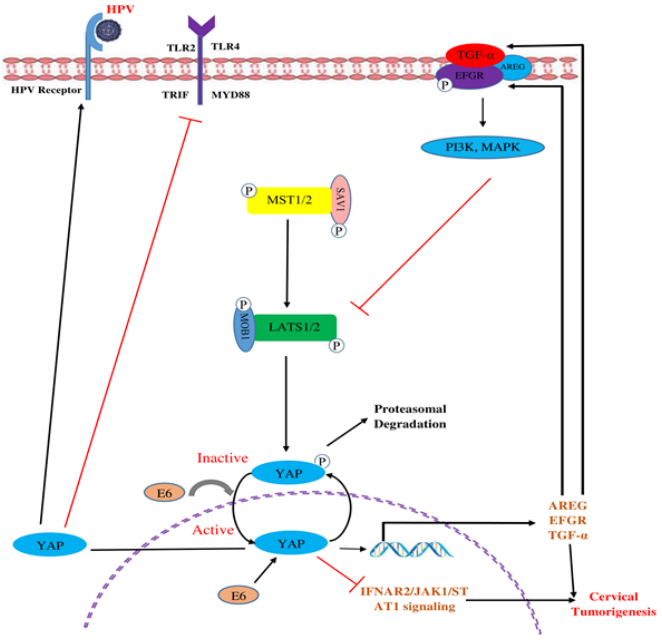
The HPV oncoproteins E6 and E7 expression can result in the suppression of some essential tumor inhibitors such as TP53 and RB1 in many types of cells [38,39]. Suppression of TP53 and RB1 extends cell life (by inhibition of death) and may lead to immortality in cervical epithelial cells, facilitating malignancy of HPV-infected cells by an oncogenic signal (including hyperactivation of YAP1) [40,41].

In epithelial cell cultures, cell-cell contact development is a key step in differentiation before the formation of distinguished apicobasal surfaces. The transcriptional co-activator YAP plays a key role in this event [42,43]. Numerous reports have shown that cell-cell contacts activate the LATS1/2 serine/threonine kinases which can phosphorylate YAP [44,45]. During phosphorylation, YAP restricts from the nucleus and it inhibits the transcription of key genes in the cell cycle [42,46]. A cervical cancer study recently demonstrated that in high-grade cervical lesions levels of nuclear YAP are increased [47].

There are some studies demonstrating that HPV16-E6 or HPV16-E6/E7 double-transgenic mice treated with estrogen may promote cervical cancer [48,49]. Related to that result, other studies report that YAP was increased in the E6 and E6/E7-induced cervical tumor cells, and YAP was localized in the nucleus of these cells [36]. The human foreskin keratinocyte(HFK) "raft" culture infected with HPV16 makes a unique model for the assessment of the HPV16 proliferation cycle [48,50].

YAP can be inactivated by two mechanisms: first, the Ser127 phosphorylation-mediated spatial adjustment (system of nuclear-cytoplasmic shuttling); and second, the Ser397 (Ser381 in YAP protein isoform 2) phosphorylation-mediated adjustment (the phosphodegron-induced degradation). These two mechanisms are applicable directly to prevent YAP oncogenic activity [51,52]. It has been observed that CK1d/ε and SOCS6 proteins are crucial for YAP degradation [52,53]. Although expression of HPV E6 in HT3 cells causes a noticeable increase in CK1d/ε and a reduction in p-YAP (S397), it suggests that the CK1d/ε pathway may not participate in the HPV E6-mediated YAP protein turning [40]. HPV E6 increases YAP protein levels, but also it upregulates the expression and activation of AREG protein in the cell culture. These observations showed a possibility that HPV E6 protein may be associated with the Hippo-ERBB signaling circle and also implicating the transformation and development of cervical cancer. Interestingly, HPV16 E6 and E7 could transcriptionally activate EGFR expression [40,54]. The HPV16 E6 makes receptor protein tyrosine kinase to signaling and facilitates the internalization of phosphorylated receptors [55] (Figure 1).

Figure 1: Schematic presentation of reciprocal interactions between HPV and Hippo signaling pathway



POLYOMAVIRUSES

Through identification of SV40 tumorigenesis in specific laboratory animals and transforms several types of cultured cells offered an opportunity to discover the molecular basis of the tumor. Viral oncogenes were introduced as key tools in the recognition of tumor inhibitor pathways in human primary cells. The p53 and p16/pRb tumor inhibitor pathways have been introduced as targets of SV40 large T. Hahn's study indicates a transformation model [56], expressed HRas V12, and a construct encoding SV40 large T to target the p14ARF/p53 and p16INK4A/pRb pathways. Recently it is discovered that SV40 small T (ST) (encoded in the large T construct) is needed without requiring large T to enhance anchorage-independent growth [57].

Murine polyomavirus also has frequently presented visions into tumorigenesis by demonstrating important modulation

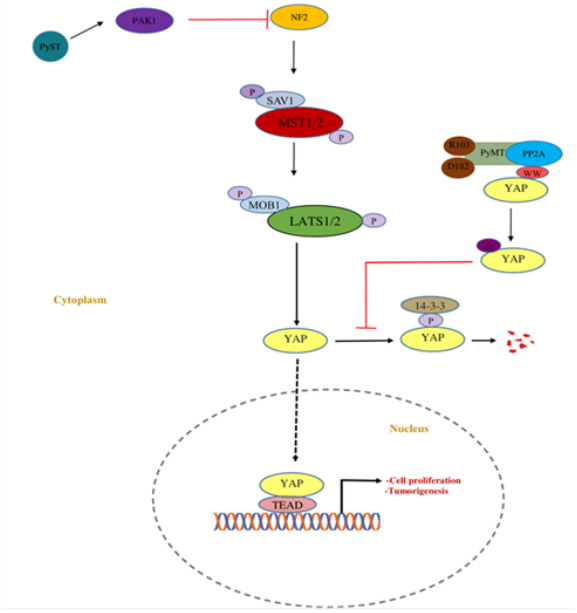
mechanisms including tyrosine phosphorylation and phosphoinositide 3-kinase (PI3K) signaling. Previous studies showed that polyomavirus small T antigen (ST) associates with YAP, an effector of the Hippo pathway, to modulate differentiation. Other studies introduced YAP as a target of middle T antigen (MT) required for transformation [58,59]. Wild-type MT via a surface containing residues R103 and D182 binds to the YAP WW domains. Mutation in each one of R103 or D182 of MT disrupts YAP binding. This happens without disturbing bindings to other signaling factors or the strength of PI3K or Ras signaling. One of the other genetic disruptions of MT binding to YAP or stopping of YAP via short hairpin RNA (shRNA) decreased MT transformation, offering that YAP has an important participation in the transformed phenotype. MT targets YAP via triggering signaling pathways that affect it and consequently bind to it. The signaling of either wild-type MT or the YAP associate MT mutant develops YAP phosphorylation at S127 and S381/397 (YAP2/YAP1) [58]. In addition to previous roles of these phosphorylated serines, MT signaling results in the reduction of YAP from the nucleus and degradation. When MT Binds to YAP, it associates YAP with protein phosphatase 2A (PP2A), resulting in the YAP dephosphorylation together with the MT complex. Furthermore, this process results in the increase of YAP in membranes. Overall, these data demonstrate that YAP develops MT transformation by mechanisms that may be due to YAP's established major transcriptional activation functions [60].

The capability of ST to discuss anchorage-independent growth brings with it its ability to inactivate the PP2A function. Suppression of PP2A may bypass the need for ST in cellular transformation models, and mutations inactivating PP2A subunits have been recognized in tumors [61,62]. PP2A has several substrates including, AKT, b-catenin, and c-Myc which are defined to be activated by ST. Although the total overexpression of these proteins only incompletely replaced ST in cellular transformation, it suggests that undefined PP2A effector pathways may be involved [62].

Hippo/MST kinase acts by a membrane-associated complex including the tumor inhibitor NF2/Merlin [63]. MST kinases enhance activating LATS kinases, which subsequently phosphorylation of the transcriptional coactivators YAP and TAZ, resulting in their inactivation of them via cytoplasmic isolation and increased degradation [64-66]. Suppression of YAP and TAZ restricts proliferation and triggers apoptosis [67].

Interestingly, it is observed that overexpression of YAP is enough to result in tumorigenesis in mice. Both SV40 ST and the Merkel cell polyomavirus ST induce endogenous YAP. Some studies provide data that ST acts through PAK1 to inactivate the NF2 tumor suppressor to alleviate the inactivation of YAP. Activation of PAK1 at the cell membrane is sufficient to bypass the requirement for ST. Restricting PAK1 activity disrupts the transforming capability of the ST protein. Therefore, ST detaches YAP from modulation via the Hippo tumor inhibitor pathway. Induction of Active YAP is required and enough to replace ST in cellular transformation. Therefore, the YAP proto-oncogene is an important mediator of the transforming properties of the ST oncoproteins [68] (Figure 2).

Figure 2: Schematic presentation of reciprocal interactions between Polyomaviruses and Hippo signaling pathway



EPSTEIN-BARR VIRUS

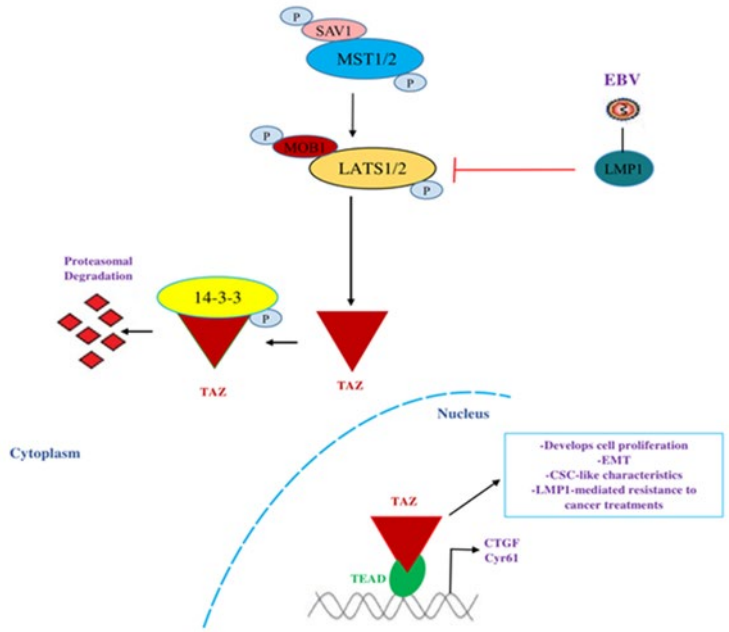
Epstein-Barr virus (EBV) is recognized as a linear dsDNA virus and a member of the human gamma-herpesvirus family that observed to correlate with various B-cell lymphomas, such as Burkitt's lymphoma, Hodgkin's lymphoma, and other tumors like nasopharyngeal carcinoma, T-cell non-Hodgkin lymphomas, and gastric carcinomas. These cancers are firmly correlated with latent Epstein-Barr virus (EBV) infection, genetic factors, and environmental factors thus EBV can regulate mechanisms affecting carcinogenesis, proliferation, death, migration of cells, epigenetic changes in lymphocyte-specific development, and induce cell immortalization. The main mechanisms related to the carcinogenic effectors of EBV consist of LMP1, LMP2, and microRNA that we will introduce bellow [69-71].

The capacity of LMP1 to immortalize and transform cells possibly depends on simultaneously regulating cellular signaling pathways which suppress apoptosis or mediate proliferative, growth factor-like effects. Furthermore, LMP1 can induce a cancer progenitor cells (CPC)-like appearance in epithelial cells, offering that LMP1-induced phenotypic alterations can be related to the promotion of cancer [72]. Therefore, the mechanism and role of LMP1 in immortalizing and transforming cells is complex and several important questions remain to be answered.

Previous studies demonstrated that LMP1 could induce actin remodeling [73]. The actin cytoskeleton is a key factor for the modulation of the Hippo pathway. These data make us investigate How LMP1 can upregulate TAZ expression by inhibition of the Hippo pathway. In a study, findings determined that the LMP1-targeted CNE1-LMP1 cells decreased TAZ expression. The target genes of TAZ are CTGF and Cyr61. Other researches demonstrate that LMP1 can trigger the mRNA expression of CTGF and Cyr61 [74]. There is the possibility that TAZ may be involved in the positive modulation of cell proliferation and survival associated with the obtaining of cancer stem cell (CSC) properties and epithelial-to-mesenchymal transition (EMT) [75, 76]. Previously, some studies have been done to determine if LMP1 can develop EMT by TAZ and define if the morphology alters. The result of these studies has shown some morphological variations from epithelial to mesenchymal phenotypes. LMP1 can develop cell proliferation, EMT, and CSC-like characteristics by TAZ and give us some visions and rationales for the LMP1-mediated resistance to cancer treatments [74].

On the other hand, it has been shown that phosphorylation of the WW domain of the transactivator TAZ by activated LATS1/2, leads to TAZ degradation [77]. Studies have shown that LMP1 can elevate the level of TAZ and increase TAZ nucleus translocation. Thus, it is possible that LMP1 could suppress LATS1/2 activation, resulting in the enhancement of TAZ stability [74] (Figure 3).

Figure 3: Schematic presentation of reciprocal interactions between EBV and Hippo signaling pathway



KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

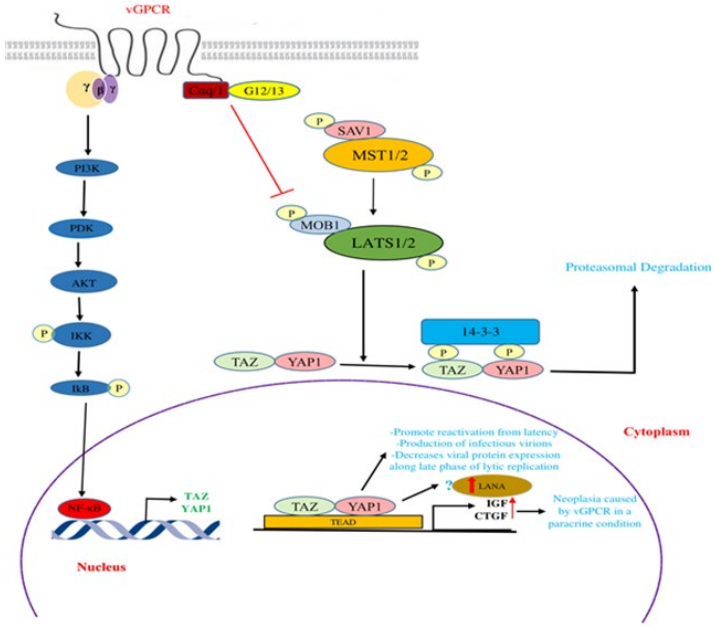
Kaposi sarcoma-associated herpesvirus (KSHV) is a double-stranded linear DNA virus of the human gamma-herpesvirus family from rhadinovirals and also an oncogenic virus that is responsible for human Kaposi sarcoma (KS) and is associated with an AIDS-defining malignancy. One of the main viral proteins of KSHV is a viral G-protein-coupled receptor (vGPCR). KSHV encodes vGPCR which is essential for the initiation and progression of Kaposi sarcoma [78-80]. Previous studies identified that YAP/TAZ, two homologous oncoproteins that can be suppressed by the Hippo tumor inhibitor pathway, activates in KSHV-infected cells in in vitro conditions, KS-like tumors in mice, and clinical human KS samples [81].

The molecular process to which vGPCR contributes in KSHV-induced oncogenesis remained uncertain but it is suggested to be involving both direct and paracrine oncogenic effects, that are activated by growth factors including VEGF and PDGF induced by vGPCR [82,83]. The KSHV protein (vGPCR) activity is via Gq/11 and G12/13 to suppress the Hippo pathway kinases Lats1/2, enhancing the activation of YAP/TAZ. Also, clearance of YAP/TAZ inhibits vGPCR-induced cell proliferation and tumorigenesis in a xenograft mice model. The transformation of cells mediated by vGPCR is sensitive to pharmacological blockers of YAP [84,85].

It is demonstrated that the Knockdown of YAP or TAZ can inhibit the vGPCR-induced CTGF expression, offering that YAP/TAZ enhances the biological functions of vGPCR. In addition to the Hippo pathway, studies have shown other signaling pathways that have also been related to the oncogenic effect of vGPCR [85]. For instance, PI3Kγ is activated by vGPCR and has a key role in vGPCR-induced sarcomas, whereas NFkB has an indirect role in vGPCR-induced paracrine neoplasia. It seems the activation of YAP/TAZ is associated with other vGPCR downstream signaling events, including PI3K and NFkB, which generally participates in KS tumorigenesis in KSHV-infected patients. It is noticeable that YAP/TAZ also upregulates the expression of several growth factors, including CTGF and insulin-like growth factor (IGF) [86,87], that can be associated with neoplasia caused by vGPCR in a paracrine condition [81, 88].

Some studies revealed that Hippo pathway activation has a key role in the promotion of the lytic cycle and transition from latency. Inactivation of the Hippo pathway modulator YAP1 may promote reactivation from latency, lytic protein increase, and production of infectious virions. Interestingly, expression of the YAP1-inactivating kinase LATS1 consequently induces reactivation from latency. Conversely, LATS1 silencing decreases viral protein expression along the late phase of lytic replication. However the detailed mechanism of switching of KSHV latent/lytic by the Hippo signaling pathway is still unclear, increased amounts of nuclear centers containing the viral latency-associated nuclear antigen (LANA) have been reported in YAP1-silenced cells [89] (Figure 4).

Figure 4: Schematic presentation of reciprocal interactions between KSHV and Hippo signaling pathway

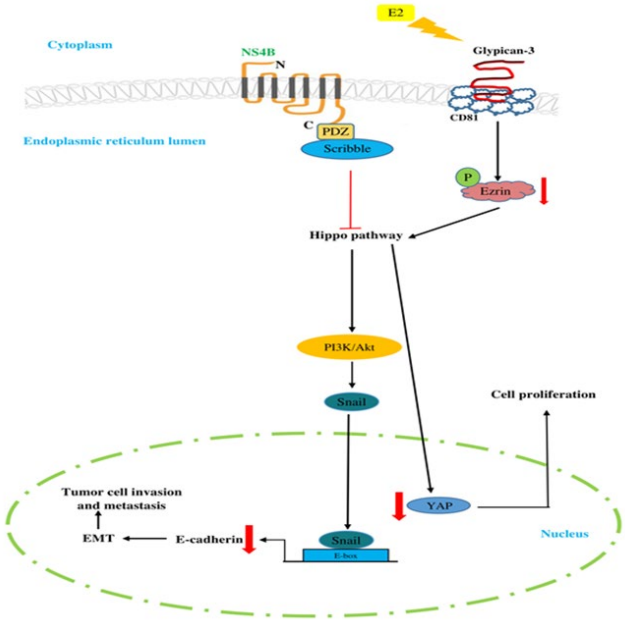


HEPATITIS C VIRUS

Hepatitis C virus (HCV), a member of the Flaviviridae family, is an enveloped RNA virus with a positive sense single-strand RNA genome [90]. Chronic HCV infection is one of the main risk factors for disease progression to metabolic disorders, fibrosis, cirrhosis, and HCC [91]. Epithelial-to-mesenchymal transition (EMT) is a physiological process by which epithelial cells lose their cell polarity and cell-to-cell junctions, and achieve migratory and invasive properties to become mesenchymal cells [92]. Previous literature reviews have shown that EMT plays a crucial role in promoting metastasis in various human cancers including HCC [93]. During the process of EMT, loss of epithelial markers, especially E-cadherin is mediated by transcriptional repressor SNAIL that binds to the E-box motif on the promoter region of E-cadherin [94]. HCV non-structural 4B (NS4B) protein is a 27kDa hydrophobic protein that role in HCC pathogenesis mediate interaction networks with cellular host proteins [95]. A study recently showed that interaction NS4B with scribble protein, a scaffold protein involved in regulating cell polarity and cell proliferation in epithelial cells, suppresses the Hippo signal pathway that subsequently results in the induction of the PI3K/AKT pathway. Induction of the PI3K-Akt pathway upregulates Snail expression via translocation to the nucleus. Finally, the up-regulation of Snail induces EMT progression by reducing E-cadherin expression which contributes to HCV-caused HCC progression, invasion, and metastasis [96].

HCVs entry to hepatocytes is recognized to be mediated by CD81 [97]. Ezrin is a member of a membrane-cytoskeleton linking protein that increases in HCC and is identified in correlation with malignant HCC [98]. In normal hepatocytes, CD81 overexpression results in the phosphorylation of ezrin and increasing nuclear YAP. As the CD81-SYK-ezrin complex causes signals to modify cell construction through protein interactions, gene expression is mediated by YAP via downstream regulation of the Hippo signaling pathway [99]. Also deregulation of the Hippo signaling pathway and increased YAP activity have been demonstrated in 50% of HCC cases [100]. In contrast since Glypican (GPC)-3 is one of the main ligands for CD81, it upregulates the expression of CD81 in HCC cells enhances activation of Hippo, a reduction in ezrin phosphorylation, and a reduction in YAP. Interestingly HCV E2 protein mimics the function of GPC3, enhances the activation of the Hippo signaling pathway, and subsequently reduces YAP and cell proliferation. Consequently, although any hepatocytes which have missing CD81 expression are considered to be resistant to HCV infection but can expand faster than the normal hepatocytes, loss of CD81 is a usual event in the promotion of hepatic neoplasia [101] (Figure 5).

Figure 5: Schematic presentation of reciprocal interactions between HCV and Hippo signaling pathway



CONCLUSION

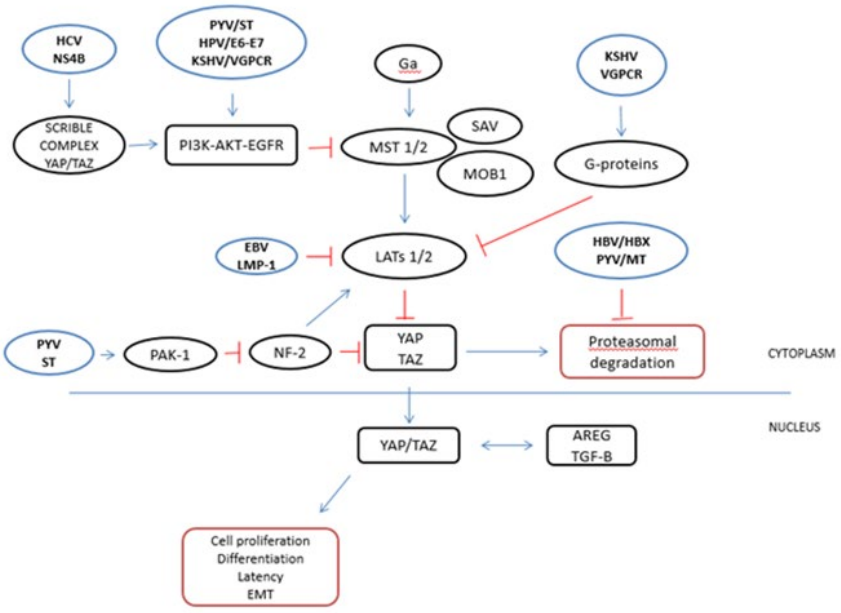
The Hippo signaling is an emerged evolutionarily conserved tumor suppressor pathway that regulates proliferation, apoptosis, differentiation, and development, whose dysregulation overexpresses YAP and TAZ as two major oncogenic downstream effectors of Hippo pathways that play a crucial role in multiple human pathological diseases, such as cancer (Table 1).

Table 1: The summary of the deregulation of the hippo signaling pathway by oncoviruses

Virus	Viral protein	Component of hippo signaling	Mechanism/Effect	References
HBV	HBx	YAP	HBx can increases YAP via the CRE-dependent HBx binding to the YAP promoter and following stimulation of YAP target genes	[27]
HPV	-	YAP1	Hyperactivation of YAP1 can be an important mediator in the persistence of HPV infection	[37]
	-	YAP1	Hyperactivation YAP1 increases the expression of HPV receptor molecules such as ITGRA6, SDC1, and EGFR	[37]
	-	YAP1	Hyperactivation of YAP1 in cervical epithelial cells may reduce viral patterns recognition TLR2 and TLR4 and adaptor proteins MYD88 and TRIF of the innate immune system	[37]
	-	YAP1	Expression of YAP may suppress the activation of transcriptional factors that are essential for the production of type I IFNs, including IRF1, IRF3, and IRF	[37]
	E6/E7	YAP	Expression and activation of main components of the type I IFN pathway, like IFNAR2, STAT1, JAK1, and IRF9, suppressed by expression of $YAPS^{127A}$	[37]
		YAP	YAP was increased in the E6 and E6/E7-induced cervical tumor cells and YAP was localized in the nucleus of these cells	[36]
PyVs		ST YAP	Polyomavirus small T antigen (ST) associates with YAP, an effector of the Hippo pathway, to modulate differentiation	[58]
		MT YAP	MT Binds to YAP, it associates YAP with protein phosphatase 2A (PP2A), resulting in the YAP dephosphorylation together with the MT complex	[60]
		ST YAP	ST acts through PAK1 to inactivate the NF2 tumor suppressor to alleviate the inactivation of YAP	[68]
EBV		LMP1 TAZ	LMP1 can upregulate TAZ expression by inhibition of Hippo pathway proteins	[74]
		LMP1 TAZ	LMP1 can develop cell proliferation, EMT, and CSC-like characteristics by TAZ	[74]
		LMP1 LATS1/2	LMP1 could suppress LATS1/2 activation, resulting in the enhancement of TAZ stability	[74]
KSHV		vGPCR Lats1/2	The KSHV protein (vGPCR) activity is via Gq/11 and G12/13 to suppress the Hippo pathway kinases Lats1/2, enhancing the activation of YAP/TAZ	[85]
		vGPCR YAP/TAZ	Activation of YAP/TAZ is associated with other vGPCR downstream signaling events, including PI3K and NF-kB	[86,87]
		- YAP1	Inactivation of the Hippo pathway modulator YAP1 may promote reactivation from latency, lytic protein increasing, and production of infectious virions	[89]
		LATS1	Expression of the YAP1-inactivating kinase LATS1 consequently induces reactivation from latency	[89]
HCV	*NS5B*	-	*Interaction NS4B with scribble protein suppresses the Hippo signal pathway that subsequently resulting in the induction of PI3K/AKT pathway*	[96]
	E2	YAP	HCV E2 protein mimics the function of GPC3, enhances the activation of the Hippo signaling pathway, and subsequently reduces YAP	[101]

The activation of YAP and TAZ contributes to cancer initiation, metastasis, and anticancer drug resistance via interactions with several signaling pathways in many human tumors. Oncoviruses either directly or indirectly by modulating a variety of cellular signaling pathways lead to the dysregulation of the Hippo signaling pathway to increase the expression of the downstream effectors TAZ and YAP which can be implicated in transformation, metastasis, and chemotherapeutic drug resistance in virus-related cancer and also to facilitate some processes involved in the pathogenesis of viral infection such as entry, persistence, escape from the immune system, and latency. However, in some cases inactivation of YAP can aid the pathogenesis of KSHV and HCV. (Figure 6).

Figure 6: Schematic presentation of reciprocal interactions between oncoviruses and Hippo signaling pathway



With these interpretations, we recommend that the overexpression of the major downstream effectors of the Hippo pathway, TAZ, and YAP can be used as prognosis markers in cancers caused by oncoviruses. Overall, Hippo signaling is a new pathway in viral infection, and in this way, better understanding interaction between oncoviruses with Hippo signaling can be used as a new therapeutic target in virus-caused cancers.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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Original Article

Relationship between Mental Health, Life Satisfaction, and Job Satisfaction of Nurses

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Abstract: *Improving the mental health of nurses and investigating the factors affecting it leads to the improvement of job performance and the quality of nursing care. Aim: The present study aimed to determine the relationship between mental health, life satisfaction, and job satisfaction of nurses in Ilam city in 2022. The present correlational study was carried out on 160 nurses working in educational hospitals of Ilam city and was selected by stratified random sampling in 2022. A four-part questionnaire was used: the first part included a question on demographic information. Minnesota Satisfaction Questionnaire (MSQ) (1977), Diener et al.'s Satisfaction with Life Scale (SWLS) (1985), and Derogatis et al.'s Symptom Checklist-90 (SCL-9) (1973) were included in the second, third and the fourth parts, respectively. Results showed no significant relationship between the total score of mental health and the total score of job satisfaction. There was a significant relationship between the total job satisfaction score and life satisfaction score (r=0.267, p=0.001). There was also an inverse and significant relationship between the total mental health score and the life satisfaction score (r=0.294, p=0.001). The collected data was analyzed using descriptive tests (frequency, mean percentage, and standard deviation) and non-parametric Spearman and Kruskal-Walli's correlation tests in SPSS ver. 22. P-value<0.05 was considered as the significance level in all tests. Job satisfaction is effective in creating motivation, efficiency, responsibility, and social accountability in the personnel of any organization. Job dissatisfaction can lead to complications such as job burnout, illness, absenteeism, the job left, leaving many nursing jobs, and the reluctance of the young workforce to attend the wards. Therefore, it is necessary to make further efforts in this field by healthcare policymakers.*

Keywords: *mental health, life satisfaction, job satisfaction*

INTRODUCTION

Mental health has an expanded definition. But every person who can deal with his/her deep issues, get along with him/herself and others, and can face his/her inevitable internal conflicts, and does not isolate him/herself from society, can be stated to have mental health [1-4]. Nurses are directly related to all social strata and touch people's problems closely. This close relationship doubles their serious responsibility toward people's health [5]. Nursing is one of the most stressful professions so the National Institute for Occupational Safety & Health has introduced it among the top 40 stressful professions [6].

There are many sources of occupational stress in the nursing work environment. Overwork, urgency, patient death, independent practice, lack of social support, poor job fit, lack of necessary specialized knowledge, unsafe workplace and rapidly changing healthcare environment are known as nursing stressors [7]. Nurses are prone to health threats in different dimensions due to long work shifts and the resulting fatigue. Nurses with no good general health are not able to provide good care such as physical and psychological support to patients. This increases the risk of mistakes and occupational accidents, and subsequent consequences affect the patient and the nurse [8].

Nurses' stress and fatigue can affect the vital and critical aspects of their performance, including problem-solving ability, decision-making ability, and creativity, and ultimately lead to disruption in medical care. Because patient safety, health and recovery depend on the safe and optimal performance of nurses [9]. Many researchers have confirmed that job-related stress can have harmful effects on the physical and mental health of individuals [10,11]. Job stress is one of

the factors affecting the health of employees. Employees' health is at risk when they face various stressors. This issue is more evident in the nursing profession [12]. The impact of job dissatisfaction on the mental health of employees has been a public concern in various societies, especially in recent years. Perceived impacts of job dissatisfaction on people's mental health from an economic point of view is also an important issue and play an important role in the quality of professional and personal life of nurses [13].

Another variable that plays a key role in improving nurses' mental health is life satisfaction. Life satisfaction is one of the mental health-related factors and refers to a person's general attitude and evaluation toward life as a whole [14]. It also reflects the balance between a person's aspirations and his/her current situation. The greater this balance, the greater the life satisfaction will be [7]. According to previous studies, this concept is in full interaction with health and there is a close relationship between physical and mental health with life satisfaction [15]. Lyubomirsky believes that people who have higher life satisfaction, use more effective and appropriate coping methods, experience deeper positive emotions and feelings and have higher general health [16]. Nurses are among the vulnerable employees of society, whose health and balance in their personal and family life is related to occupational variables. Work-family conflict causes psychological symptoms such as higher stress, depression, chronic physical problems, increased physical complaints, lower life satisfaction, low quality of family life, and low energy levels [17]. Suzuki et al. stated that nurses were in 27th place in the evaluation of 130 stressful jobs sorted according to their reference to doctors for health problems [18].

Improving the mental health of nurses and investigating the factors affecting it leads to the improvement of job performance and the quality of nursing care. Since nursing is one of the sensitive professions and nurses spend an important part of their lives with people and patients, their mental health leads to optimal job performance and guarantees the health and improvement of many patients and people [19].

Having job satisfaction, life satisfaction and mental health leads to an improvement in the quality of patient care and increasing productivity and improving nursing services, therefore, conducting relevant studies and using their results in various aspects of the nursing profession can have useful results in care, education, management, therapy and also the personal life of a nurse [20]. Therefore, the present study aimed to determine the relationship between mental health, life satisfaction, and job satisfaction of nurses in Ilam city in 2022.

MATERIALS AND METHODS

The present correlational study was carried out on 160 nurses working in educational hospitals of Ilam city and was selected by stratified random sampling in 2022. Inclusion criteria included working in one of the above-mentioned educational hospitals and having informed consent to participate in the study. Failure to complete one of the questionnaires, an incomplete questionnaire, and unwillingness to cooperate were among the exclusion criteria. A four-part questionnaire was used: the first part included a question on demographic information. Minnesota Satisfaction Questionnaire (MSQ) (1977), Diener et al.'s Satisfaction with Life Scale (SWLS) (1985), and Derogatis et al.'s Symptom Checklist-90 (SCL-9) (1973) were included in the second, third and the fourth parts, respectively. Demographic information of nurses included age, gender, years of work experience, marital status, education level, hospital, ward, position, income level, employment status, number of children, and home type.

Minnesota Satisfaction Questionnaire (MSQ) consists of 19 items and 6 subscales of the payment system, type of job, achievement opportunities, organizational atmosphere, leadership style, and physical conditions. MSQ is scored based on a 5-point Likert scale, ranging from "Completely Disagree" (Score 1) to "Completely Agree" (Score 5). To calculate the overall score of the questionnaire, the scores of all the items are added together. Scores 19-38, 38-57, and 38-57 indicate poor, moderate, and very good job satisfaction, respectively. This questionnaire has been used in many types of research in the world [21-26]. Its internal consistency reliability was measured $\alpha = 0.87$.

Diener et al.'s Satisfaction with Life Scale (SWLS) is a 5-item instrument designed to measure a person's cognitive and overall judgment of life. This instrument is scored based on a 7-point Likert scale ranging from 1 (Completely disagree) to 7 (Completely agree). The overall score of this scale is calculated by summing the answers and the possible score range is 5 and 35. A higher score indicates higher levels of life satisfaction. This questionnaire has also been used in many types of research in the world [27-32]. The Cronbach's α reliability was 0.86.

Derogatis et al.'s Symptom Checklist-90 (SCL-9) can be completed within approximately 25 minutes. This questionnaire is the short form of SCL-90 and was extracted by Najarian & Davoudi based on the original form and through exploratory factor analysis. It consists of 25 items and 9 subscales of somatization (6 questions), obsession-compulsion (3 questions), interpersonal sensitivity (3 questions), depression (2 questions), anxiety (3 questions), phobia (3 questions), paranoid thoughts (1 question), psychosis (3

questions), hostility (1 question). SCL was answered according to its original form and based on a 5-point Likert swale ranging from None or Rarely (0), A little (1), To some extent (2), A lot (3), Very much or Strongly (4). The overall score of general psychological injuries was extracted. Higher scores indicate more damage [30]. The Cronbach's alpha reliability of the tool was calculated $\alpha = 0.94$.

The collected data was analyzed using descriptive tests (frequency, mean percentage, and standard deviation) and non-parametric Spearman and Kruskal-Walli's correlation tests in SPSS ver. 22. P-value<0.05 was considered as the significance level in all tests.

Ethical considerations: The present study was approved by the Ethics Committee of Ilam University of Medical Sciences with the code IR.MEDILAM.REC.1400.229. The contents of the Nuremberg and Helsinki declarations and the 26 research ethics codes were observed.

RESULTS

The results showed that most of the participants were female (61.2%), aged 26-29 (40%), single (52.5%), childless (75%), owning their own house (72.5%), and had 6-10 million-toman income (70%). Also, the majority of participants had a bachelor's degree (88.8%), less than 6 years of work experience (70%), were ward nurses (91.2%), were permanent nurses (45%), and worked in ICUs (45%).

Table 1 shows the correlation matrix of job satisfaction, life satisfaction, and mental health. This table determines the relationship between job satisfaction, life satisfaction, and mental health of research subjects. The table shows no significant relationship between the total score of mental health and the total score of job satisfaction. There was a significant relationship between the total job satisfaction score and life satisfaction score (r=0.267, p=0.001). There was also an inverse and significant relationship between the total mental health score and the life satisfaction score (r=0.294, p=0.001).

Kruskal-Walli's test shows the relationship between job satisfaction, life satisfaction, and mental health according to demographic characteristics (Table 2). This table shows that the highest frequency of the variables of work experience, education level, medical training center, hospital ward, income level, and employment status are related to less than 3 years (p=0.017, df=4, k=11.98), BA (p=0.014, df=1, k=6.07), Ayat-Allah-Taleghani hospital (P=0.004, df=2, k=11.03), ICUs (P=0.001, df=2, k=55.91), 6-10 million tomans(P=0.022, df=2, k=7.62), and contractual employment (P=0.001, df=2, k=14.37), which are all had a statistically significant relationship with job satisfaction. The highest frequency of gender, marital status, and employment status were related to females (P=0.037, df=1, k=4.34), married individuals (P=0.034, df=1, k=4.47), and contractual individuals (p=0.032, df=2, k=6.88), which all had a statistically significant relationship with life satisfaction. Also, the female gender (k=11.75, p=0.001, df=1) had a statistically significant relationship with mental health.

DISCUSSION

Mental health affects health-related variables related to [4,33-35]. The results of the present research showed no significant relationship between each of the mental health components and the total score of job satisfaction. There was also no significant relationship between the total scores of job satisfaction and mental health. However, there was an inverse and significant relationship between the two components of job satisfaction and some mental health components, that is between the payment system with physical complaints, interpersonal sensitivity, phobia, and the overall mental health score, and also between the type of job with obsessive-compulsive and paranoid thought.

Kahe et al. revealed a statistically significant relationship between overall job satisfaction with employees' mental health, and people with high job satisfaction obtained a better mental health score (below average) (p=0.02). In other words, the lower the level of mental health, the lower the job satisfaction and vice versa [36].

Table 1: Correlation matrix of job satisfaction, life satisfaction, and mental health (Spearman correlation)

Variable			***Correlation***	
Variable	**Life satisfaction**	**Mental health**	**Meaningful**	
Job Satisfaction	0/267	-0/063	r	*1*
	*0/001	0/428	P	-
Life satisfaction	1	-0/294	r	
	-	*0/001	P	
Mental health		1	r	
		-	P	

Table 2: Relationship between job satisfaction, life satisfaction, and mental health according to demographic characteristics (Kruskal Walli's)

Variable		Mental health				Life satisfaction				Job Satisfaction			
		P	df	K	Mean	P	df	K	Mean	P	df	K	Mean
Gender	Man	0/001	1	11/75	64/73	0/037	1	4/34	70/92	0/262	1	1/25	75/34
	Female				90/48				86/56				83/77
Age	Less than 25 years	0/420	3	2/82	79/84	0/209	3	4/53	76/97	0/181	3	4/87	85/39
	26-29 years				86/58				87/72				86/63
	30-34 years				79/29				66/21				75/57
	More than 34 years				69/50				82/90				65/83
Marital status	Single	0/64	1	0/213	82/11	0/034	1	4/47	73/14	0/071	1	3/26	86/79
	Married				78/72				88/63				73/55
Number of children	0	0/357	3	3/23	82/43	0/647	3	1/65	78/47	0/202	3	4/61	85
	1				62/61				93/5				66/72
	2				82/13				81				65/5
	3				91/17				80/83				71/83
Type of house	Rental	0/227	1	1/46	87/68	0/927	1	0/008	79/95	0/836	1	0/043	81/73
	Personal				77/78				80/71				80/03
Work experience	Less than 3 years	0/105	4	7/64	89/33	0/985	4	0/37	81/09	0/017	4	11/98	94/17
	6-3 years				83/14				79/43				82/29
	9-6 (9-12) years				60/93				86/36				65/5
	< 3 years, > 12 years				50/83				74/5				53/5
	6-3 years				74/14				79/93				63/71
Level of Education	Masters	0/198	1	1/65	82/18	0/423	1	0/64	81/54	0/014	1	6/07	83/71
	MSc				67/28				72/28				55/17
Section	Public section	0/265	3	13/45	83/08	0/254	3	13/63	68/24	0/001	2	55/91	64/24
	Special section				76/95				89/79				91/39
	Emergency room				85/4				79/3				66/75

Veysey et al. showed an inverse relationship between the general health questionnaire and job satisfaction and a direct relationship with the increase in cases of stress [37]. In her study in teaching hospitals of Hormozgan, Mastaneh et al. (2013) also showed a significant negative correlation between mental health pay satisfaction ($p<0.005$) and type of work ($P<0.01$) [38].

Sarafis et al. also showed a significant relationship between the stress management domain of health-promoting lifestyle and the payment system domain of job satisfaction ($p< 0.005$) [39].

Ghasemipirbalooti et al. found a statistically significant relationship between mental health and job satisfaction of the employees of Shahrekord University of Medical Sciences ($p=0.001$) [40].

Mei et al. (2015) investigated the effect of psychological characteristics of employees on their performance and job satisfaction and the indirect effect of feelings on their relationships. They concluded a positive and direct relationship between job satisfaction with psychological characteristics and positive feelings of employees [41].

Many studies have shown a direct relationship between mental health and job satisfaction [42-47].

The results of this study show an inverse and significant relationship between life satisfaction and each of the components of mental health except for the "paranoid thought" component. The results also show that the level of mental health of nurses increases and mental injuries decrease with increasing life satisfaction scores. Salimi also showed in research titled "Investigating mental health and its relationship with job burnout and life satisfaction of employees" that there is a significant relationship between mental health and life satisfaction.

The consistency between the results of the present research with Salimi's study is attributed to the correlation between mental health and life satisfaction. That is, life satisfaction prerequisite for mental health, and if there is no satisfaction, negative mood and despair dominate the person and make him/her susceptible to all kinds of mental illnesses [48].

In a study on nurses in Ahvaz government hospitals, it was found that life satisfaction is correlated with high mental health (Birgani et al.). The higher the level of life satisfaction,

the more likely a person is to experience positive emotions and feelings [49]. Geravand also showed in a study that mental health has a direct effect on life satisfaction.

Changes in mental health and emotions can lead to changes in life satisfaction and a person's attitude [50]. The results of various research show a significant relationship between mental health and life satisfaction. The results of Ferguson's study indicated the interaction between mental health and life satisfaction [51].

In a study of the level of job satisfaction of nurses in hospitals affiliated with Kashan University of Medical Sciences and related factors, Tagharobi et al. showed a statistically significant relationship between the level of education, major stress in the last six months, the workplace, the level of life satisfaction, mental health satisfaction health, pay satisfaction, satisfaction with the number of employees in each shift, satisfaction with the feedback of patients and their families, satisfaction with the treatment and performance of doctors, satisfaction with the treatment and performance of head nurses and colleagues, satisfaction with the treatment and performance of nursing office managers, satisfaction with workplace amenities and happiness with job satisfaction [52].

As was pointed out by Grant, nurses are among the most vulnerable working group in society, and their health and personal-family life balance are strongly related to occupational variables. Work and family life conflict and subsequent stress cause psychological symptoms such as higher stress, depression, chronic physical problems, increased physical complaints, lower life satisfaction, low quality of family life, and low energy levels in these people [17].

CONCLUSION

According to the results of the present study, nurses' life satisfaction score increases with the increase in their job satisfaction score. But there is no significant relationship between the total mental health score and the total job satisfaction score. However, there is an inverse and significant relationship between mental health and life satisfaction. On the other hand, the level of mental health of nurses increases, and mental injuries decrease with the increase in life satisfaction scores. Considering the vital role of nurses in achieving the goals of the health system and public health, neglecting the job dissatisfaction of nurses may have consequences such as reducing productivity, reducing the level of responsibility, reducing the level of quality of their performance, reducing life satisfaction and increasing the number of psychological injuries. Job satisfaction is effective in creating motivation, efficiency, responsibility, and social accountability in the personnel of any organization. Job dissatisfaction can lead to complications such as job burnout, illness, absenteeism, the job left, leaving many nursing jobs, and the reluctance of the young workforce to attend the wards. Therefore, it is necessary to make further efforts in this field by healthcare policymakers.

Acknowledgments
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Ethics approval and consent to participate
IR.MEDILAM.REC.1400.229
Informed written consent was obtained from all participants included in the study. All participants were informed about the purpose of the study and assured of confidentiality and anonymity. The respondents were informed of their freedom to withdraw from the study at any time.

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Original Article

Survey the Effect of Educational Intervention Based on Mobile Short Message Service (SMS) on Self-Care in Patients with Type 2 Diabetes Referring to the Diabetes Clinic in Khorram Abad City

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Abstract: *Introduction: Self-care behaviors are very important to control type 2 diabetes. The current study was conducted aiming at determining the effect of the educational intervention based on mobile short message service on the self-care behaviors in patients with type 2 diabetes in Khorram Abad. Methods: This study was a semi-experimental study on 191 patients with type 2 diabetes (45case and 45 controls). Data collection tools included a demographic information form, International Physical Activity Questionnaire, Self-Care Scale, and Self-Efficacy Scale. Three to four educational messages were sent daily through mobile phones as an educational intervention. Data were analyzed with SPSS V24 at a significance level of 0.05. Findings: The data analysis indicated that the average score of self-care, self-efficacy, and blood sugar was significantly higher in the case group than in the control group before and after the intervention (P<0.001). However, in terms of the physical activity variable, this relationship was not statistically significant in the case and control groups. Conclusion: Educational interventions to empower diabetic patients by strengthening their self-care can be an effective way to improve the health of diabetic patients. Therefore, it is suggested to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.*

Keywords: *self-care, self-efficacy, physical activity, blood sugar, type 2 diabetes*

INTRODUCTION

Diabetes is the most common disease caused by metabolic disorders, which is mainly characterized by a chronic increase in blood sugar and disturbed carbohydrate, fat, and protein metabolism. In general, diabetes is a multifactorial disorder diagnosed by a chronic increase in blood sugar or hyperglycemia (according to diagnostic criteria) and is caused by a disorder in insulin secretion or function or both [1]. Retinal damage and blindness, peripheral neuropathy, leg pain, stroke, peripheral vascular problems, end-stage renal disease, and limb amputation are among the most serious complications of diabetes [2]. The most common type of diabetes is type 2 diabetes, which accounts for approximately 90-95% of diabetes cases and is usually associated with metabolic disorders, weight gain, and physical inactivity [3]. About 422 million people worldwide suffer from diabetes, the majority of whom live in low- and middle-income countries, and in 2019, diabetes was introduced as the direct cause of 1.5 million deaths [4]. According to the International Diabetes Federation (IDF), the number of diabetic patients will reach 439 million people in 2030, and the prevalence of diabetes in developing countries will be 69% [5]. More than 3 million people with diabetes live in Iran [6]. By 2030, Iran will be one of the most prevalent regions in the world in terms of diabetes [7]. Paying attention to the chronic and costly nature of this disease for the general health of society and the creation of a large financial burden, it is essential to pay serious attention to this problem and its consequences [8]. According to the results obtained from the studies, the long-term complications of this disease, including neuropathy, nephropathy, and retinopathy can be prevented, or at least the occurrence of these complications can be postponed if blood sugar is controlled [9,

10]. Many studies have found physical activity and exercise to be effective in reducing blood sugar levels and controlling diabetes [11,12]. The World Health Organization considers education to be the basis of diabetes treatment. Therefore, self-care is one of the important topics of diabetes treatment. Self-care is a learned regulation function in humans, which is based on the ability of people to perform care actions on themselves [13]. It includes the behaviors of following a proper diet, taking medicine on time, self-monitoring of blood sugar, performing regular physical activity, and physical care of the feet [14]. Studies show that 67% of patients with type 2 diabetes do not monitor their blood sugar according to the recommendations. Also, only 19-30% of diabetic patients use the recommended physical activities and compliance with nutritional behaviors in these patients is 65%, and only 7% of diabetic patients fully adhere to all aspects of self-care behaviors [15, 16]. Self-efficacy is also known as a strategy to improve self-care in diabetic patients, which results in self-confidence and adherence to care recommendations to control blood sugar in these patients [17].

Nowadays, to follow up and educate diabetic people, new communication systems are required to direct the focus of care and treatment from the clinic to the patient's daily life. Researchers have pointed out the use of mobile phone service as a method of education and follow-up in chronic diseases, that short message is one of the main functions of mobile phone in the education of diabetic patients [18,19].

Considering the importance of self-care in controlling the blood sugar of diabetic patients and the value of self-efficacy as one of the self-care approaches, it seems necessary to design educational interventions on the platform of mobile phones and in the form of short messages as a relatively new educational method in controlling the disease of diabetics. Thus, the present study was conducted to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad.

MATERIALS AND METHODS

The present study was a semi-experimental intervention research with a pre-test and post-test design. The sample size was specified as 90 people who were selected from the research population of patients with type 2 diabetes referring to the diabetes clinic in Khorramabad, Lorestan province. The participants were selected according to the inclusion criteria using random sampling. Then, they were divided into case and control groups using a simple randomization method (45 ones in each group). The inclusion criteria include informed consent to participate in the research, having a minimum level of literacy and understanding of the content sent through text messages, having a fixed personal mobile number to receive educational SMS, lack of any serious diabetic complication, and not having special diets because of specific diseases or physical problems limiting physical activity. Exclusion criteria include suffering from serious complications of diabetes during the study, suffering from diseases that limit physical activity or nutrition, and the patient's unwillingness to receive educational messages.

Data collection tools in this study included a demographic information form, International Physical Activity Questionnaire (IPAQ), a Self-Care Scale developed by Tubert, and Self-Efficacy Scale. Demographic information included: age, height, weight, gender, educational level, employment status, marital status, and type of treatment. The IPAQ with seven items measured the duration of physical activity in the last week in four categories: moderate physical activity, intense physical activity, walking, and sitting activities. According to the scoring protocol in this questionnaire, the intensity of physical activity based on the ratio of energy consumption in terms of metabolic equivalent of task (MET) in the last seven days is classified into three levels: low, medium, and high. It was proposed by the World Health Organization and the Centers for Disease Control and Prevention in 1998 to measure the physical activity of people aged 15 to 69 years and its validity and reliability have been confirmed in 12 countries worldwide, including Iran [20,21]. A self-care questionnaire was used in this study that included 15 items in the areas of diet compliance, physical activity, blood sugar monitoring, insulin injection or diabetes pill consumption, foot care, and smoking, which was translated and standardized by Hamdzadeh [22,23]. The self-efficacy questionnaire, which is one of the constructs of the standard Health Belief Model Questionnaire, validity and reliability of which have been confirmed [24].

After presenting a letter of introduction from the Iran University of Medical Sciences and obtaining the necessary permits, the researcher referred to a diabetes clinic in Khorram Abad and selected 90 people from among 200 eligible people using random sampling. People who were willing to participate in the study and met the inclusion criteria were assigned to the case and control groups (45 in each group) using a simple randomization method. After random allocation, blood sugar samples were taken in all groups four days before the start of the intervention. In the first session, the researcher explained the purpose of the meeting to the participants, and written informed consent was obtained. Then, the personal information form and questionnaires were completed by the case and control groups. The case group received an educational program in the form of short educational messages via mobile phone, for four weeks and on average, at

least three to four messages per day related to the educational content, including exercise, self-monitoring of blood sugar, diet, insulin therapy, medication use, and foot care. For example, short messages about physical activity and blood sugar monitoring were "for people with diabetes, walking is the best exercise" or "it is better for people with diabetes to measure their fasting blood sugar after waking up". To improve self-efficacy, encouraging messages about adherence to self-care were used.

Finally, after two months, the same questionnaires (demographic, physical activity, self-care, and self-efficacy scales) were distributed and completed by the participants, and the blood sugar levels of the two groups were measured again. During this period, the control group did not receive any educational intervention.

However, to comply with the ethical principles, an educational CD containing self-care training was given to the control group after completing the research. The data of the two groups before and after the intervention were entered into SPSS version 24 software and analyzed using Wilcoxon, Mann-Whitney, Chi-square test, independent t-test, and paired t-test at a significant level of 0.05.

RESULTS

The participants' average age was 51.01 ± 0.967, and the average height and weight were 164.57 ± 1.031 and 75.69 ± 1.101, respectively.

Before the implementation of the educational program, the case and control groups were matched in terms of demographic variables, including age, height, weight, gender, education, employment status, marital status, and type of treatment, and no statistically significant difference was observed between the two groups (Table 1).

Table 1: Comparison of the absolute and relative frequency distribution of demographic variables in case and control groups

Variable		Case		Control		P value
		No.	%	No.	%	
Gender	Female	31	69	26	57/7	0.796
	Male	14	31	19	42/2	
Employment	Unemployed	5	11	2	4	0.686
	Housewife	26	58	24	53	
	Self-employed	9	20	9	20	
	Public	5	11	10	22	
Education	Illiterate	5	11	4	9	0.479
	Primary school	17	38	10	22	
	Secondary school	9	20	5	11	
	High school	4	9	11	24	
	High school diploma	4	9	10	22	
	Higher education	6	9	5	11	
Marital status	Single	2	4	1	2	0.800
	Divorced	1	2	0	0	
	Widow	9	20	5	11	
	Married	33	73	39	87	
Type of treatment	Insulin	21	47	21	47	0.920
	Oral medication	20	45	22	49	
	Diet	4	9	2	4	
	Exercise	0	0	0	0	

As observed in Table 2, there is no statistically significant difference in terms of the average score of physical activity, self-care, self-efficacy, and blood sugar between the case and control groups before the intervention, while after the intervention, the average scores of these variables, except for physical activity (P=0.8), was significantly higher in the case group compared to the control group (P<0.001). In the control group, before and after the intervention, no significant difference was observed in the average scores of physical activity, self-care, self-efficacy, and blood sugar (P>0.05).

Table 2: Comparison of the average scores of physical activity, self-care, self-efficacy, and blood sugar, before and after the intervention in case and control groups

Scale	Group	Case	Control	p-value
		Mean ± SD	Mean ± SD	
Physical activity	Before intervention	677.5±954.938	307.5±461.75	0.6
	After intervention	903.75±973.125	655±925.625	0.8
	p-value	0.8	0.4	_
Self-care	Before intervention	2.31±0.813	2.38±0.844	0.12
	After intervention	3.38±0.688	2±0.906	0<0001
	p-value	0<001	0.22	_
Self-efficacy	Before intervention	2.7±0.4	2.7±0.3	0.15
	After intervention	3.7±0.7	2.7±0.7	0<0001
	p-value	0<001	0.286	_
Blood sugar	Before intervention	180±80.5	185±51	0.8
	After intervention	170±87.5	180±70.5	0<001
	p-value	0.009	0.491	_

Table 3: Comparison of blood sugar scores before and after the educational intervention in the case group

Method of treatment	Blood sugar level before the intervention	Blood sugar level after the intervention	Level of change
Insulin	227.09	217.92	-9.17
Oral medicine	173.48	170.65	-2.83
Diet	181	180.44	-0.56
Exercise	194	194.5	0.5
Medium	193.89	190.88	-3.02

Table 3 indicates that the blood sugar level in all patients with different treatment methods (insulin, oral medication, diet) in the case group reduced after the educational intervention compared to before intervention, except for the patients who only used exercise to control their blood sugar.

DISCUSSION

The present study was conducted aiming to determine the effect of the educational intervention based on mobile phone short messages on self-care in patients with type 2 diabetes referred to the diabetes clinic in Khorramabad. The research findings suggest that the educational intervention through mobile phone short messages improved self-care in the case group, which is consistent with findings by Abaza et al. [25] and Agbede et al. [26] on the effect of the intervention based on mobile phone short messages in the self-care of diabetic patients and the findings by Yari et al. [27] and Rahnavard et al. [28] concerning SMS-based and self-care education of diabetic patients. Krishna et al. [29] reported that technologies such as mobile phones and text messages, which are currently part of people's daily lives, have the potential to influence self-care and improve people's health. The approach of health education through SMS to promote self-care in diabetic patients is more welcomed than traditional approaches that require reading pamphlets or attending lectures [25,30].

In this study, self-efficacy also there was a significant difference in the case group in terms of self-efficacy after the educational intervention. Katz and Nordwall [31] and Peymani et al. [32] also found that interventions based on mobile phone messages improve self-efficacy and self-care. Similar studies [33,34] regarding the self-care of diabetic patients also indicate a significant difference in the average score of self-efficacy and self-care in the case group compared to the control group after the educational intervention. These studies are consistent with our work in that increasing self-efficacy causes the adoption of self-care behaviors in diabetic patients. Self-efficacy is an important prerequisite for behavior change [35]. An individual with low self-efficacy is less likely to make an effort to perform a new health behavior or to change the behavior he is used to [36]. Researchers believe that self-efficacy has a suitable framework for understanding and predicting and patient's commitment to self-care behavior in the treatment of diabetes [37].

The results of the present study showed that education through mobile phone short messages does not affect increasing physical activity in the case group, which is in line with the findings by Bakhshian et al[38] regarding the effect of education through mobile phone short messages on self-care of patients with type 2 diabetes. It is also in agreement with the findings by Kim et al. [39] on adherence to diabetes control recommendations, while our findings are not consistent with the study of Paul et al. [40] regarding the use of software that can be installed on mobile phones to improve the amount of daily walking in patients with a history of stroke and the study Martha et al.[41] concerning mobile phone SMS intervention on diabetes self-care activities, cardiovascular disease risk awareness, and food choices among type 2 diabetic patients. This discrepancy can be due to the lack of sports facilities and parks in the current study or the addition of chronic diseases to the patients participating in this research, as well as social and cultural factors that prevent people from doing physical activities in public environments. In the present study, since the number of women is more than men, the problem of social and cultural factors could be more marked.

The use of mobile phone messages in the current study showed a significant positive effect on the reduction of blood sugar in the case group after the educational intervention, which is consistent with the findings of similar studies in this field [42,43,44]. In patients suffering from diabetes, education is as valuable as medicine, exercise, and diet, because the treatment will never be effective unless the patient is well aware of the nature of his disease and takes positive steps to deal with it.

CONCLUSION

The results of this study demonstrated that educational interventions based on mobile phone short messages can be effective in enhancing self-care in patients with diabetes. Educational interventions aimed at empowering diabetic patients by strengthening self-care in them can be an effective way to improve the health of patients with diabetes. Therefore, it is recommended to use patient empowerment programs, especially with a self-care approach, to improve the health of patients.

Conflicts of interest and sources of funding

The personal interests of the authors were not related to the results of this research.

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Authors' contribution

All authors were responsible for the study. EA, MM, and TD were accountable for the study Conceptualization and led the writing of the paper. BI conducted the Literature review and assisted in writing the paper. MM and OS performed the Statistical analysis, assisted in interpreting the data, and wrote the paper. OS, FSI, and EA assisted with the interpretation of the results and drafting of programmatic Implications. FB and MM were responsible for data collection and coordination of the study. TD co-led the conceptualization, supervised all aspects of writing the paper, and provided extensive comments on the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Vice President of Research and Technology of Iran University of Medical Sciences with code IR.IUMS.REC.1397.501. Informed consent was obtained from all individual participants included in the study before conducting.

Consent to publish

All participants consented verbally to the publication of the interview data.

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Original Article

A Comparative Study of Patients' Trust in Physicians and Nurses in COVID-19 and Non-COVID-19 Wards in a Reference Hospital in Tehran, Iran

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Abstract: *Trust in health care professionals is considered as a basis for patients' care. This study aimed to compare the level of patients' trust in physicians and nurses in both general and COVID-19 wards in a reference hospital in Tehran during the pandemic. This was a cross-sectional descriptive study. The study population comprised patients from Baqiyatallah Hospital in 2022. The data collection tool was the scale of patients' trust in nurses and the Wake-Forest physician trust scale. Data analysis was performed using SPSS-26 software. The level of patients' trust in physicians in COVID-19 wards was significantly lower than in Non-COVID-19 wards (P<0.001), but there was no difference between the two groups in the trust of nurses. The lowest level of trust was in the receiving of required information from nurses, which had a lower score compared to other questions in the questionnaire, and the average amount of receiving required information for patients in general wards was significantly lower than that of patients in COVID-19 (P=0.006). Trust in nurses and physicians in both COVID-19 and general wards was optimum. During the COVID-19 pandemic, the medical staff worked tirelessly and despite the difficulties and complications of the pandemic, patients have high trust in physicians and nurses.*

Keywords: *trust; nurses, physicians; COVID-19; coronavirus*

INTRODUCTION

Physicians and nurses, as the main members of the medical staff, take on different roles, considering the physical, psychological, and social needs of patients. They may act as educators, counselors, mentors, informers, advocates, and act as experts. To play such roles, physicians and nurses need to establish a good relationship with their patients [1,2]. Trust forms the basis of the relationship between the physician or nurse [3] and patient and provides the conditions for the physician or nurse and patient to work together for a better care plan [4]. The relationship between trust and health outcomes is different in individual studies [5]. For example, in a sample of patients with diabetes, trust in a healthcare professional was positively associated with objective and mental health outcomes (glycemic control, health-related quality of life, and patient satisfaction] [6].

The hypothetical relationship between trust and health outcomes is also reinforced by the ethical claims derived from trustworthy and patient-centered relationships in clinical settings [7]. Studies show that patients who have more trust in their therapist are more satisfied with the care and are more likely to follow treatment recommendations [8,9]. Patients' experiences of trust in nursing depend on nurses' knowledge, and the commitment to conversation to build and develop relationships [10]. Therapeutic staff must receive appropriate training to build a bond of trust between themselves and the patient to establish a trustable relationship between themselves and the patient [11]. Trust between patient and therapist is essential to reduce patients' anxiety and build the capacity to control them [12]. The COVID-19 epidemic has created special conditions in the relationship between the medical staff and patients [13]; furthermore, the need for social distancing and the use of protective equipment on the

one hand, as well as an increasing number of patients, especially those with severe illnesses, epidemics, illnesses and dementia and lack of time to provide medical care, on the other hand, has created a different kind of doctor-nurse relationship with the patient that may affect patients' trust [14]. Considering the importance of patients 'trust in physicians and nurses in adhering to treatment and the effectiveness of medical care, this study was designed to compare the level of patients' trust in physicians and nurses in general and COVID-19 wards in a reference hospital in Tehran during the pandemic.

MATERIALS AND METHODS

This study was descriptive and cross-sectional. The study population consisted of patients admitted to both the general and COVID-19 wards of Baqiyatallah Hospital, Tehran, Iran in 2022. Inclusion criteria included the age range of age: 20-70, hospitalization for more than three days in hospital wards, having no serious psychological disorders, and submitting consent to participate in the study. The data collection tools included the patient's trust scale in the nurse and the Wake-Forest physician trust scale. The Nurse Trust Scale has been validated by Nouripour et al [15]. The Physician Trust Questionnaire has been validated by Forati et al [16].

The scale of patient trust in the nurse contains five questions about Responding to the need, Usefulness of measures, Trust in measures and Practice of treatment" and Giving sufficient information to the patient by the nurse with the scales Never, Rarely, Sometimes, Most of the time, Usually, Always". The Physician Trust Questionnaire includes nine questions about Trust in Skills, Treatment Selection, and Physician Decisions on a scale of "Strongly Agree, Agree, Neither Agree Nor Disagree, Strongly Disagree and Disagree".

Patients with more than three days of hospitalization were randomly selected from the ward list. First, patient satisfaction with the study was obtained and then a questionnaire about patients' trust in physicians and nurses was filled in by an interviewer. Data analysis was performed using SPSS-26 software. Descriptive analysis was performed with mean, standard deviation, frequency, percentage, and analytical analysis depending on the type of data with t-test and Chi-square test.

RESULTS

Four hundred patients participated in this study. Descriptive values related to the age of these patients by group and in general are displayed in Table 1. The mean age of patients in the COVID-19 group was 53.3 years and in the Non-COVID-19 group was 53.8 years. This study included 257 male patients (64.3%). In addition, the sex distribution was the same in the two groups of patients with COVID-19 and Non-COVID-19 and no significant difference was observed in the sex distribution of the two groups (P=0.132).

Table 1: Descriptive characteristics of the patients admitted to Covid-19 and non-Covid-19 wards

Variable		COVID-19	Non-COVID-19	P Value
Age (Mean ±SD]		5.3 ± 14.53	3.8 ± 17.53	0.11
Sex	Male	120	137	13.0
	Female	79	65	
Education	Less than a Bachelor's degree	99 (54.1 %)	147 (8.72 %)	<0.001
	Bachelor degree	57 (1.31 %)	49 (3.24 %)	
	Senior	22 (0.12 %)	5 (5.2 %)	
	PhD	5 (7.2 %)	1 (5.0 %)	

In total, 198 patients (49.5%) with COVID-19 and 202 (50.5%) Non-COVID-19 patients were considered in this study. The overall mean of trust in nurses in the COVID-19 group was not significantly different from that of the Non-COVID-19 group.

Most people in both COVID-19 (85.3%) and Non-COVID-19 (86.6%) groups reported that nurses were available, most of the time or always when they needed them.

Most patients in both COVID-19 (99.4%) and Non-COVID-19 (97.4%) groups believed that the nurse's activity was useful to them, most of the time or always. The mean usefulness of therapeutic activities in patients in general wards was 0.1 higher than in patients in COVID-19 wards but this difference was not significant (P>0.05).

Most patients in both COVID-19 (99%) and Non-COVID-19 (95%) groups believed that they trusted the care of nurses always. The average level of trust in medical care for patients in general wards was 0.1% lower than that of patients in COVID-19 wards. But the difference was not significant (P>0.05).

In the COVID-19 group, 93% of patients often or always followed the advice of the nurses, while in the Non-COVID-19 group, 96.5% of patients most of the time or always followed the statements and recommendations of the nurses. The mean rate of treatment of patients with therapeutic recommendations in general wards was significantly higher (0.4) than patients admitted to the COVID-19 ward (P<0.001).

In the COVID-19 group, most patients (71.8%) believed that most of the time, or always, nurses provided enough accurate information about their disease. While in the Non-COVID-19 group, about half of the patients (55%) believed that most of the time or always the nurses provided them with sufficient accurate information about their disease. The average amount of information required for patients in general wards was 0.3 less than for patients admitted to the COVID-19 ward. There was a significant difference in the amount of information required for patients in general wards and COVID-19.

Table 2: Descriptive values related to the trust variables of the patients admitted in the Covid-19 and non-covid-19 wards

Variable	COVID-19			Non-COVID-19		
	SD ± Min	Min	Max	SD ± Min	Min	Max
Responsibility for patient needs	4.6 ± 1.1	2	6	4.6 ± 1.1	2	6
The usefulness of therapeutic activities	8.2 ± 0.5	3	6	8.3 ± 0.5	1	6
Reliability of medical care	3.5 ± 8.0	3	6	2.5 ± 1.1	1	6
Following the recommendations	5±0.9	3	6	4.5±0.9	2	6
Patients' access to the necessary information	4.2± 1.3	1	6	3.9± 1.6	1	6
Satisfaction with the nurse	7.0 ± 9.4	2.6	6	7.0 ± 9.4	2.6	6
Satisfaction with the physicians	3.5±0.3	2.8	4.3	3.7±0.3	2.2	4.5

DISCUSSION

Patients' trust in nurses and physicians is important and at the same time complex phenomenon that to achieve it, some professional characteristics must be created and strengthened and some ethical principles must be observed [17]. In the study of patients' trust in nurses of Baqiyatallah Hospital, Tehran, Iran, we used the questionnaire of patients' trust (P=0.006). The results of the study assessing the patients' trust in the physicians showed that most patients participating in the study in both groups of COVID-19 (92.4%) and Non-COVID-19 (97.5%) had a high level of trust in physicians. Considering the mean difference, it can be said that the average trust in physicians in COVID-19 patients was significantly lower by 0.14 than in Non-COVID-19 patients (P<0.001).

Ravdin and Cabral nurses, which was validated by Nouripour et al [15]. This questionnaire had five questions, and in what follows, we will review the results obtained from the questions.

The level of responding to patients' needs and being available to them when needed, the level of use of treatment activities, and the level of trust in medical care in both general and COVID-19 wards were desirable. The rate of follow-up to treatment recommendations was favorable in both groups, but the mean rate of follow-up to treatment recommendations of patients in general wards was significantly higher than patients admitted to the COVID-19 ward. Studies have shown that there is a positive relationship between the level of trust in the nurses and the level of treatment adherence [18]. Trust in the health care specialist has been proposed as a basis for effective and basic treatments for patient-centered care [19-21]. However, the novelty of COVID-19 and the fact that there is still no definitive cure for the disease is a problem noted in the study by Baker [22]. The lack of definitive treatment and protocol for this disease and frequent changes in health protocols during this period may reflect scientific uncertainty; meanwhile, the uncertainty of scientific evidence reduces patients' trust to follow the recommendations of medical staff compared to other diseases and overshadows their treatment adherence.

In this study, the lowest level of trust was in the average amount of information required for patients, which had a lower score compared to other questions in the questionnaire, and the average amount of information required for patients in general wards was 0.3 less than patients in the COVID-19 ward. Nurses in both the COVID and general wards have the lowest level of patients' trust in providing the necessary information. In Ozaras and Abaan study, the use of medical terms or terms that the patient does not fully understand, the lack of anticipation or understanding of patients' information needs, and neglecting responsibilities and factors related to nursing work such as busyness, etc. have been mentioned as important causes in reducing the provision of the necessary information to patients [23]. It seems that to improve the provision of information to patients, some programs should be implemented in the hospital and the nursing department.

The level of patients' trust in physicians in COVID-19 wards was significantly lower than in Non-COVID-19 patients. Gupta et al. showed that the pandemic coronavirus disease 2019 (COVID-19) has posed new threats to the trust in medical staff [24]. Factors such as the high prevalence and involvement of doctors during the epidemic peaks, as well as the specific way of communicating with the patient (personal protective equipment and social distancing, etc.] and that the patient does not even know and see the face of the treating physician well, are involved in this issue [25]. Identifying the causes of this difference requires further study and investigation of the issue from different respective.

CONCLUSION

Trust in nurses and physicians in both COVID-19 and general wards was optimum. This finding indicates that during the COVID-19 pandemic, the medical staff worked tirelessly and despite the difficulties and complications of the pandemic, patients have high trust in physicians and nurses.

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Original Article

Covid-19 Risk Perception in a Sample of Iranian Population

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Abstract: *How people behave in an emergency depends on many social, cultural, and contextual factors. This study aimed to determine the COVID-19 risk perception in Iranian people. This study was descriptive. The study population was the assistant of patients who had come to Baqiyatallah Hospital in 2021. The number of participants was 500. The study tool was the COVID-19 risk assessment questionnaire. Data were analyzed using SPSS-22 software. The mean risk perception score was 2.78(±0.6) on a 7-point scale. The lowest and highest mean of the predictors of risk perception in COVID-19 was trust in government and personal efficacy, respectively. There was a significant difference between men and women in the mean of collective efficacy (P= 0.032). Social knowledge (p=0.007), trust in science (p=0.001), and trust in medical professionals (p=0.050) in positive direct experience people were significantly more than in people with negative direct experience of COVID-19. The most important predictors of risk perception in the COVID-19 epidemic were gender, education, social knowledge, and trust in science (P<0.05). People's perception of the risk of COVID-19 depends on several various factors, the most important factors were gender, level of education, social knowledge, and people's trust in science.*

Keywords: *COVID-19; coronavirus; risk perception*

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and has spread around the world quickly, emerging as a significant challenge of the current century for public health, the economy, markets, and policies in many countries [1].

The coronavirus outbreak was declared a public health emergency of international concern by the World Health Organization on 30 January 2020. Today, most people around the world have understood the outcomes of COVID-19 infection and they have accepted the need for hand hygiene and social distancing to prevent its spread. But, some of them ignore or delay hygienic actions, government laws, and social distances in public places, or homes. The fact that individuals in the same situation of common challenge act so differently indicates that the persons' risk perception relating to this new and chronic challenge is strongly different [2].

How people behave in an emergency depends on their understanding of the severity and depth of the risk and the extent of their vulnerability, but many social, cultural, and contextual factors may influence their risk perception [3]. Little is known about how people perceive the risk associated with emerging infectious diseases compared to other areas of risk. Risk perception leads to the adoption of preventative health behaviors so misperception of COVID-19 risk in this condition can lead to negative consequences of pandemic control [4].

Personal experience with the virus, individualistic and prosocial values, hearing about the virus from friends and family, trust in government, science, and medical professionals, personal knowledge of government strategy, and personal and collective efficacy were all significant predictors of risk perception, in the first global assessment of public risk perception of COVID-19 [5].

Previous studies have examined differences in risk perception of COVID-19 in diverse populations and races. Health workers reported higher risk perception, level of worry, and knowledge as related to COVID-19 infection compared to the general population [6]. In some studies, females were found to perceive risks higher, complied more with the government's guidelines, and coped better than males in response to COVID-19 [7]. But Self-reported anxiety over the pandemic is a crucial predictor in explaining risk perceptions in all age and gender groups [8]. And overall public initial concerns and trust can play an essential role in improving the perceived risk of a pandemic and increasing public participation in adopting preventive measures [9]. This study aimed to determine the risk perception score and its related factor in Iranian people.

MATERIALS AND METHODS

This study was descriptive-analytical. The study population was the assistant of patients who had come to the clinic of Baqiyatallah Hospital in 2021. The inclusion criteria were 15 to 80 years old, no serious and acute illness, and consent to participate in the study. The number of participants in the study was 500, with a 5% alpha error and 20% beta error. The study tool was the COVID-19 risk assessment questionnaire, which was designed by Dryhurst [10] using previous studies to identify all dimensions of risk perception covering affective, cognitive, and temporal-spatial dimensions to provide a holistic measure of risk perception. The questionnaire included items on COVID-19 experience, demographic variables (age, gender, and education) knowledge, both personal and social knowledge, social amplification, prosociality, individualism, trust in l three domain of science, medical professionals, and government, personal efficacy, and collective efficacy. The validity of the COVID-19 risk assessment questionnaire was assessed using face and content validity approaches. The impact score of each item was assessed to evaluate the face validity. The questionnaire was distributed among 10 technical experts who were asked to evaluate items based on the mentioned criterion. Then the importance of each item was calculated using the impact score formula and the results were compared with the standard score (1.5), and items whose scores were higher than the standard score were included in the questionnaire. Next, the content validity of the instrument was analyzed using the Content Validity Ratio (CVR) and Content Validity Index (CVI). 10 respondent experts also participated in this phase. Experts were asked to group items as "essential", "essential but not useful", or "not essential" to calculate CVR. Their responses were calculated based on the CVR formula and the results were compared with Lawshe's Table. Numbers higher than 0.6 were approved. CVI survey was done based on the Waltz and Basel content validity index [11]. A questionnaire was distributed among the experts who were asked to rank 24 items based on a four-part Likert scale as compatible with 3 criteria of relevancy, simplicity, and clarity. (For example, unrelated items, partially related items, related items, and fully related items were scored 1, 2, 3, and 4, respectively). Items higher than 0.6 were approved according to the CVI score. Cronbach's alpha method was used to assess the questionnaire's reliability. Data collection was started after the standardization of the questionnaire.

Patients completed the study questionnaire, after written consent. The questionnaire was filled out using the interview method and by a trained interviewer. All information obtained from patients was confidential and the study received an ethics code from the ethics committee of Baqiyatallah Hospital. Primary data were analyzed using SPSS-22 software. Descriptive data analysis was performed using mean and standard deviation in quantitative data and frequency and percentage in qualitative data. Analytical analysis was performed according to the normality of the data with parametric and non-parametric tests. Linear and logistic regression were used to measure the relationship between variables according to the type of data.

RESULTS

The validity and reliability of the instrument of this study

In the first step, the COVID-19 risk assessment questionnaire was validated. Tables 1 and 2 show that the scores of all items of the questionnaire were higher than the criterion score (i.e. 1.5) and experts approved the quantitative face validity of all items. Moreover, the results of CVI indicate that all items of the questionnaire obtained a score higher than 0.6, and content validity was considered appropriate by experts. The reliability of the questionnaire based on Cronbach's alpha was 0.79, 0.69, and 0.77 for risk perception, individualism, and trust in science, respectively.

Study findings

In the study samples, 500 people participated, of whom 253 (50.6%) were women and 247 (49.4%) were men.

The mean age of participants was 38.9 (±11.7) and 147 (29.4%) of the people have experienced the disease. The descriptive characteristics are listed in Tables 3 and 4.

The mean risk perception score was 2.78(±0.6) on a 7-point scale. The lowest mean of the predictors of risk perception in COVID-19 was in trust in the government and the highest mean was in personal efficacy (Table 4).

Table 1: Results of calculating quantitative face validity (impact score) of items of the designed instrument

Item	Frequency	Importance	Impact score
Q1: How worried are you personally about the following issues at present? Coronavirus/COVID-19	0.7	4.4	3.08
Q2: How likely do you think it is that you will be directly and personally affected by the following in the next 6 months? - Catching the coronavirus/COVID-19	0.6	4.1	2.46
Q3: How likely do you think it is that your family and friends in the country you are currently living in will be directly affected by the following in the next 6 months? Catching the coronavirus/COVID-19	0.6	3.6	2.16
Q4: How much do you agree or disagree with the following statements? – The coronavirus/COVID-19 will NOT affect very many people in the country I'm currently living in	0.5	3.6	1.8
Q5: How likely do you agree or disagree with the following statements? - I will probably get sick with the coronavirus/ COVID-19	0.7	3.8	2.66
Q6: How much do you agree or disagree with the following statements? - Getting sick with the coronavirus/COVID-19 can be serious	0.6	3.3	1.98
Q7: How much do you feel you understand the government's strategy to deal with the coronavirus/COVID-19 pandemic?	0.6	3.5	2.1
Q8: To what extent do you think scientists have a good understanding of the coronavirus/ COVID-19?	0.5	3.6	1.8
Q9: Have you ever had, or thought you might have, the coronavirus/COVID-19?	0.6	3.1	1.92
Q10: Have you come across information about coronavirus/COVID-19 from: - Friends and family	0.6	3.4	2.04
Q11: To what extent do you think it's important to do things for the benefit of others and society even if they have some costs to you personally?	0.7	2.2	1.54
Q12: The government interferes far too much in our everyday lives.	0.8	2.6	2.2
Q13: How much do you trust the country's politicians to deal effectively with the pandemic?	0.6	2.5	1.5
Q14: How much do you trust each of the following? – Scientists	0.7	3.3	2.31
Q15: How much do you trust each of the following? - Medical doctors and nurses	0.6	3.2	1.92
Q16: To what extent do you feel that the personal actions you are taking to try to limit the spread of coronavirus make a difference?	0.6	3	1.8
Q17: To what extent do you feel the actions that your country is taking to limit the spread of coronavirus make a difference?	0.7	3	2.1

Table 2: Content validity index of questionnaires through CVR and CVI

Item	CVR	CVI		
		simplicity	perspicuity	relevance
Q1: How worried are you personally about the following issues at present? Coronavirus/COVID-19	1	0.9	0.9	0.9
Q2: How likely do you think it is that you will be directly and personally affected by the following in the next 6 months? - Catching the coronavirus/COVID-19	0.6	0.9	0.8	0.8
Q3: How likely do you think it is that your family and friends in the country you are currently living in will be directly affected by the following in the next 6 months? Catching the coronavirus/COVID-19	0.8	0.7	0.8	0.8
Q4: How much do you agree or disagree with the following statements? – The coronavirus/COVID-19 will NOT affect very many people in the country I'm currently living in	0.8	0.8	0.8	0.9
Q5: How likely do you agree or disagree with the following statements? - I will probably get sick with the coronavirus/ COVID-19	0.6	0.9	0.9	0.7
Q6: How much do you agree or disagree with the following statements? - Getting sick with the coronavirus/COVID-19 can be serious	0.8	0.8	0.8	0.7

Q7: How much do you feel you understand the government's strategy to deal with the coronavirus/COVID-19 pandemic?	0.9	0.8	0.7	0.9
Q8: To what extent do you think scientists have a good understanding of the coronavirus/COVID-19?	0.7	0.7	0.8	0.8
Q9: Have you ever had, or thought you might have, the coronavirus/COVID-19?	0.9	0.8	0.7	0.7
Q10: Have you come across information about coronavirus/COVID-19 from: - Friends and family	0.8	0.9	0.9	0.9
Q11: To what extent do you think it's important to do things for the benefit of others and society even if they have some costs to you personally?	0.6	0.7	0.8	0.7
Q12: The government interferes far too much in our everyday lives.	0.6	0.6	0.6	0.7
Q13: How much do you trust the country's politicians to deal effectively with the pandemic?	0.9	0.7	0.7	0.8
Q14: How much do you trust each of the following? – Scientists	1	1	1	0.9
Q15: How much do you trust each of the following? - Medical doctors and nurses	1	1	1	1
Q16: To what extent do you feel that the personal actions you are taking to try to limit the spread of coronavirus make a difference?	0.7	0.8	0.7	0.9
Q17: To what extent do you feel the actions that your country is taking to limit the spread of coronavirus make a difference?	0.9	0.7	0.7	0.9

Table 3: Descriptive characteristics of the participants in the study of risk perception of Covid-19

		Frequency	Percent
Gender	Female	253	50.6
	Male	247	49.4
	Total	500	100.0
Education	Under Diploma	37	7.4
	Diploma	149	29.8
	BSc	178	35.6
	MSc	91	18.2
	PhD	42	8.4
	Total	497	99.4
Direct experience with the disease	Yes	147	29.4
	No	353	70.6
	Total	500	100.0

Table 4: The scores of the risk perception questionnaire in participants in the Covid-19 risk perception study

		Number	Minimum	Maximum	Mean	Std. Deviation
Age		497	15	78	38.97	11.731
Risk perception		500	1	4	2.78	0.60669
Personal knowledge		500	1	7	4.78	1.50084
Social knowledge.		500	1	7	4.34	1.43105
Social amplification	Yes	426			85.2*	
	No	74			14.8*	
Prosociality		500	1	7	5.33	1.39061
Individualism		500	1	5	2.73	0.80308
Trust in government		500	1	5	2.20	1.071
Trust in science		500	1	5	3.73	0.73161
Trust in medical professionals		500	1	5	3.94	0.814

Personal efficacy	500	1	7	5.35	1.23006
Collective efficacy	500	1	7	4.02	1.20094

**: Percent*

Next, we ran a Spearman's rho to assess the correlation of the predictors that play a role in COVID-19 risk perception (Table 5). Social knowledge, trust in science, and trust in medical professionals were all significantly associated with risk perception refer to Table 3.

Table 5: The statistics of the predictors of COVID-19 risk perception

		Age	Risk perception	Personal knowledge	Social knowledge	Prosociality	Individualism	Trust in government	Trust in science	Trust in medical professionals	Personal efficacy	Collective efficacy
Age	Correlation Coefficient		.006	.000	.010	.092*	.030	.096*	-.005	.054	.006	.086
	Sig. (2-tailed)		.889	.999	.825	.040	.498	.033	.917	.229	.886	.055
Risk perception	Correlation Coefficient	.006		.044	-.266**	.009	-.078	.018	-.227**	-.156**	-.005	-.006
	Sig. (2-tailed)	.889		.329	.000	.833	.082	.694	.000	.000	.915	.891
Personal knowledge	Correlation Coefficient	.000	.044		-.061	.158**	-.218**	-.069	-.022	.100*	.296**	.069
	Sig. (2-tailed)	.999	.329		.171	.000	.000	.123	.618	.025	.000	.122
Social knowledge	Correlation Coefficient	.010	-.266**	-.061		-.028	.065	.026	.250**	.079	-.039	.097*
	Sig. (2-tailed)	.825	.000	.171		.532	.149	.567	.000	.076	.385	.029
Prosociality	Correlation Coefficient	.092*	.009	.158**	-.028		-.338**	-.031	.131**	.173**	.316**	.069
	Sig. (2-tailed)	.040	.833	.000	.532		.000	.486	.003	.000	.000	.125
Individualism	Correlation Coefficient	.030	-.078	-.218**	.065	-.338**		.052	-.061	-.134**	-.359**	-.122**
	Sig. (2-tailed)	.498	.082	.000	.149	.000		.243	.170	.003	.000	.006
Trust in government	Correlation Coefficient	.096*	.018	-.069	.026	-.031	.052		.088*	.074	-.022	.339**
	Sig. (2-tailed)	.033	.694	.123	.567	.486	.243		.048	.098	.623	.000
Trust in science	Correlation Coefficient	-.005	-.227**	-.022	.250**	.131**	-.061	.088*		.407**	.121**	.128**
	Sig. (2-tailed)	.917	.000	.618	.000	.003	.170	.048		.000	.007	.004
Trust in medical professionals	Correlation Coefficient	.054	-.156**	.100*	.079	.173**	-.134**	.074	.407**		.249**	.179**
	Sig. (2-tailed)	.229	.000	.025	.076	.000	.003	.098	.000		.000	.000
Personal efficacy	Correlation Coefficient	.006	-.005	.296**	-.039	.316**	-.359**	-.022	.121**	.249**		.277**
	Sig. (2-tailed)	.886	.915	.000	.385	.000	.000	.623	.007	.000		.000
Collective efficacy	Correlation Coefficient	.086	-.006	.069	.097*	.069	-.122**	.339**	.128**	.179**	.277**	
	Sig. (2-tailed)	.055	.891	.122	.029	.125	.006	.000	.004	.000	.000	

** Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).*

All predictors were analyzed based on gender variables (the Mann-Whitney U test was used due to abnormal distribution). There was a statistically significant difference between men and women in the mean of collective efficacy (P= 0.032) and the rest were not.

Specifically, people who have had direct personal experience of COVID-19 were different in some predictors of risk perception from those who had no direct experience of disease and social knowledge (p=0.007), trust in science (p=0.001) and trust in medical professionals (p=0.050) in positive direct experience people were significantly more than in people with negative direct experience of COVID-19.

We investigated the determinants of risk perception in regression models. The correlations of demographic variables with risk perception were assessed and gender, as well as education, were significantly associated with risk perception of COVID-19, such that males perceive less risk compared to females (Table 6) and the most important predictors of risk perception in COVID-19 epidemic were gender, education, social knowledge and trust in science (Table 7).

Table 6: Regression output of demographic variables of risk perception in COVID-19

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.991	0.142		21.078	0.000
	Age	-0.001	0.002	-0.017	-0.371	0.711
	Gender	0.115	0.055	0.095	2.081	0.038
	Education	-0.118	0.026	-0.205	-4.452	0.000

a. Dependent Variable: Risk perception

Table 7: Regression output of predictors of risk perception in COVID-19

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	4.141	.339		12.226	.000
Age	-.001	.002	-.018	-.419	.675
Gender	.116	.053	.096	2.187	.029
Education	-.111	.026	-.193	-4.219	.000
Personal knowledge	.021	.018	.053	1.181	.238
Social knowledge	-.099	.019	-.234	-5.303	.000
Direct experience	-.031	.058	-.024	-.544	.586
Social amplificatio	.033	.074	.020	.448	.654
Prosociality	-.001	.021	-.001	-.029	.977
Individualism	-.061	.037	-.081	-1.636	.103
Trust in government	.024	.026	.043	.935	.350
Trust in science	-.113	.042	-.136	-2.697	.007
Trust in medical professionals	-.068	.037	-.091	-1.843	.066
Personal efficacy	-.016	.025	-.033	-.661	.509
Collective efficacy	.017	.024	.033	.690	.490

a. Dependent Variable: Risk perception

DISCUSSION

Risk perception is important in determining health-protective behavior. Perception of risk in critical experiences like epidemics is influenced by social, cultural, and contextual factors. Previous experiences such as SARS, influenza, and swine flu epidemic showed that the strategies performed and the results of activities often depend on recognizing the predictors of risk perception and coordinating programs with the important predictors [12]. It should be considered the management of risk perception predictors directs by governments in all programs and activities. The risk perception model recommends in COVID-19 epidemics consist of clusters of variables that correspond to the cognitive tradition (e.g. people's knowledge and understanding of risks), the emotional and experiential tradition (e.g. personal experience), the social-cultural paradigm (e.g. the social amplification of risk, cultural theory, trust, and values), and relevant individual differences (e.g. gender, education, ideology). The mean risk perception score was 2.78(±0.6) in this study which is slightly different from the average risk perception score in other countries. The study of risk perception in ten countries found that the risk perception score of COVID-19 epidemics varied between 4.78 and 5.45 on a 7-point scale, and it is relatively high in countries of the U.K., Spain, and North America. Therefore, in this study, the score of risk perception was significantly lower than in all ten countries. High-risk perceptions may enhance the public's active response to the epidemic. Risk perception is a dynamic process and it changes over time. In this study, more than a year had passed from the beginning of the epidemic, which can be an influential factor in the perception of risk.

In demographic factors, gender was the significant predictor of risk perception. The risk perception score of women was higher than men on all the components of risk perception. Other studies reported the same result and it seems that gender was included as the moderator of risk perception and protective behavior [13]. In similar crises of the past decades, women are constantly seen to be more precautionary behaviors than their male counterparts. Another basic demographic predictor is the level of education of the individual, especially in positive experience persons. In other studies, a high level of educated is more likely to improve the preparedness level and adherence to COVID-19 preventive and control guidelines [14], and poor education was

significantly associated with lower risk perception [15], similar to our findings.

The level of education could affect knowledge as well as social knowledge; therefore social knowledge might be an important predictor of risk perception, which was observed in this study. Although studies on COVID-19 have reported associations between social knowledge and risk perception [16], some of them did not find this result [17]. Social knowledge is acquired through socialization and lived experience; however educational guidelines in the media at the national level could affect social knowledge, especially in critical situations such as the COVID-19 epidemic. The basic role of social knowledge in predicting the level of risk perception can be considered in the planning and implementation of national protocols.

This study found that scientific sources were the most trusted sources for COVID-19 information. Similar results were reported in Kwok's study on the COVID-19 pandemic [18] and the prior outbreak of SARS in 2003 [19] and the influenza A (H1N1) pandemic in 2009 [20].

Trust in science and experts in this study was greater than trust in government. The lowest mean of the predictors of risk perception was trust in the government. Trust in the government has been found to influence people's willingness to be accepting preventive activities [21], social limitations [22], and vaccination programs [23]. When people lack the necessary knowledge to evaluate the risk of a hazard, trust becomes an important cue regarding whom to believe and behavior [24]. People need to rely on scientists, professionals, and government agencies to listen and realize the information and the people's trust in these sources might be an important factor contributing to people's acceptance of the difficult precautionary measures needed to eradicate a pandemic [25].

This study might be consisting of some limitations. It should be noted that the study population was limited to those who were referred to the hospital. These people may not be a perfect example of the general population, and it is better to do such research at the national level and with diverse populations. The duration of the research was limited, and it is better to do this research several times.

CONCLUSION

People's perception of the risk of COVID-190 depends on several various factors, the most important of which were gender, level of education, social knowledge, and people's trust in science. It is recommended that studies be performed with larger and heterogeneous populations and longer time.

Acknowledgments

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REVIEW

Carcinoid Heart Disease – A 2022 Retrospective

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Abstract: *We aim to introduce a 2022 update on CHD (carcinoid heart disease) considering a multidisciplinary perspective. This is a narrative mini-review. We searched English, full-length papers (PubMed). Inclusion criteria: original articles regardless of the level of statistical significance. We identified 44 papers and manually selected those with CHD, as follows: 8 original studies, 1 case series (N=9 patients), 16 case reports (N=1 patient/paper), and a total of 1,030 patients on published studies. The most remarkable results are the longest period of enrolment of 3-4 decades; CHD ratio among carcinoid syndrome of 37%; 30-day mortality post-cardiac surgery of 12%; median survival in CHD from 1.3 to 3.9 years (more than 2 years if valve intervention is provided); most useful prognostic markers: 5HIIA, NT-pro-BNP, but, potentially, chromogranin A. The specific protective role of somatostatin analogs against developing CHD is yet to be determined. CHD in the absence of carcinoid syndrome/liver metastasis may be related to ovarian NEN (neuroendocrine neoplasia) with a better outcome if prompt intervention. Remarkably, 3 guidelines are released regarding CHD on behalf of ENETS (European Neuroendocrine Tumor Society), and ESC (European Society of Cardiology). CHD still represents a most challenging entity situated at the crossroads of surgery, cardiology, oncology, endocrinology, and gastroenterology.*

Keywords: *carcinoid heart disease, neuroendocrine, serotonin, neuroendocrine neoplasia, carcinoid syndrome, 5HIIA, valve, tricuspid, echocardiography*

INTRODUCTION

The complex field of neuroendocrine neoplasia (NEN), a rare condition with an annual incidence of 2 to 5.1 cases per 100,000 individuals, represents one of the most spectacular domains with massive recent progress from a multidisciplinary perspective [1,2]. The two main direct cardiac complications of NENs' evolution are either heart metastasis or carcinoid heart disease (CHD), also, recently named carcinoid valvular heart disease [3,4]. Primary cardiac NENs are mostly uncommon [5]. While myocardial and pericardial NENs' spreading is unusual (representing less than 1-3% of NENs-related metastases), CHD represents the hallmark of a very severe disease, typically embracing a very poor outcome [6,7]. The advances in cardiac surgery and interventional once-cardiology which have been registered within the latest years improved the overall CHD prognostic, as recently seen in other cardiac surgical procedures [8,9].

CHD is related to hormonally active NENs that increase overall morbidity and mortality; nevertheless, valve intervention might associate with numerous benefits, but carriers of such interventions display additional risks, including the need for post-operative pacing or connected infectious risks [10,11]. The statistical confirmation that the surgical approach to CHD improves survival is currently based on observational data rather than controlled studies [12].

The pathogenesis of CHD is still poorly understood. However, the presence of CHD is closely correlated with abnormal levels of biomarkers (blood and urinary neuroendocrine markers, also, named vasoactive substances which are more than 40 types, despite not testing all of them in daily practice), especially serotonin, and its urinary metabolite 5-HIIA (5-hydroxy-indole acetic acid), but, also, chromogranin A, neuron-specific enolase, etc. which serve as diagnostic tools and prognostic markers as well as part of the surveillance protocols [13-15]. Novel biomarkers like those based on mRNA

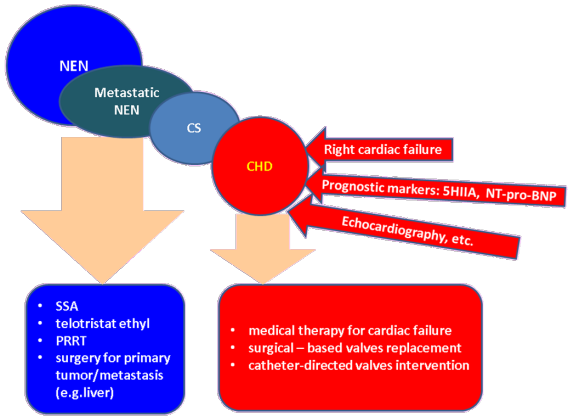
transcript are currently under development for daily practice implementation [13]. Cardiac imaging is essential for recognizing CHD, from traditional ultrasound to advanced techniques such as speckle-tracking echocardiography, 68 DOTA peptide PET-CT (Positronic Emission Tomography – Computed Tomography) scans, and cardiac magnetic resonance or CT [12,16-18].

CHD almost exclusively targets the right cardiac side with consecutive heart failure, but in the early stages, the complication might be underdiagnosed [19]. The left side of the heart is usually not affected (unless abnormal intra-cardiac shunts) because the fact that the main contributor of CHD, namely serotonin (5-hydroxytryptamine), but, also, histamine, bradykinins, and tachykinins, are enzymatically inactivated into pulmonary bloodstream [19]. Remarkably, in 2018 three cases of quadruple valve replacement were reported in addition to the prior four concerning CHD, which represents exceptional situations in NEN-CHD pointing out that the left heart side may be affected, as well [20].

Usually, at the moment when CHD is identified, the patients already have clinically manifested NEN in terms of traditional carcinoid syndrome (flush, diarrhea, wheezing, loss of appetite and malnutrition, non-specific abdominal pain or cramps, hypotension, telangiectasia, edema, etc.) that is presented in correlation with liver metastases, grossly affecting 30-40% of all NENs [21]. Most patients with CHD are aged between 50 and 70 years (males being slightly more affected than females), and present chronic fatigue and dyspnea, independent of carcinoid syndrome (but may be initially misdiagnosed as carcinoid syndrome or even carcinoid crisis) [19]. The most frequent type of NEN causing CHD is gut-derivate (noting that the small intestine is the most frequent NEN up to 65-67% followed by lung NEN of 25%) and approximately one out of five or two cases (depending on the studied population) is prove to develop CHD which finally influence the prognostic even more than the primary tumor growth itself (in some cases) [12,22]. One meta-analysis from 2021 (including 9 studies, N=416 adults with NEN, mean age of 63 years, 53% males) showed that 75% of CHD – related NENs originate from the small intestine and colon, and 98% of them have synchronous hepatic metastases. Tricuspid regurgitation was confirmed in 97% of individuals of different severities, respective 72% concerning pulmonary valve, while right heart failure was identified in 48% of all cases. After having a valve replacement (99% of tricuspid, and 59% of pulmonary valve), a 30-day mortality of 9% was identified (with a median post-surgery survival of 3 years) which points out an acceptable safety profile of valves intervention in CHD [23].

Overall, the multidisciplinary management of CHD includes cardiologic therapy for heart failure, the control of serotonin excess by using high doses of somatostatin analogs such as octreotide or lanreotide (and/or telotristat ethyl) that act on tumor growth as well, peptide receptor radionuclide therapy in addition to surgery for primary tumor and metastases, especially at the liver site (if feasible) to reduce the tumor burden, and, finally, surgical – based valves replacement or catheter-directed valves intervention in selected patients, depending on various criteria that take into consideration the underlying NEN, the heart status, and the access to cardiac surgery or interventional cardiology [24-28]. Globally, patients with carcinoid syndrome (thus metastatic NENs) and CHD have a more severe prognostic when compare to those without CHD, knowing that NEN individuals with clinically manifested carcinoid syndrome have a more severe prognostic versus those carcinoid free [28,29]. Valve intervention in CHD improves long-term outcomes despite the current peri-operatory mortality rate being considered at 10-20% [29]. Of note, there is no specific medication for CHD other than the medical approach for the originating tumor and associated hormonal panel (NEN's management), on one hand, and cardiologic therapy for heart failure in addition to interventional procedures targeting the affected valves [28-30] (Figure 1).

Figure 1: Sneak peek – carcinoid valvular heart disease



NEN = neuroendocrine neoplasia; CS = carcinoid syndrome; CHD = cardiac heart disease; 5HIIA = 5-hydroxyindoleacetic acid; NT-pro-BNP = NT-pro-brain natriuretic peptide; SSA = somatostatin analogues; PRRT = peptide receptor radionuclide therapy

MATERIALS AND METHODS

Aim

Our purpose is to introduce a 2022 update on CHD considering a multidisciplinary perspective.

Method

This is a narrative mini-review of literature. We searched English, full-length papers published in 2022 concerning journals indexed in PubMed (by using the terms "carcinoid

heart disease"). Inclusion criteria are represented by original articles particularly/specifically addressing CHD (in vivo) regardless of the level of statistical significance (original studies or case reports/series). Exclusion criteria: papers considering heart metastasis in NENs or primary cardiac NENs.

According to mentioned methodology, we identified 44 papers in 2022, and manually selected those with CHD, as follows: 8 original studies, 1 case series (N=9 patients), 16 case reports (N=1 patient/paper), a total of 1,030 patients on published studies, in addition to 8 reviews, 3 guidelines (one with duplicate). We excluded 3 articles concerning non-CHD, one in vitro study, and 3 papers regarding withdrawal (Figure 2).

CLINICAL STUDIES PUBLISHED IN 2022 CONCERNING CHD (accessed via PubMed)

We identified 8 original studies in 2022 (Table 1). A multicenter, retrospective study on 275 patients analyzed the clinical presentation and the outcome in NENs, confirming that more than one-third of the individuals with carcinoid syndrome are affected by CHD. All the patients had carcinoid syndrome (mean age of 57 years, aged between 21 and 84 years; metastatic disease was found in 94.6% of cases). CHD was identified in 37.3% of them (N=104) by using echocardiography, with the tricuspid valve being the most affected cardiac site, single or in combination with other valves. CHD correlated with a poor prognostic in individuals with carcinoid syndrome (similarly, male sex was found to be a risk factor); median survival in the studied population was of 9 years) [31].

Figure 2: The articles included according to the methodology of research

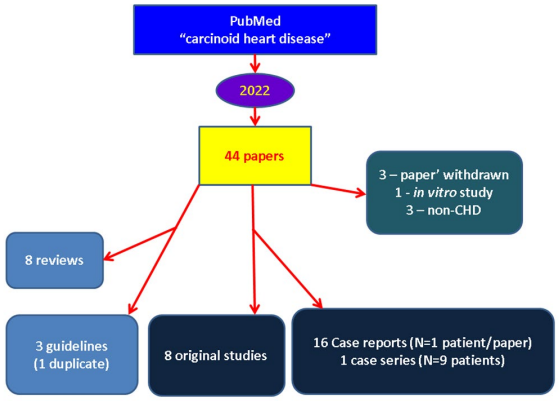


Table 1: Original studies published in 2022 considering carcinoid heart disease

First author/ Reference number	Type of Study	Number of patients	Results
Bergsten J [32]	retrospective (1986 - 2019)	60	30-day mortality (after cardiac surgery)=12% median post-operatory survival = 2.2 years
Levy S [34]	retrospective (1984 - 2021)	84	median survival since CHD diagnostic varying from 1.3 to 3.9 years
Levy S (bis) [35]	validation (case-control) study (1984 - 2021)	114	NT-pro-BNP has the best predictive value for CHD
Kostiainen I [36]	cross-sectional cohort study	65	low rate of CHD (5%) highly sensitive value of echocardiography (not BNP)
Shah HA [33]	retrospective study (2010 - 2019)	17	a 35% decline rate of 5-HIIA in association with liver function improvement
Macfie R [39]	retrospective study (2001 - 2021)	282	overall survival is lower in CHD *versus* non-CHD (N1=40 *versus* N2=242, p<0.001) valve replacement improves survival in CHD + inoperable liver metastasis
Konsek-Komorowska SJ [38]	retrospective study	108	chromogranin A inversely correlates with overall survival (p<0.05) in patients with carcinoid syndrome overall survival is lower in CHD *versus* non-CHD + carcinoid syndrome (67.22 versus 73 months)
Fijalkowski R [31]	retrospective study	275	prevalence of CHD 37.3% among patients with carcinoid syndrome overall survival is lower in CHD *versus* non-CHD

Abbreviations: CHD=cardiac heart disease; BNP=brain natriuretic peptide; N=number of patients

Bergsten J. et al. studied 60 patients (between 1986 and 2019, a total of 3 decades) with a median age of 64 years with CHD and cardiac surgery (N1=42 persons underwent both tricuspid and pulmonary valve replacement; N2=18 individuals had only tricuspid valve replacement with/without pulmonary valvotomy). The procedures (which exclusively used bioprosthetic valves) associated a 30-day mortality of 12%; and a median post-operatory survival of 2.2 years (a maximum of 21 years; statistically significantly higher in N1 versus N2, respective of 3 versus 0.9 years, p=0.02). A most useful tool of prognostic was echocardiography, either as a pre-operatory assessment (but with the limitation of underestimating

pulmonary involvement) or post-operatory surveillance. Additional poor prognostic markers are high pre-surgery levels of 5-HIIA and NT-pro-BNP (NT-pro-brain natriuretic peptide). Overall, the double valve is superior to single tricuspid replacement [32]. Moreover, a retrospective UK study (between 2010 and 2019) on 17 patients with CHD who became candidates for valve replacement surgery had a single valve procedure (3/17) versus double valve (13/17), respective triple valve (1/17). Post-operatory, a 35% decline of 5-HIIA witnesses the improvement of general status in association with hepatic function recovery which suggests an indirect benefit of cardiac surgery [33].

Levy S. et al. introduced a cohort study on 84 patients with CHD (58% males) followed for 4 decades (between 1984 and 2021) at Netherlands Cancer Institute; the median age of NEN confirmation was of 62.3 years with a consecutive CHD diagnostic after a median of 1.1 years and an associated median survival varying from 1.3 to 3.9 years (considering the patients who were most recently admitted when valve replacement surgery was more often applied). Prognostic markers are NT-proBNP (positively correlated with tricuspid regurgitation), respective to the implementation of valve replacement surgical procedures (which increases the survival rate) [34]. The same researchers conducted a validation (case-control) study on CHD – associated biomarkers. 114 individuals (N1 with CHD versus N2 without CHD) showed similar age (median age of N1 – 57.9 years, respective of N2 – 59.7 years, p=0.29). Among studied assessments like circulating serotonin, activin A, sST2 (soluble suppression of tumorigenicity 2), CTGF (connective tissue growth factor), and NT-pro-BNP had the best predictive value for CHD in individuals with high serotonin [35].

As opposed to these results, we mention a cross-sectional cohort study on 65 patients with small intestinal NETs (neuroendocrine tumors) (45% with carcinoid syndrome and 5% with CHD). The results showed that both categories had a statistically significant increase in hepatic tumor load, serum levels of chromogranin A, and urinary 5HIIA when compare to the patients without carcinoid syndrome ($p<0.005$). However, the authors identified a lower rate of CHD (5%) than expected in persons with carcinoid syndrome (30-50% – according to data from the literature). Moreover, they did not confirm the predictive value of proBNP but identified the highly sensitive value of echocardiography in subjects with elevated chromogranin A and 5HIIA [36].

Another classical biomarker in NENs is chromogranin A, very sensitive, but less specific [37]. A retrospective study focusing on blood chromogranin A values (N=108 patients with carcinoid syndrome; N'=84 individuals were followed-up and 27 of them complicated with CHD) showed that its increased levels inversely correlate with overall survival ($p<0.05$) in patients with carcinoid syndrome. Also, the presence of CHD is statistically significantly associated with a reduced overall survival (67.22 versus 73 months in patients with carcinoid syndrome but CHD-free) [38].

Some authors consider the protective effect of somatostatin analogs like octreotide for CHD, but a large study on 282 patients with carcinoid syndrome (between 2001 and 2021) did not sustain this observation. Overall survival was lower in patients with CHD versus non-CHD (N1=40 versus N2=242, $p<0.001$). Considering the patients with inoperable hepatic metastasis, the survival rate was lower in patients without undergoing valve replacement when compared to the individuals with CHD and cardiac surgery or the individuals CHD-free [39]. Similarly, we mention a case series of 9 subjects with NEN who followed for 39 months; initially, the patients were CHD-free and started therapy with somatostatin analogs (median time from NEN diagnostic to therapy initiation of one month; median time from starting the medical treatment to CHD confirmation of 23 months) [40]. As far as we currently know, we cannot predict that using somatostatin analogs is a protection factor against CHD, but, of course, this medication has a proven effect in controlling the hormonal and tumor burden [41].

INTERESTING CASE REPORTS

We identified according to our methodology 16 case reports (a total of 16 patients with CHD). We mention the case of a 70-year-old female with torrential tricuspid regurgitation due to CHD regarding an ovarian teratoma with neuroendocrine differentiation. The subject had a heterotopic tricuspid valve replacement with a stable disease within the following 6 months. Mostly particular, the patient did not experience carcinoid syndrome, and neither imaging assays proved any metastatic NEN (including hepatic) after gynecological surgery [42]. Similarly, another case of a large insular primary ovarian NEN was reported on a 40-year-old female admitted for virilization syndrome in association with unexpected CHD – related heart failure; the panel of increased neuroendocrine markers improved after gynecological intervention, but CHD aggravated even in the absence of liver metastasis requiring valve replacement within 6 years [43]. This scenario is extremely unlikely among the typical evolution of NENs, neither ovarian NEN is recognized as a common source of serotonin over-production. However, ovarian carcinoid tumors are among the rarest forms of CHD in the absence of carcinoid syndrome and/or liver metastases, thus their early removal massively improves the survival rate and does not follow within the general frame concerning the very poor outcome in CHD-NENs [44-46].

adequate diagnostic/recognition when she was identified with CHD and had cardiac surgery. The identification of primary NEN was after the intervention for CHD [47]. This case brings out at least two atypical aspects: CHD in young adults, as prior mentioned, which is rather atypical unless a clear genetic background is present, and CHD diagnostic before confirmation of primary NEN [48]. Generally, one-third of NENs remain with unknown primary sources [49]. Theoretically, younger patients with CHD and NEN might have a better post-cardiac surgery prognostic, but further statistical evidence is needed.

A 44-year-old male with neurofibromatosis type 1 had a 6-year history of gastric NET with hepatic metastasis. He was admitted for sensory neuronopathy and a meningocele. After glucocorticoid treatment was empirically initiated, additional decompressive laminectomy was done, but the patient soon developed CHD with a fatal outcome within 3 months (due to pneumonia) [50]. Of note, currently, we do not know the potential role of glucocorticoids (probably recommended for incidental co-morbidities) in aggravating CHD. Moreover, there are no consistent data to point out that individuals with genetic syndromes underlying NEN have a higher risk of developing CHD [51].

As consideration amid the recent COVID-19 pandemic, we mention one case of a 55-year-old male who delayed the presentation and was admitted for severe CHD which was confirmed synchronously with a high serotonin-producing primary NEN [52]. Another case brings out the particular aspects of cardio-oncology practice following valve replacement in subjects with CHD [53]. This is the case of a 58-year-old female who experienced a carcinoid crisis peri-operatory [54]. Another aspect concerns the post-surgery risks, for instance, a 77-year-old woman was offered a successful ablation therapy for recurrent atrial flutter developed after the surgical procedure of replacing the tricuspid valve [55]. Moreover, another case of an 81-year-old male introduces the idea of consecutive interventions in CHD considering the right side of the heart (severe pulmonary regurgitation was successfully approached by trans-catheter implantation 4 years after initial surgery for tricuspid) [56].

DISCUSSIONS

Pathogenic traits of CHD

Hedinger syndrome, tricuspid insufficiency in addition to pulmonary stenosis due to CHD still represents a challenging condition nowadays [57]. CHD is a progressive condition in subjects with carcinoid syndrome where the right side of the heart is transformed in terms of fibrosis, degeneration, inflammation, desmoplastic reactions causing valvar dam-age (stenosis or regurgitation), and cardiac insufficiency [58,59]. The formation of plaque-like deposits (myo-proliferative plaques) on endocardial areas of the valves and subvalvular system impairs the blood flow dynamics [60-62]. Similarly to CHD, mesenteric fibrosis might develop in individuals with carcinoid syndrome [60]. Common pathogenic factors are described like TGF-β (Transforming growth factor beta) or PDGF (platelet-derived growth factor), etc [60].

The clinical approach of CDD

The main predictors of CHD are an advanced stage of NEN with high levels of circulating serotonin but mostly increased 24-hour urinary 5HIIA in addition to the presence of carcinoid syndrome and other clinical features [63-65]. Other collateral elements may involve particular cardiac anatomy, for instance, patent foramen ovale is reported in half of the individuals with CHS (twice more frequently than in the general population) which causes a right-to-left shunting [66,67]. Intra-cardiac shunt explains the left heart involvement in CHD as one case was published in 2022 with four valves involvement [68,67].

Since initially, CHD is subclinical or completely asymptomatic, a strategy of periodic check-ups is necessary, including performing differential diagnostics with other complications of NENs [60,63,69]. Cardiac imaging represents a strong point in CHD management. Ultrasound is useful for serial evaluations and early assessment; CT exams might show various elements like early contrast opacification of inferior cave vein as an indicator of tricuspid regurgitation; modern imaging procedures like 68Ga-DOTATATE PET are helpful for adequate positive diagnostic, especially for cases with a low index of suspicion, when a differentiation with myocardial metastasis or non-NEN incidental conditions is needed [70-72]. One case from 2022 introduced 203Pb-VMT-α-NET Scintigraphy as a new alternative to PET/CT [73].

Full blown picture of heart valve disease – associated clinical manifestations and related cardiovascular and pulmonary complications of NENs including carcinoid crisis represents a sign of advanced disease and prompt multi-disciplinary intervention is required [74,75]. The overall experience with cardiac surgery for CHD is limited when compared to many of the non-CHD causes, thus there are still some areas of open discussion like predictors of successful intervention, the timing of the procedure, criteria of candidates selection, the type replacement (single or double valve) [76-79].

Key messages on behalf of highly specialized medical and surgical societies

In 2022 three papers of major importance were released concerning CHD. European Neuroendocrine Tumor Society (ENETS) published excellent “guidance” regarding carcinoid

syndrome and CHD. We mention a few major aspects: NT-proBNP remains the most important marker of CHD at the first-ever diagnostic (in patients who experience high serotonin status with or without clinical manifestation like carcinoid syndrome) and during follow-up. A cutoff value of 260 pg/mL indicates further investigations with echocardiography. In terms of ultrasound, the transthoracic approach is indicated, stress echocardiography might not bring additional information; while inadequate ultrasound data (including the evaluation of pulmonary vein damage) or planning for cardiac surgery may require supplementary imaging procedures like cardiac CT or magnetic resonance. Once identified, CHD requires serial NT-proBNP assays and cardiac ultrasound every 6 to 12 months, noting that sudden progress might occur anytime. Surgery (bioprosthetic valves are advised) should be done in severe CHD with more than 1 year of expected post-surgery survival; some specific criteria are recommended considering the adequate timing of intervention which should not be delayed. Double valve (tricuspid and pulmonary) replacement is better than a single one. Minimally invasive procedures are not yet studied enough [80]. Additionally, ENETS introduced a "synoptic" concerning the major role of echocardiography in CHD [81]. A collaboration of ESC (European Society of Cardiology) with other societies provided a guideline on cardio-oncology, also, addressing CHD. We mention some aspects, such as, for instance, echocardiography is recommended in patients with clinical evidence of CHD, carcinoid syndrome, and high BNP followed by a check-up every 3 to 6 months (level IB). BNP is assessed at baseline and every 6 months (level IB). A specific preparation is required considering the surgical/interventional risk of triggering a carcinoid crisis. Valve replacement, as in the ENETS guide-line, is recommended in patients with an expected survival of more than 1 year after intervention if they have symptomatic CHD or asymptomatic CHD (tricuspid or pulmonary) but with progressive dysfunction (level IC, respective IIaC) or severe mitral and/or aortic involvement in symptomatic cases (level IC) [82,83].

CONCLUSIONS

CHD represents a most challenging entity situated at the crossroads of surgery, cardiology, oncology, endocrinology, and gastroenterology. Until a few years ago, CHD was synonymous with a very severe form of NEN/carcinoid syndrome, with an expected poor outcome and limited resources. Recent cardio-oncology progress in addition to multiple shaped interventions for NEN allows a new level of approach in CHD to the benefit of the patients. However, many aspects are still open issues even in 2022.

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All authors have read and agreed to the published version of the manuscript.

List of Abbreviations
5-HIIA = 5-hydroxyindoleacetic acid
NEN = neuroendocrine neoplasia
CHD = carcinoid heart disease
NT-pro-BNP = NT-pro-brain natriuretic peptide
PET-CT = Positronic Emission Tomography – Computed Tomography
TGF = Transforming Growth Factor
PDGF = platelet-derived growth factor
NET = neuroendocrine tumor
NEN = neuroendocrine neoplasia
sST2 = soluble suppression of tumorigenicity 2
CTGF = Connective Tissue Growth Factor
ENETS=European Neuroendocrine Tumor Society
ESC=European Society of Cardiology

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REVIEW

Collision Tumors of the Thyroid

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Abstract: *Collision tumors, rare neoplasms underlying two distinct histological types of carcinomas (with different origins) separated by normal tissue, are described in many organs including the thyroid gland, but to a lesser extent. We aim to overview the current published data concerning collision tumors of the thyroid (CTT). This is a narrative review considering papers published on PubMed through a search starting from the terms "collision" and "thyroid". Inclusion criteria are full-length articles, the English language, the timeline from inception to December 2022, and original studies with different levels of statistical evidence. We followed the main types of thyroid carcinomas: differentiated thyroid carcinoma of papillary and follicular type, anaplastic/ undifferentiated/poorly differentiated carcinoma, respectively medullary thyroid carcinoma. We identified 92 papers based on the mentioned key words criteria. We excluded 54 papers (reviews or non-thyroid CTT or other topics outside our purpose). We included in the final analysis 38 papers: 34 case reports (1 patient per paper), 2 case series (2 individuals per each article), one case series of 3 subjects, and a single study of retrospective type (including 21 subjects) – a total of 62 patients on published cases which represents the largest study on published cases to our knowledge (according to our methodology).*

Keywords: *collision, thyroid, thyroid cancer, papillary carcinoma, follicular carcinoma, medullary thyroid cancer, thyroidectomy, pathological report*

INTRODUCTION

Collision tumors, rare neoplasms underlying (at least) two distinct histological types of carcinomas (with different origins) separated by normal tissue (and no mixture areas), are described in many organs (for instance, lung, stomach, breast, and ovary, etc.) including the thyroid gland, but to a lesser extent than seen in others sites (affecting less than 1% of all thyroid malignancies) [1-3]. The most common combination is papillary and medullar carcinomas, but any type of primary thyroid malignancy is reported in collision with a secondary spreading to the thyroid from a non-thyroid primary cancer or another primary type [4-6].

Collision tumors of the thyroid (CTT) are regarded as challenging in terms of recognition, poor outcome elements, and particular behavior in addition to the fact that there are still areas of the unknown regarding this chapter on thyroid neoplasms [7-9]. A distinction with composite tumors should be done (where the same mutation causes histologically different entities) [10-12]. Also, metachronous tumors (syndromic or not) represent a different category [13-14].

We aim to overview the current published data concerning CTT.

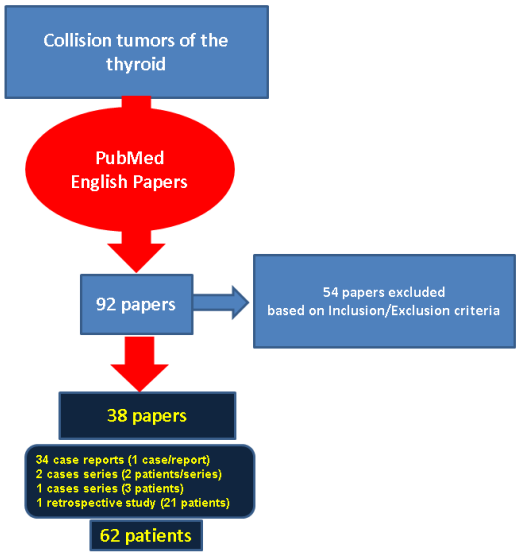
MATERIALS AND METHODS

This is a narrative review considering papers published on PubMed through a search starting from the terms "collision" and "thyroid". Inclusion criteria are full-length articles, the English language, the timeline from inception to December 2022, and original studies with different levels of statistical evidence. Exclusion criteria are non-thyroid collision tumors, non-English papers, and reviews. We followed the main types of thyroid carcinomas: differentiated thyroid carcinoma of papillary and follicular type, anaplastic/undifferentiated/ poorly differentiated carcinoma, respectively medullary thyroid carcinoma.

RESULTS

We identified 92 papers based on the mentioned key words criteria. We excluded 54 papers (reviews or non-thyroid CTT or other topics outside our purpose). We included in the final analysis 38 papers including 34 case reports (1 patient per paper), 2 case series (2 individuals per each article), one case series of 3 subjects, and a single study of retrospective type (including 21 subjects) – a total of 62 patients on published cases which represents the largest study on published cases to our knowledge (according to previously mentioned methodology) (Figure 1).

Figure 1: The method of research considering collision tumors of the thyroid



Papillary thyroid carcinoma

Papillary thyroid carcinoma is the most frequent histological type in CTT, but, generally as a single entity, it represents the most common thyroid neoplasia [15]. Apart from the most often association of medullary thyroid neoplasia, follicular malignancy is reported as well [16-19]. The combination with thyroid metastasis (typically from lung, breast, or renal cancers, etc.) is reported at the level of case reports (the spreading may be by local extension or via blood mainstream) [20-21]. For example, a case from 2007 introduced a CTT consisting of metastasis from lung adenocarcinoma and papillary thyroid cancer in a 63-year-old female [20]. A case report from 2021 included a 60-year-old female who was identified with the same carcinoma and metastasis from osteosarcoma within a CTT. This is the first case with bone origin as the primary source reported in a CTT [21]. The combination with a second primary cancer which is not originating from the thyroid is also reported. We identified a single case of a 67-year-old male who was diagnosed with a CTT associated a papillary thyroid cancer (with lymph node metastases) and parathyroid carcinoma clinically manifested with primary hyperparathyroidism [22]. The co-existence of a squamous cell carcinoma was reported, as well, originating from different sites or of unknown origin [23-27]. In 2004, a most unusual association with metastatic liposarcoma was published (this represents a most unique combination) [28].

Follicular thyroid carcinoma

This is the case of a 62-year-old female with follicular carcinoma (with capsular and vascular invasion) in association with a mucoepidermoid carcinoma and distant metastasis. Immunohistochemistry report might identify the mucoepidermoid differentiation of follicular malignancy over a different histological type (like mucoepidermoid malignancy) [29]. Hürthle cell carcinoma (regarded as a type of follicular group of differentiated thyroid cancers) is reported in less than 3% of thyroid malignancies, with the same ratio (of 3%) being reported for medullary thyroid carcinoma, as well. Their combination was identified on a 70-year-old female as a CTT (of note, the medullary cancer was intra-thyroidal, while the lymph nodes extension was derivate from Hürthle cell carcinoma) [30].

Interestingly, one retrospective study on 8 patients diagnosed with a primary thyroid malignancy identified the co-presence of the second histological type, meaning a follicular thyroid adenoma in association with papillary thyroid carcinoma (7/8), respectively medullary thyroid carcinoma (1/8) [31]. However, the current definition of CTT includes two malignant tumors, either primary or secondary, not a benign one like a follicular adenoma (which cannot be differentiated from a follicular carcinoma through the cytological report and it requires histological assessment) or a non-tumor – related cyst [31,32].

Medullary thyroid carcinoma

CTT may concern the thyroid gland and/or associated lymph nodes [33,34]. For instance, there is a case published in 2021 involving a 27-year-old male presenting CTT at lymph nodes (central compartment) from papillary thyroid cancer and non-syndromic medullary thyroid cancer. The growth speed was evaluated and the authors suggested that it may be similar to what we prior know concerning each of the two malignancies [35]. Generally, collision metastasis (mostly at cervical lymph nodes) is rarely reported in the absence of a thyroid tumor itself. We identified two papers concerning collision metastasis from papillary thyroid cancer and breast malignancy in female subjects [36,37]. In another case, a 73-year-old male was admitted for collision metastasis (cervical lymph nodes) from squamous cell carcinoma of nasal origin and B-cell lymphoma [38]. A previous report from 2009 included a lymph node spreading from a primary follicular

malignancy and a squamous cell carcinoma of unknown origin [39]. Moreover, we mention the case of a 47-year-old male with collision metastasis from oral tongue–related squamous cell carcinoma and accidentally detected thyroid papillary carcinoma [40].

However, we currently do not have enough statistical evidence to compare the evolution in CTT versus non – CTT cases. The largest study particularly addressing CTT is a retrospective analysis that included the experience from 2009 to 2019 (a 10-year experience) on a tertiary care cancer. CTT (medullary + papillary) represented 4.7% of the medullary cancer series (N=21 individuals, the sex ratio of 1; mean age of 45.33 years, range between 26 and 77 years). 66.66% of the papillary cancers were situated at the right thyroid lobe; 85.7% of them were of micro-papillary type. The mean size of medullary cancer was three times larger than differentiated thyroid tumors. The contribution to lymph node spreading (which was confirmed in 76.2% of cases) was done by medullary type (87.5%), papillary cancer (12.5%), respectively both malignancies (12.5%) [41].

Anaplastic, undifferentiated, and poorly differentiated thyroid carcinoma

In 2022 Gupta RK. et al. reported the case of a 55-year-old female with a most unusual CTT: anaplastic thyroid malignancy (osteoclastic variant) and primary squamous cell carcinoma, each affecting a different thyroid lobe in addition to distant metastases at the level of bone, muscle and lymph nodes. However, according to WHO (World Health Organization) 2022, these two types are parts of the same malignancy spectrum thus their combination is nowadays regarded as a CTT-like lesion [42].

The "late anaplastic shift" theory concerns the identification of anaplastic thyroid carcinoma on a subject with a long history of differentiated thyroid carcinoma (either papillary or follicular) [43]. However, the synchronous identification of a differentiated tumor in association with a poorly differentiated cancer respecting a border area between them is consistent with the current concept of CTT. Here we mention the case published in 2021 by Vlad M. et al. (a 56-year-old female admitted amid the COVID-19 pandemic with a post-thyroidectomy confirmation of anaplastic cancer on the left lobe and lymph nodes spreading and a papillary micro-carcinoma on the right lobe) [44].

The presence of two highly aggressive malignancies in CTT makes the prognostic extremely severe. There is the case of a 63-year-old female confirmed with a triple CTT: poorly differentiated carcinoma, papillary carcinoma (classical and hobnail variant – this is the first case report underlying this subtype in CTT), and oncocytic (Hürthle cell) carcinoma. The hobnail pattern has been reported in anaplastic and poorly differentiated thyroid malignancies, representing a sign of a very aggressive profile with rapid evolution [45].

DISCUSSION

Pathogenic loops in collision

The field of thyroid surgery is extremely complex, while the domains of pathological reports underlying thyroid neoplasia are also extremely vast and dynamic [46-48]. The collision phenomenon has been described at many levels within multiple organs of the human body [49]. "Chance" theory is one pathogenic hypothesis [41]. CTT might be underdiagnosed, especially micro PTC [41]. Fine needle aspiration (cytological report) might not identify both tumors. However, immunohistochemistry and molecular studies might help the diagnostic [50].

Whether different immune or genetic co-morbidities might act as a trigger or epigenetic elements for CTT development there is still an open question. We mention a case of CTT (papillary and medullary neoplasia) in a 46-year-old female with multiple sclerosis and prior therapy with interferon. This is the first case of CTT on a patient with this neurologic condition (published in 2020) and there is still missing information about a potential pathogenic loop [51].

Common genetic background of both tumors underlying CTT is reported in some cases. For instance, there is the case of a 69-year-old woman with Hürthle cell carcinoma (on the right lobe) and multi-centric papillary cancer (on both thyroid lobes), associated with BRAF mutations (V600E gene) [52].

Also, in 2010, a case of non-multiple endocrine neoplasia medullary cancer in addition to papillary malignancy underlying CTT was reported in a female carrying a particular RET mutation (familial medullary thyroid cancer) [53]. A RET somatic point mutation in association with mixt medullary–papillary (including a follicular variant of papillary cancer) neoplasia was described in a 59-year-old male (a case published in 2007) [54]. Earlier data also suggest this theory [55]. Moreover, the co-presence of granulomatous inflammation in a case of papillary–medullary malignancy might represent the hallmark of common pathways [56].

Early reports of CTT

Of historical note, we identified the first report regarding CTT on PubMed in 1977 (non-English paper) [57]. The first English paper concerning a single case with medullary and papillary neoplasia is dated 1989 [58]. The first case series (2 out of 3 cases) on PubMed with the same mix of neoplasia is published in 1994 [59]. The first CTT with collision metastasis published in a PubMed – indexed journal dates from 1996 [60]. The first

triple CTT (on PubMed) was reported on a 27-year-old female with papillary, follicular, and medullary carcinoma [61].

The literature-based analysis

Overall, we identified 62 cases on published data concerning CTT, thus we may say that this particular entity remains at the level of case reports. However, paying attention to this most unusual neoplasia might improve the prognostic and help early detection of at least one of the tumors. With one exception, all cases included at least one differentiated thyroid malignancy, either papillary or follicular (including 3 cases of Hürthle cell/oncocytic carcinoma). Five reports were consistent with the concept of collision metastasis concerning local lymph nodes (near the thyroid). 16/38 articles included medullary thyroid cancers. Based on these data, following differentiated (papillary being the most frequent) and medullary types, elements of squamous cell carcinoma originating from different organs were more frequent than anaplastic/poorly differentiated carcinoma. As mentioned, the most uncommon elements in CTT were: metastatic liposarcoma and osteosarcoma, respectively, and parathyroid carcinoma. All reported cases are adults (we did not identify any pediatric cases). The age varied between 26 and 88 years. More than half of the papers included female patients, but the single study we have so far reported an equal prevalence between males and women (Table 1).

Table 1: Published cases on collision tumors of the thyroid according to our methodology; the cases are displayed from the most recent starting with 2022 (please see references in the text according to first column indices)

First author, Number, Reference number	Studied population	DTC	ATC	MTC	Other types of carcinoma
Vijayvergiya G., 1, [29]	1 case (62-y, F)	FTC			mucoepidermoid carcinoma
Yeap ZH., 2, [30]	1 case (70-y, F)	Hürthle cell carcinoma		MTC	
Vlad M., 3, [44]	1 case (56-y, F)	micro-PTC	ATC		
Li H., 4, [35]	1 case (27-y, M)	PTC		sporadic MTC	
Toyoshima MTK., 5, [45]	1 case (63-y, F)	oncocytic carcinoma classical and hobnail PTC	poorly differentiated carcinoma		
Koufopoulos N., 6, [21]	1 case (63-y, F)	PTC		MTC	metastasis from osteosarcoma
Foppiani L., 7, [22]	1 case (67-y, M)	PTC			parathyroid carcinoma
Thomas A., 8, [41]	retrospective (N=21, F/M=1)	PTC		MTC	
Feng JW, 9, [16]	1 case (40-y, F)	PTC + FTC			
Sirbu AM., 10, [51]	1 case (46-y, F)	PTC		MTC	
Pishdad R., 11, [17]	1 case (79-y, M)	PTC + FTC			
Dikbas O., 12, [18]	1 case (35-y, F)	PTC		MTC	
Alhanafy AM., 13, [33]	1 case (73-y, F)	PTC			moderately differentiated squamous cell carcinoma
Sinno S., 14, [52]	1 case (69-y, F)	PTC + Hürthle cell carcinoma			
Fulciniti F., 15, [50]	1 case (#)	PTC			mucoepidermoid carcinoma
Gao Q., 16, [36]	1 case (59-y, F)	PTC			breast cancer ##
Ryan N., 17, [23]	1 case (88-y, F)	PTC			squamous cell carcinoma
Gurkan E., 18, [6]	1 case (44-y, M) 1 case (77-y, M)	PTC		MTC	
Plauche V., 19, [19]	1 case (62-y, F)	PTC + FTC			
Wang X., 20, [24]	1 case (55-y, M)	PTC			laryngeal squamous cell carcinoma
Kataria K., 21, [56]	1 case (35-y, M)	PTC		MTC	
Zeng H, 22, [37]	1 case (49-y, F)	PTC			breast cancer ##
Sadat Alavi M., 23, [34]	1 case (25-y, M)	PTC		MTC	

Kakarala K., 24, [38]	1 case (73-y, M)				squamous cell carcinoma low-grade B cell lymphoma ##
Warman M., 25, [25]	1 case (84-y, F)	PTC			squamous cell carcinoma
Gul K., 26, [53]	1 case (F, #)	PTC		MTC	
Jacobson AS., 27, [26]	1 case (#)	PTC			squamous cell carcinoma
Mattioli F., 28, [39]	1 case (#)	FTC			squamous cell carcinoma ##
Lim YC., 29, [40]	1 case (47-y, M)	PTC			squamous cell carcinoma##
Rekhi B., 30, [54]	1 case (59-y, M)	PTC		MTC	
Nabili V., 31, [20]	1 case (63-y, F)	PTC			metastasis from lung adenocarcinoma
Walvekar RR., 32, [27]	1 case (65-y, F)	PTC			squamous cell carcinoma
Rossi S., 33, [55]	1 case (61-y, F) 1 case (27-y, M) 1 case (34-y, M)	PTC		MTC	
Brandwein-Gensler M., 34, [28]	1 case (86-y, F)	PTC			metastatic liposarcoma
Wu CJ., 35, [61]	1 case (27-y, F)	PTC + FTC		MTC	
Pastolero GC., 36, [60]	1 case (#)	PTC		MTC	
Lax SF., 37, [59]	2 cases (#)	PTC		MTC	
Gero MJ., 38, [58]	1 case (#)	PTC		MTC	

We currently do not know if any data concerning the age or the sex of the subjects should be regarded as risk factors. In addition to the lack of poor survival indices (which are relevant in metastatic cases, those with late diagnostic, and anaplastic/ poorly differentiated tumors), the underlying mechanisms are controversial and we need further histological, molecular, and genetic studies.

CONCLUSIONS

The current limits of the CTT field are correlated with the small size of published data until the present time. We still need markers of poor prognostic. Awareness is essential for early detection with a consecutive potential improvement of the outcome.

Conflicts of interest and sources of funding
The authors declare no conflict of interest.

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Authors' contribution
Conceptualization, A.D., and M.C.; methodology, M.C.; software, A.P.C.; validation, A.D., E.P., and M.C.; formal analysis, D.T.; investigation, A.P.C., D.T.; resources, A.D., M.C.; data curation, E.P.; writing—original draft preparation, M.C.; writing—review and editing, A.D., A.P.C, D.T., M.C.; visualization, M.C.; supervision, A.D.; project administration, A.D., M.C.
All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Diagnosis of Chronic Kidney Disease in the Elderly – A Challenge for the Practitioner

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Abstract: *The recent increase in life expectancy is the main argument for a better understanding of the pathophysiological mechanisms underlying aging. These, once known, can provide possible links to therapies to prevent aging or slow down the process. Normal aging is associated with a progressive decrease in the glomerular filtration rate. Accurate estimation of GFR in the elderly is under the suspicion of multiple errors mainly due to sarcopenia and decreased protein intake. Differentiation between chronic kidney disease and the physiological decline of GFR might be a challenge in clinical practice and this has consequences on the evolution and treatment of the numerous comorbidities of the elderly. The current trend to use non-invasive diagnostic techniques explains the need to identify a serological marker to help differentiate between decreased GFR secondary to kidney aging or the development of chronic kidney disease.*

Keywords: *elderly, normal kidney aging, chronic kidney disease*

INTRODUCTION

Globally, there is a progressive aging of the population.

According to the United Nations Report, 1 in 11 people was over 65 years old in 2019 and it is estimated that in 2050, 1 in 6 people will be this age [1].

The aging process affects the whole body and is characterized by structural changes and functional decline in all organs, leading to an increased incidence of many diseases considered "specific to the elderly". Among these changes, the aging-associated alterations in both structure and function of the kidney are particularly important, having consequences on the evolution and treatment of the numerous comorbidities of the elderly.

Accurate quantification of kidney function in the elderly is difficult, both overestimating and underestimating the glomerular filtration rate (GFR) being possible with currently available serum creatinine-based calculation formulas.

AGING KIDNEY PROCESS

An accurate description of histological changes in the senile kidney, i.e. changes attributed to advanced age per se, is difficult because the elderly usually accumulate important comorbid burdens with impact on the kidney structure and function: diabetes, hypertension, heart failure, etc. Many of the terms that describe physiological changes in the aged kidney superimpose with terms that describe renal pathological processes: nephrosclerosis, tubulointerstitial sclerosis, glomerulosclerosis, arteriosclerosis, etc.

Macroscopically, starting from the 4th decade of age, the kidneys begin to decrease in size and weight progressively, so that around the 7th decade there is a reduction by about 20-30% of kidney weight compared to the age of 40 years [2]; in the elderly without kidney disease, the decrease in kidney size is symmetrical. Also, with age, the kidney's cortex becomes thinner and rougher, the number and size of benign cysts

increases, and the pelvis is enlarged with more fatty infiltration. The scarring is more advanced when associated with aortic and renal atherosclerosis (Figures 1 – 5) [3].

Microscopically, starting with the 5th decade of life, there is a progressive decrease in the total number of nephrons due to sclerosis of all nephron compartments [3]: glomerulosclerosis, tubulointerstitial sclerosis, and arteriosclerosis.

The prevalence of glomerulosclerosis increased from 25% in the 3rd decade of life to 82% in the 8th decade [4], the main cause being arteriosclerosis of the small intrarenal arteries.

Figure 1: 87 years old male: granular surface with increased scarring

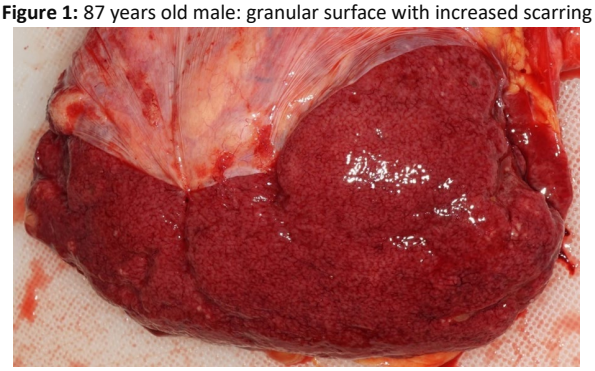


Figure 2: Same case, yellow arrows: small stones, thinning of the cortex

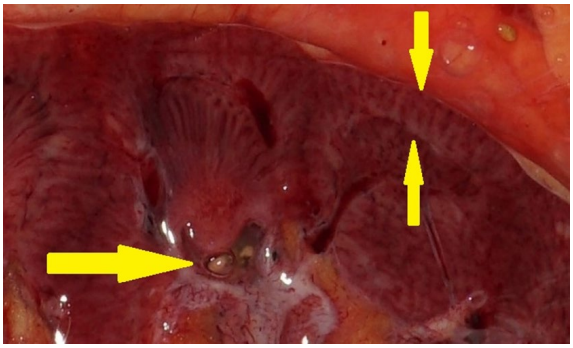


Figure 3: Same case, yellow arrows: glomerulosclerosis (10X HE)

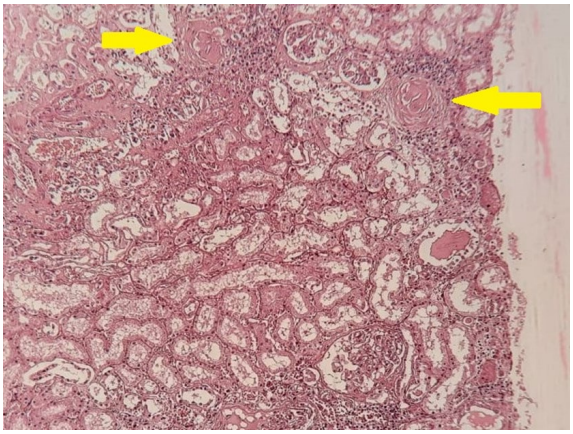


Figure 4: Same case, yellow arrows: arterial wall thickening (10X HE)

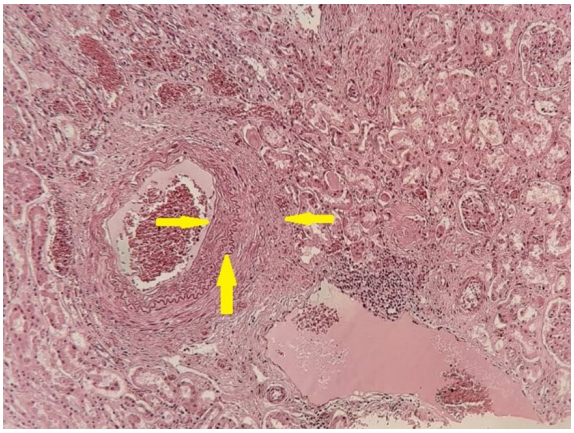
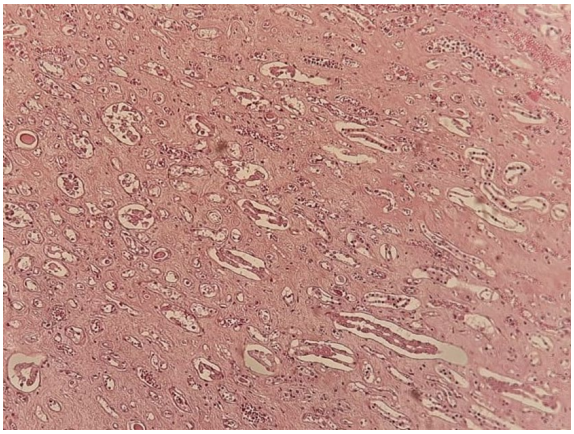


Figure 5: Same case: interstitial sclerosis (10X HE)



The hallmark of the functional decline of the senile kidney is a progressive decrease in the glomerular filtration rate (GFR) [5]. The decline in GFR begins around the 4th decade of life; between the 8th and 9th decades, the GFR is over 45% lower than at the age of 40 [3,6].

ESTIMATED GLOMERULAR FILTRATION RATE (eGFR) IN ELDERLY

There is no consensus regarding the optimal equation for the estimation of GFR in the elderly. Formulas used in clinical practice in adults – MDRD (Modification of Diet in Renal Disease, CKD-EPI (Chronic Kidney Disease – Epidemiology Collaboration), Cockroft-Gault are all based on serum creatinine levels and they have not been all validated for individuals older than 70 years.

Creatinine-based equations for GFR estimation have several drawbacks in older individuals [7]. Due to age-associated sarcopenia, there is a reduced release of creatinine from the muscles, therefore also a reduced urinary excretion of creatinine [8]. In elderly diabetics, especially in the late-elderly group (75 years or older), it has been noted a more decreased

serum creatinine when compared with non-diabetics and this observation was explained by the increased loss of muscle mass promoted by diabetes [9]. Also, the elderly tend to have a reduced intake of proteins and a decreased basal metabolism, therefore, they have supplementary causes for the reduced production of creatinine [10]. Increased metabolism of creatinine by intestinal microorganisms may be an additional cause for reduced levels of serum creatinine, as there are proven age-related alterations of gut microbiota [11]. Formulas using the patient's weight besides serum creatinine give more errors due to increased fat percentage in the organism of the elderly. Several researchers had concluded that both MDRD [12] and CKD-EPI [13] formulas tend to overestimate GFR in the elderly, while Cockroft Gault's [14] formula underestimates it [15,16].

Serum creatinine alone is often used in clinical practice not especially for estimation of GFR, but for monitoring the patient during treatment with a potential nephrotoxic agent (aminoglycosides for example) or a potential ischemic renal insult (cardiac surgery for example). Besides the fact that this conduct is not correct due to the late and modest increase of serum creatinine level compared to the moment and degree of kidney insult, in the elderly, it is a source of supplementary severe misjudgments. The elderly have often lower basal serum creatinine and during an injury renal process, an increase of serum creatinine remains in the range of the normal values of the laboratory and this result may be misinterpreted as a stable state, i.e. no iatrogenic. Moreover, the creatinine increase may be blunted by intensive hydration of the patient applied to minimize the kidney insult.

Formulas based on both serum creatinine and serum cystatin C have been proposed to overcome the disadvantages of formulas based only on creatinine values [17]. Nevertheless, serum cystatin C is influenced not only by kidney function, but also by several diseases often present in the elderly, such as atherosclerosis, inflammatory estates, or obesity [18,19].

Several alternative equations have been developed for older individuals. The HUGE formula (Hematocrit Urea GEnder) was developed in 2011 by a group of researchers from Spain to differentiate patients without CKD from those with CKD [20]; this formula was validated in some studies [21,22].

L = 2.505458 - (0.264418 x hematocrit) + (0.111800 x blood urea); 1.383960 is added for the male patient. For L <0, CKD is excluded; for L> 0, it is classified as CKD.

In 2012, the Berlin Initiative Study (BIS) introduced two new formulas based on serum creatinine and serum creatinine plus serum cystatin C, respectively, for estimating GFR in people over 70 years of age [23].

BIS 1: eGFR = 3736 x serum creatinine-0.87 x age-0.95; in women, the result is multiplied by 0.82.

BIS 2: eGFR = 767 x cystatin C-0.61 x serum creatinine-0.40 x age-0.57; in women, the result is multiplied by 0.87.

Although these formulas have been validated in some studies, there are no large trials to conclude their superior performance, therefore meanwhile guidelines recommend using the same equation as in the general adult population.

NORMAL KIDNEY AGING VERSUS CKD IN ELDERLY

Reported data on CKD prevalence in the elderly is impressive. A highly cited source in the literature is a meta-analysis performed by Hills and collab. which comprises more than 100 reports from all continents; according to this meta-analysis, the prevalence of CKD in all stages in the general population is 13.4% (11,7-15.1%), but increases to 27.6% (range 26.7-28.5%) in the 6th decade and 34.3% (31.9-36.7%) in the 7th decade of life. The same source also revealed increased CKD stage 3-5 in the elderly: from 10.6% in the general population (9.2-12.2%) to 11.3% (8.1-14.5%) in the 6th decade and respectively to 27.9% (16.4-39.3%) in the 7th decade [24]. Nevertheless, a large proportion of the geriatric population meets the KDIGO criteria for CKD even without markers of renal impairment [25,26]. Regardless of the formulas utilized to estimate GFR, an important question needs to get answered in the face of an older patient with abnormal GFR and no markers of kidney disease. Is it CKD without markers of kidney disease or is it normal kidney aging? Several reasons require a response to this question. Establishing a diagnosis of CKD imposes close monitoring of the patient and adequate preparation for conservative or dialysis treatment. Most of the patients aged >65 years have estimated GFR between 45-60 mL/min/1.73m^2; older patients with estimated GFR between 45-60 mL/min/1.73m^2 have, in comparison with younger adults, decreased risk to progress to end-stage renal disease (ESRD), but increased risk to die from other diseases until they reach ESRD [27,28]. Close nephrological monitoring of all these patients would be a significant burden for physicians, and also for the health systems. On the other hand, CKD, even oligosymptomatic, is associated from early stages with increased risk for cardiovascular events and cardiovascular mortality; specific complications of CKD, such as acidosis or increased parathormone (PTH) have early onset and need to be controlled.

There are several subtle clues allowing the differentiation between the physiologic decrease of GFR and CKD (Table 1).

Serum urea and creatinine increase late in the course of CKD in all patients, but especially in elderly patients. Low serum creatinine levels are observed due to decreased basal

metabolism, reduced protein intake, and sarcopenia [3], therefore CKD may be present with normal values of serum creatinine in the elderly. However, the aging kidney excretes increased amounts of urea, due to the reduced density of urea channels in the collecting tubes [29]; a normal serum urea level is associated with normal aging [15].

Table 1: CKD versus normal kidney aging

	CKD	***Aging kidney***
Nitrogen waste products in the blood	Increased	Normal
Albuminuria	Present	Absent
Anemia	Present (erythropoietin deficiency)	May be present (nutritional anemia)
Acid-base balance	Metabolic acidosis	Normal
Ca-P metabolism	Hypocalcemia decreased active vitamin D, increased PTH	Possible hypocalcemia, normal PTH, normal active vitamin D

No anemia should be present as in healthy older populations erythropoietin (EPO) production is normal or even increased secondary to increased erythrocyte turnover, increased EPO resistance, or as a response to occult digestive blood losses [30,31]. Nevertheless, in clinical practice, the elderly have often nutritional mild/moderate anemia that may be misinterpreted as CKD-associated anemia. Reduced secretion of EPO in CKD is associated with normocytic and normochromic anemia, while nutritional anemia is often hypochromic and microcytic [32], but they may coexist.

No abnormalities of mineral metabolism are present in healthy older individuals. Renal tubular excretion of calcium, magnesium, and phosphorus is not altered in healthy elderly [5,33]. The elderly are prone to hypomagnesemia, but this seems to be mainly due to reduced dietary intake, decreased intestinal absorption of magnesium, and/or laxative abuse [5]. Although the elderly present more often with mild hypocalcemia, it occurs mainly secondary to native vitamin D deficiency through reduced sun exposure or decreased skin synthesis of vitamin D [34]. In contrast with CKD, there is no deficiency of active vitamin D, nor an increased synthesis of parathormone in healthy elderly.

Under basal conditions, the kidneys of the elderly have a normal capacity for the excretion of acids and the acid-base balance does not undergo significant changes even with a normal- or hyper-protein diet [35]. However, the renal reserve of the elderly is reduced and a rapidly installed high acid load may be difficult to be compensated by the senile kidney; the elderly are highly vulnerable to the side effects of acute metabolic acidosis (uncontrolled diabetes, sepsis, acute kidney injury).

On the other hand, the presence of albuminuria is a strong argument for CKD even if the patient has other comorbidities that may explain this finding (systemic atherosclerosis, diabetes, cardiac failure). Albuminuria is associated with an increased risk of progression of CKD [36], therefore applying antiproteinuric measures might have the same benefits in the elderly as in younger adults, i.e., attenuating the progression of CKD and reducing cardiovascular events [37]. Nevertheless, overzealous administration of RAAS inhibitors in the elderly is associated with an increased risk of side effects (hypotension, hyperkaliemia, acute kidney injury) in the presence of reduced GFR and systemic atherosclerosis or other comorbidities; another reason why these drugs are no longer needed in elderly without proteinuria is the fact that they become useless, not having time to install their renoprotective effect over time [38,39]. The elderly are prone to hyperkaliemia even in the absence of reduced GFR due to the low sensitivity of distal tubules to aldosterone [31,40].

Taking into account that the elderly have a higher risk of death due to various complications than to progress to ESRD, there is a need to assess individual risk for these outcomes to analyze treatment options and make early plans for conservative treatment (diet, prevention, and control of uremic syndrome with medication) or renal replacement therapy. CKD has an extremely heterogeneous presentation in the elderly; between an independent full-functioning person without cognitive impairment and without major comorbidities at one pole to a frail, immobilized in-bed individual with severe cognitive dysfunction, multiple disabling comorbidities and total dependence for medical care at the other pole, there are numerous clinical pictures. One such equation was proposed by Tangri et al in 2011 who developed the Kidney Failure Risk Equation (KFRE) using variables like age, sex, estimated GFR, albuminuria, serum calcium, serum phosphate, serum bicarbonate, and serum albumin to predict progression toward ESRD after 1, 3 and 5 years respectively for patients with CKD stages 3 to 5 [41]; a variant using only 4 variables (age, sex, estimated GFR and albuminuria) may be used, but the results offer less accuracy [41]. This model has not been designed especially for older individuals, but it has been validated both for patients < 65 years and those above this age and for various races [42,43]. Calculation of this score is

available on the internet at http://www.qxmd.com/Kidney-Failure-Risk-Equation. KFRE is recommended by European Renal Best Practice Group to be used in elderly with an estimated GFR < 45 mL/min/1.73 m^2 to assess progress toward ESRD [44]. Nevertheless, a lot of old individuals are placed in the range of GFR between 45-60mL/min/1.73 m^2 and have no markers of kidney disease (albuminuria) [45]; there are no available validated prediction models for this situation and members of European Renal Best Practice Group opine for the urgent need of such prediction models.

Regarding the prediction of death, in the literature there are numerous scores specially designated for the old population and most of them also include, besides demographic variables (age, gender) and comorbidities (heart failure, diabetes, active neoplasia, anemia, history of stroke, chronic obstructive pulmonary disease, etc.), a marker of kidney dysfunction such as estimated GFR or urinary albumin/creatinine ratio [46-49]. These models estimate the risk of death at periods ranging from 3 months to 5 years. The European Good Practice Guideline recommends the use of the Bansal score in current practice for assessing the risk of mortality of non-fragile, un-institutionalized elderly people with CKD >stage 3b [44,47]; this score uses 9 variables - age, sex, GERD, urinary albumin/ creatinine ratio, smoking, diabetes, heart failure, history of stroke; according to this predictive model, the 5-year mortality varies between 3.8% for individuals with lower scores to 83.6% for individuals with all 9 items reached. Nevertheless, again, none of these models took into analysis the particular situation of individuals with normal kidney aging, i.e. those patients without markers of kidney damage and GFR between 45-60 mL/min/1.73m^2. We found in the literature just one publication, that of Van Pottelbergh and collab. who analyzed mortality of very old (>85 years) individuals with GFR> 60 mL/min versus those with GFR between 45-60 mL/min [50]. According to the authors of this paper, the combination of the baseline estimated GFR and the estimated GFR slope over 3 years can be used to assess the risk of death; in the subgroup of patients having estimated baseline GFR between 45-60 mL/min only those with a GFR decrease ≥ 5 mL/min/year had an increased risk of death, while among patients with baseline GFR > 60 mL/min, a decrease of ≥ 3mL/min/year was associated with increased risk of death. The same authors explained that these changes are a reflection of cardiovascular damage. Also, the authors share the opinion of a lot of others those older adults with GFR between 45-60 mL/min should not be classified as moderate stage 3 CKD.

CONCLUSIONS

Aging associates the functional decline of all tissues and organs secondary to degenerative structural changes. Normal kidney aging is a complex process that is difficult to be differentiated in clinical practice from chronic kidney disease with contemporary available criteria; both share the reduced glomerular filtration rate as the main feature. Nevertheless, it is important to make this differentiation.

Establishing a diagnosis of CKD imposes periodic nephrology consultation for monitoring the progression of the disease, treatment of complications, early planning of conservative or dialysis treatment, and also adjustment of treatment of multiple comorbidities of the elderly. CKD has multiple complications and requires often interdisciplinary care: cardiologists, neurologists, psychiatrists, nutritionists, etc. On the other hand, proving that reduced GFR is only a sign of normal aging and that there are no markers of kidney damage allows gerontologists to provide holistic care for slowing the aging process and minimizing the incidence of high-risk diseases in the elderly.

Thus, misclassifying an elderly person as a patient with CKD involves an unnecessary psychological burden for the patient and his family, as well as unnecessary medical care and increased financial expenses.

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The authors declare no conflict of interest.

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Authors' contribution
Conceptualization, D.R., C.D., I.A.V., G.E.L.; methodology, A.L.C., R.F.T.; software, M.O.D.L.; investigation, I.A.V., D.R., C.D.; writing—original draft preparation, A.L.C., F.P.; writing—review and editing, S.I., I.A.V.; supervision, A.L.C., D.I.
All authors have read and agreed to the published version of the manuscript.

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