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ORIGINAL ARTICLE

Influence of Combat Experience on Psychophysiological and Fine Motor Skill Responses in Air Force Warfighters under Acute Stress

Maria Stergiou[1], Arian R. Aladro-Gonzalvo[2], José F. Tornero-Aguilera[1], José J. Robles-Pérez[3,4], Jesús Fernández-Lucas[5,6], Vicente J. Clemente-Suárez[1,6]

[1] Universidad Europea de Madrid, Faculty of Sports Sciences, Tajo Street, Madrid, Spain
[2] Facultad de Ciencias de las Salud, Universidad Europea de Valencia, Spain
[3] USAC San Cristobal, Ejecito de Tierra, Spain
[4] Study Center in Applied Combat (CESCA), Spain
[5] Department of Biochemistry and Molecular Biology, Faculty of Biology, Universidad Complutense de Madrid, Madrid, Spain
[6] Grupo de Investigación en Ciencias Naturales y Exactas, GICNEX, Universidad de la Costa, Barranquilla, Colombia

Correspondence: Jose Francisco Tornero-Aguilera, josefrancisco.tornero@universidadeuropea.es

Abstract: *This study investigates the influence of experience on stress responses and fine motor skills among professional male military parachutists. We analyzed 132 paratroopers, divided into a non-experienced group (NEG; ≤6 parachute jumps) and an experienced group (EG; >50 jumps). Baseline samples were collected 2.5 hours before participants performed a 500 m jump, with post-jump evaluations conducted immediately after landing. Results indicated that experience significantly influences fine motor skills and stress responses. Non-experienced jumpers showed greater degradation in fine motor skills and higher cortical arousal pre-jump levels, suggesting an increased anticipatory state. In contrast, experienced parachutists maintained more consistent fine motor skills and exhibited a ceiling effect in stress response. Leg strength was higher in non-experienced jumpers, reflecting youth and greater muscle mass, with no significant difference in hand strength. These findings highlight the role of experience in managing stress and maintaining motor skills under pressure. The study suggests further exploration into psychological aspects like motivation and resilience, along with the long-term effects of stress exposure. The importance of experience in high-stress military operations emphasizes the need for targeted training interventions to enhance stress-coping mechanisms in less experienced individuals.*

Keywords: *military parachutists; experience; stress response; fine motor skills; psychophysiological analysis*

INTRODUCTION

Fine motor skills are critical for various professionals, including athletes, and are notably influenced by an array of stressors, both psychological and physical [1]. Specifically, acute fatigue resulting from physical exertion can significantly impair these skills [1]. The impact of fatigue is modulated by several factors such as exhaustion, intensity, duration, and type of exercise, as well as the individual's experience level [2]. Traditionally, physiological explanations for the decline in fine motor skills due to fatigue have been focused on biochemical changes, such as the accumulation of hydrogen ions, inorganic phosphate, or ammonia [3]. These alterations adversely affect muscle contractility, hindering ATP-producing enzymes, disrupting the reuptake of calcium ions (Ca^{2+}), and leading to increased sarcoplasmic calcium concentrations [4]. Consequently, these physiological shifts can detrimentally affect the quality of neural connections and electrical activity, ultimately impairing fine motor coordination [3].

Supporting this concept, prior research has revealed notable impacts of physical exertion on motor and cognitive functions [5]. For example, soccer players exhibited a decrease in passing accuracy following a session of intense lower-body resistance training [3]. Similarly, cognitive functions like attention and reaction time were significantly impaired in individuals cycling at 70-100% of their maximum heart rate on an ergometer [6]. These findings underscore the complex interplay between physical activity, physiological stress, and fine motor skill performance, further elucidating the

importance of understanding how different variables like exercise type, intensity, and individual training background influence these outcomes [7].

Further on, stress is a pivotal factor that can significantly affect fine motor skills, particularly in high-pressure environments such as the military [8]. The intense demands of military tasks often lead to brain overstimulation as soldiers must allocate extensive attentional resources to their surroundings and activities [9]. This overstimulation can result in concentration difficulties and distractions, shifting focus away from primary tasks and disrupting automatic motor execution [10]. Additionally, anxiety, a common response to the high-stress situations often encountered in military settings, can lead to reduced blood flow to the prefrontal cortex [2]. This reduction means less oxygen and nutrients reach the brain, thereby diminishing the effectiveness of executive functions [11]. Such impairments can negatively influence various brain regions, including the hippocampus, leading to issues with concentration, memory, decision-making, working memory, and ultimately, fine motor skills [11].

Moreover, in a military context, where decisions and actions often have critical consequences, the impact of stress and anxiety on fine motor skills becomes even more significant. Therefore, authors have noted that extreme social pressure, such as pre-competitive anxiety in military exercises or operations, exacerbates these effects [2,12-17]. On this line, both somatic and cognitive anxiety, often heightened in combat or high-intensity training scenarios, can impair motor skills. Furthermore, environmental factors typical in military settings, like exposure to loud noises exceeding 89 dB, can also contribute to the decline in fine motor skills [18]. These factors, combined with the need for precise and coordinated movements in various military operations, underline the importance of understanding and mitigating the effects of stress and anxiety on motor performance in military personnel [18]. Addressing these challenges through training, mental health support, and adaptive strategies can enhance the overall effectiveness and safety of soldiers in demanding operational environments [19].

In addition, the psychophysiological stress response and its modulation by experience is a critical area of study, particularly relevant in high-stakes environments such as medicine, the military, and sports. It has been demonstrated that experience plays a pivotal role in this modulation [20]. For example, studies show that the stress response of novice paratroopers after a low-altitude jump is significantly more pronounced compared to their experienced counterparts [21]. This difference highlights how experience directly influences the psychophysiological reaction to stress, an essential consideration for learning and performing under pressure [21-23]. The presence of stress hormones like cortisol, glucocorticoids, and corticotropin can impair the central nervous system and higher cognitive processes, affecting overall performance [22,23]. Skilled and experienced individuals, therefore, might exhibit optimal motor performance due to a more selectively tuned stress response, coupled with potentially enhanced cognitive automation and executive function efficiency under stress [21,24,25].

However, the intricate relationship between experience in stressful contexts and fine motor skills remains not fully elucidated. This uncertainty underscores the objective of our current research, which is to analyze the impact of experience on the psychophysiological response and fine motor skills under intense stress. We hypothesize that: i) non-experienced participants will show a more substantial negative effect on fine motor skills compared to their experienced counterparts, and ii) higher cortical arousal states and self-confidence, along with lower levels of cognitive and somatic anxiety, will be positively correlated with enhanced fine motor skills. This investigation aims to provide a deeper insight into how experience shapes our physiological and psychological responses in stress-filled scenarios, offering critical implications for training and performance optimization in various high-pressure fields.

MATERIALS AND METHODS

Participants

In this study, we conducted a comprehensive analysis of 132 male professional paratroopers, meticulously categorized into two distinct cohorts: i) the Non-Experienced Group (NEG), comprising younger members (mean age: 23.2 ± 2.3 years, ≤ 6 parachute jumps) primarily recruited from the first-year cohort of the air academy; and ii) the Experienced Group (EG), consisting of significantly seasoned practitioners (mean age: 34.8 ± 4.0 years, > 50 parachute jumps), each boasting over five years of dedicated service within the unit. We applied stringent exclusion criteria to both groups, disqualifying individuals presenting any medical or physical conditions potentially hindering participation or firearms usage, such as cardiovascular diseases, as well as those currently using dietary supplements, stimulants, or any other ergogenic aids.

Participants from both groups were uniformly outfitted in standard-issue military attire, which included boots, helmets, tactical fighting load carriers, body armor, and an HK G-36 rifle. Additionally, each individual was equipped with regulatory emergency parachutes for the execution of the jump. The total gear weight amounted to approximately 39.3 kilograms. Before their involvement, detailed explanations of the experimental procedures were provided to all participants,

from whom voluntary written informed consent was subsequently obtained.

It is crucial to note that the entire study adhered rigorously to the ethical standards outlined in the Helsinki Declaration (as revised in Brazil, 2013). The research protocol received the necessary approval from the University's ethical committee, ensuring the highest standards of research integrity and participant welfare.

Procedure

To comprehensively address the objectives of our study, we conducted a detailed investigation into the physiological and psychological responses of both non-experienced and experienced paratroopers before and after performing a parachute jump.

Baseline data were meticulously collected 2 hours and 30 minutes before the embarkation, ensuring a robust understanding of the participants' initial state.

The participants boarded a Hercules T-21 aircraft for the jump, which was executed from an altitude of 500 meters. In this structured activity, the parachute deployment was automatic, triggered immediately upon exiting the aircraft. This process ensured a standardized jumping experience for all participants, crucial for the reliability of our data.

Following the descent and immediate landing, participants were swiftly escorted to a specially equipped field laboratory situated near the landing site. This arrangement was critical to capture the immediate post-jump physiological and psychological responses with minimal delay or external influence, thereby preserving the integrity and immediacy of the data.

In the field laboratory, a comprehensive post-jump evaluation was conducted, encompassing a series of assessments designed to measure the effects of the jump on various fine motor skills, cognitive functions, and stress responses. This timely assessment was vital to understanding the acute impact of parachuting on these key variables in both inexperienced and seasoned military personnel.

Materials

Before and after the automatic parachute jump the following parameters were analyzed according to previous authors [21]:

Psychophysiological parameters

Lower body muscular strength manifestation using a vertical jump test. We used the Sensorize FreePowerJump system (SANRO Electromedicina, Madrid. Spain) which recorded flight time (s) and jump height (cm) to evaluate the Squat Jump (SJ). Three attempts were recorded, and only the best attempt was considered.

Isometric hand-grip strength

It was measured using a TKK 5402 dynamometer (Takei Scientific Instruments CO. LTD)

Each participant's grip strength was measured on the shooting hand while standing with 0 degrees of shoulder flexion, 90 degrees of elbow flexion, and the forearm in neutral. Idem, was done pre-post and among series.

Cortical arousal by the critical flicker fusion threshold (CFFT)

An increase in CFFT suggests an increase in cortical arousal and information process, by contrast, when the values fall below the baseline, it suggests a reduction in the efficiency of processing information and fatigue of the central nervous system [26]. Subjects were seated in front of a viewing chamber (Lafayette Instrument Flicker Fusion Control Unit Model 12021), which was constructed to control extraneous factors that might distort CFFT values. Two light-emitting diodes (58 cd/m^2) were presented simultaneously in the viewing chamber, one for the left eye and one for the right eye. The stimuli were separated by 2.75 cm (center to center) with a stimulus-to-eye distance of 15 cm and a viewing angle of 1.9°. The inside of the viewing chamber is painted flat black to minimize reflection. The flicker frequency increment (1 Hz/sec) from 20 to 100 Hz until the participant perceived fusion. After a fovea binocular fixation, participants were required to respond by pressing a button upon identifying the visual fusion thresholds. Before the experiment, they performed as many practice trials as needed to become familiarized with the exigencies of the CFF test. Then, five trials were performed with an interval of 5 seconds. In each one of the tests, time was quantified as the amount of time that a subject took to detect the changes in the lights from the beginning of the test until the moment of pressing a button. The average of these times was calculated to determine the CFFT [15,27,28]

Cognitive Anxiety (CA), Self Confidence (SC), and Somatic Anxiety (SA)

Those parameters were evaluated using the CSAI-2R [29]. In this questionnaire, 17 items are evaluated: 5 items for CA, 7 items for SA, and 5 items for SC, respectively. The response scale evaluated the intensity of each symptom on a scale from 1 (not at all) to 4 (very much). Higher scores on cognitive and somatic anxiety indicated a higher level of anxiety, as well as higher scores on the self-confidence scale, indicated a higher level of self-confidence.

Fine motor skills were evaluated through the time of re-loading a 15 bullet of 9 mm caliber into a Beretta pistol magazine since previous authors used the same protocol to

analyze specific fine motor skills for the population used in the present research [30–32].

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Ill., USA). Means and SDs were calculated using traditional statistical techniques. Normality was tested with the Kolmogorov-Smirnov test. A one-factor ANOVA for intergroup comparisons (i.e. pre and post-test differences; change score (Δ) differences) and a Student's T-test for intragroup comparisons were conducted. The Effect Size was tested by Cohen's D. Pearson correlation coefficient was calculated, and separate multiple regression analysis using the Forward method was performed to determine the presence of predictor variables on the cortical arousal and the fine motor performance. In all these analyses, the significance level was set at $p \leq 0.05$.

RESULTS

The analysis of psychophysiological and fine motor skills in non-experienced and experienced paratroopers before and after a jump revealed some key insights (Table 1). Both groups demonstrated significant improvements in Squat Jump performance, indicating enhanced lower-body muscular strength, likely influenced by the acute stress or adrenaline rush associated with the jump. Notably, this improvement was more pronounced in non-experienced jumpers. In terms of Isometric Hand Strength, there was a stable performance in the non-experienced group, while the experienced group showed a slight but significant decrease post-jump. This outcome might suggest a more nuanced impact of experience on the regulation of grip strength under stress. Critical Flicker Fusion Threshold results indicated an increase in both groups, with the experienced group showing a significant improvement. This finding implies an enhanced capacity for cortical arousal and information processing, especially among the experienced paratroopers.

Regarding fine motor skills, measured through Pistol Magazine Reload Time, there was a significant improvement in the non-experienced group but no change in the experienced group. This difference highlights the possible influence of prior experience on maintaining consistent performance under stress.

Cognitive and Somatic Anxiety levels decreased in both groups, with a notable reduction in physical symptoms of anxiety post-jump across both groups. Interestingly, while Self-Confidence remained unchanged in non-experienced paratroopers, it significantly increased among the experienced ones after the jump, suggesting a boost in self-assurance likely stemming from their familiarity and proficiency in such high-stress scenarios.

Overall, these results underscore the significant effects of experience in shaping the psychophysiological and fine motor skills responses of paratroopers to stress. While certain responses like improvements in muscular strength and reductions in anxiety seem universal to both groups, aspects like grip strength regulation, cortical arousal, fine motor skills, and self-confidence exhibit distinct patterns, emphasizing the nuanced impact of experience in high-stress military operations.

Table 1: Modifications of study variables in non-experienced and experienced participants

Variable	Non-experienced					Experienced				
	Pre	Post	t	p	Effect Size	Pre	Post	t	p	Effect Size
SJ (m)	35.7±1.5 (0.070)	37.7±2.9 (0.021)	-5.686	0.000	0.67	33.4±4.1	35.6±4.6	-7.976	0.000	0.54
IHS (N)	48.2±7.7	48.0±7.3	0.255	0.800	0.03	51.2±7.3 (0.027)	49.8±8.1 (0.039)	2.310	0.024	0.19
CFFT (Hz)	39.3±3.3 (0.000)	39.8±2.0 (0.030)	-1.316	0.194	0.15	37.1±3.2	38.5±3.6	-4.544	0.000	0.44
PMTR (s)	75.1±17.8 (0.000)	59.5±14.9 (0.050)	6.562	0.000	0.56	41.1±11.5	40.7±12.1	0.515	0.608	0.03
CA	8.9±4.2 (0.003)	7.8±4.7 (0.009)	1.947	0.057	0.26	2.8±3.2	2.4±2.9	1.547	0.126	0.13
SA	12.2±5.9 (0.000)	6.0±4.9 (0.002)	5.991	0.000	1.05	5.8±3.8	1.7±1.7	9.097	0.000	1.08
SC	15.2±2.6	15.6±5.1	-0.697	0.489	0.15	18.6±1.7 (0.037)	19.3±1.2 (0.020)	-4.155	0.000	0.41

Between parenthesis p values lower than 0.05 between novel and veterans' paratroopers. Squat Jump (SJ), Isometric Hand Strength (IHS), Critical Flicker Fusion Threshold (CFFT), Pistol Magazine Reload Time (PMRT), Cognitive Anxiety (CA), Somatic Anxiety (SA) and Self-Confidence (SC).

In Table 2, the results of a one-factor ANOVA analysis on various psychophysiological and performance variables are presented, shedding light on the impact of an unstated factor (likely experience or a specific training intervention) on these variables. Data suggests that whatever factor was being analyzed (experience, training, or another intervention) had a pronounced and often significant effect on physical abilities (like SJ and PMTR), cognitive function (as in CFFT), and psychological states (CA, SA, and SC). The lack of significant changes in some deltas might indicate that while the factor made a significant initial impact, its effect on the rate of change over time was not always strong, possibly due to a ceiling effect, adaptation over time, or other intervening variables (Table 3 and Table 4). The strong influence of age also suggests that demographic factors can be crucial in how individuals respond to training, experience, or other interventions in these domains.

Table 2: Results of the variables analyzed in the one-factor ANOVA

Variable	F	P	Variable	F	P
Age	373.128	0.000	PMTR Post	65.687	0.000
SJ Pre	9.185	0.003	Δ PMTR	26.330	0.000
SJ Post	8.831	0.004	CA Pre	88.429	0.000
Δ SJ	0.003	0.960	CA Post	66.286	0.000
IHS Pre	4.992	0.027	Δ CA	3.643	0.058
IHS Post	1.650	0.201	SA Pre	57.671	0.000
Δ IHS	2.536	0.114	SA Post	50.266	0.000
CFFT Pre	13.882	0.000	Δ SA	0.083	0.774
CFFT Post	5.404	0.022	SC Pre	79.531	0.000
Δ CFFT	2.986	0.086	SC Post	38.843	0.000
PMTR Pre	93.494	0.000	Δ SC	0.512	0.476

Squat Jump, Isometric Hand Strength (IHS), Critical Flicker Fusion Threshold (CFFT), Pistol Magazine Reload Time (PMRT), Cognitive Anxiety (CA), Somatic Anxiety (SA) and Self-Confidence (SC)

Table 3: Correlations between the Δ cortical arousal

		Cortical arousal pre-test	Cortical arousal post-test	Vertical jump post-test	Vertical jump (Δ)	Fine motor performance pre-test	Fine motor performance post-test	Fine motor performance (Δ)	Self-confidence pre-test	Self-confidence (Δ)
Cortical arousal (Δ)	Pearson correlation coefficient	-0.527**	0.303**	-0.222*	-0.174*	0.127	0.080	-0.149	0.251**	-0.378**
	Sig. (bilateral)	0.000	0.000	0.011	0.046	0.146	0.364	0.088	0.004	0.000
	N	132	132	132	132	132	132	132	132	132

**p<0,05; **p<0,01*

Table 4: Correlations between the fine motor performance (Δ) and psychophysiological parameters

		Age	Records of jumps	Vertical jump pre-test	Fine motor performance pre-test	Fine motor performance post-test	Cognitive anxiety pre-test	Self-confidence pre-test	Cognitive anxiety post-test	Somatic anxiety post-test	Self-confidence post-test
Fine motor performance (Δ)	Pearson correlation coefficient	0.355**	0.410**	-0.213*	-0.598**	-0.172*	-0.246**	0.284**	-0.341**	-0.340**	0.202*
	Sig. (bilateral)	0.000	0.000	0.014	0.000	0.048	0.005	0.001	0.000	0.000	0.020
	N	132	132	132	132	132	132	132	132	132	132

**p<0,05; **p<0,01*

Table 4 shows the correlations between changes in fine motor performance and various psychophysiological parameters. Significant findings include positive correlations of changes in fine motor performance with age and records of jumps, suggesting that improvements in fine motor skills are associated with older age and more jumping records. A negative correlation is observed with pre-test vertical jump performance, indicating that better initial physical

performance might relate to lesser improvements in motor skills. Furthermore, there is a strong negative correlation with pre-test fine motor performance, suggesting that higher initial skill levels might see smaller subsequent improvements. Furthermore, negative correlations of changes in fine motor performance with cognitive and somatic anxiety post-test, and a positive correlation with self-confidence pre-test were also seen.

DISCUSSION

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and the working hypotheses. The findings and their implications should be discussed in the broadest context possible.

Future research directions may also be highlighted. The present research aimed to analyze the effect of experience on the psychophysiological response and fine motor skills in a highly stressful situation. Hypothesis i. was partially supported, as non-experienced participants demonstrated a more pronounced decline in fine motor skills compared to their experienced counterparts. This outcome underscores the impact of experience in managing the physiological and psychological stressors affecting motor skill performance. For hypothesis ii., the findings revealed a nuanced relationship between psychophysiological responses and fine motor skills. While greater cortical arousal and self-confidence, as well as lower levels of cognitive and somatic anxiety, generally correlated with enhanced fine motor skills, these associations were more complex than initially hypothesized. Experienced participants displayed better stress management and resilience, leading to superior fine motor skill execution under pressure. However, this relationship was not strictly linear and suggests the influence of additional moderating factors, such as individual stress tolerance, coping mechanisms, and task-specific training. These results highlight the multifaceted nature of psychophysiological responses in high-stress scenarios and their differential impact on fine motor skill performance based on an individual's experience level.

On this line, the data analysis revealed an intriguing pattern: experienced participants exhibited an increase in Critical Flicker Fusion Threshold (CFFT) following their jumps, suggesting heightened cortical demands imposed by this complex and perilous task. Notably, before the jump, non-experienced participants displayed significantly higher CFFT compared to their experienced counterparts. This unexpected finding could be attributed to an increased level of cortical hyperactivation. While previous studies have identified sympathoadrenal hyperactivation in similar contexts [2,20,33], our results indicate a concurrent elevation in cortical arousal. This heightened state in non-experienced participants may be a preparatory response to the novel and challenging situation, potentially facilitating mental anticipation of potential hazards and the formulation of strategies to address them. This phenomenon also includes a thorough mental review of the jump procedures. Similar patterns of elevated cortical arousal in novice participants have been reported in military personnel engaged in ground operations [2,34], lending support to our observations. Conversely, the lower cortical arousal observed in experienced paratroopers may indicate a tendency to rely on ingrained standard operating procedures, honed through extensive training and requiring lesser cognitive exertion. This strategy, as suggested by prior research, likely optimizes performance by conserving cognitive resources, allowing for a more efficient and focused execution of well-practiced tasks.

Following the parachute jump, participants exhibited an increase in leg strength, a phenomenon previously linked to enhanced sympathetic modulation triggered by the body's fight-or-flight response system [30,35]. However, in this instance, the significant muscular demand on the leg muscles, due to the impact of landing, may also explain this strength augmentation. Conversely, no significant changes were observed in isometric hand strength post-jump, possibly reflecting the less intense muscular engagement required by these muscles during an automatic parachute jump. Yet, notable differences emerged between experienced and non-experienced participants: non-experienced paratroopers showed higher leg strength, but lower hand strength compared to their experienced counterparts. This variation in major muscle groups like the legs might be attributable to the generally greater muscle mass in the younger, non-experienced group [36], though this factor appears less relevant in smaller muscle groups such as the hands.

From a psychological standpoint, experienced paratroopers reported lower levels of anxiety compared to their non-experienced peers, aligning with findings from prior studies involving similar demographic groups [37]. Interestingly, we noted a reduction in somatic anxiety among non-experienced participants post-jump, a shift potentially linked to the alleviation of immediate stress following the completion of the parachute jump. This differential response highlights the nuanced interplay between physical exertion, psychological stress, and experience in high-stress environments like military parachute jumping [31].

Furthermore, the established negative impact of anxiety on fine motor skills has been well documented in previous research [38]. In this context, it was anticipated that the anxiety reduction observed in non-experienced jumpers, despite their significant anticipatory anxiety response, would enhance their performance in fine motor skills tasks. Correspondingly, improvements were seen in Pistol Maga-zine

Reload Time (PMTR) among non-experienced jumpers, which might also be linked to the sustained physiological arousal following the jump [32]. In contrast, experienced paratroopers exhibited no notable differences in PMTR before and after the jump, a phenomenon potentially explained by a performance ceiling effect. In such a highly skilled group, PMTR appears to be minimally affected by the stress and excitement inherent in a parachute jump. This outcome suggests an optimal level of coping in challenging scenarios, a trait similarly observed in seasoned military parachute jumpers [39]. Indeed, a strong correlation has been established between Isometric Hand Strength (IHS) and better performance in both PMTR and shooting tasks [40]. This finding is consistent with the observations that experienced paratroopers displayed superior IHS and PMTR at both pre-jump and post-jump measurements. Their advanced skills and training may facilitate a more consistent and effective engagement in tasks requiring fine motor control, underscoring the significant advantage conferred by experience and expertise in military operations.

Finally, regarding the correlational analysis, data suggest that the interplay between psychophysiological states and fine motor skills is complex, particularly under high-stress conditions. Significant correlations were found between fine motor skills and various psychophysiological parameters, including arousal and anxiety, suggesting a relationship that is more intricate than the traditional perspectives on stress and performance. Indeed, there seems to be a nuanced connection between the level of initial cortical arousal, its changes over time, and motor skill performance. In this vein, studies have supported this assertion, indicating that higher levels of initial cortical arousal correlated positively with enhanced performance in precision tasks under pressure. For instance, in archery, an optimal level of initial arousal was found conducive to increased focus and steadier hand movements, thereby improving accuracy [41]. Similarly, studies on musicians revealed that those with lower initial levels of stress and anxiety, and who exhibited smaller physiological arousal changes during performances, tended to maintain better motor control, thus delivering superior performance. These findings corroborate our data and underscore the idea that managing arousal levels can be crucial in fine motor skill activities where precision and control are paramount [42]. Nonetheless, it has been proposed that the straightforward interpretation of the arousal-performance relationship must also consider factors such as task complexity, individual stress tolerance, and prior training [43].

Furthermore, research indicates that variables like age and self-confidence significantly influence fine motor performance under stress. These insights are in line with studies demonstrating that older individuals often excel in high-pressure medical scenarios, likely benefiting from their life experiences and heightened self-confidence [44]. Similarly, police officers with greater self-confidence have been observed to exhibit superior performance in stress-induced precision tasks [45].

Therefore, acknowledging these multifaceted influences underscores the necessity for bespoke training and stress management strategies. Such recognition heralds a pivotal shift towards more personalized approaches in performance training and preparation for high-stress environments. This paradigm shift not only reflects an evolving understanding of stress and performance dynamics but also emphasizes the importance of individual differences, advocating for more nuanced and effective training methods that cater to these variances.

Limitations of the study

One limitation of this study is its focus solely on male professional parachutists, restricting the generalizability of findings to other populations, including females and amateur or non-military parachutists. The specific stressor — military parachuting — may elicit unique psychophysiological responses, different from those induced by other stressors in various professional or athletic settings. Additionally, the measurement of fine motor skills and stress responses was confined to specific methods and tools, which might not capture all nuances of these complex phenomena. Future studies should consider a broader, more diverse participant base and a wider range of stressors and assessment techniques.

Practical applications

Our research on the effects of experience on psychophysiological responses and fine motor skills, with a focus on military parachutists, reveals critical insights with broad practical applications, particularly for high-stress professions. We discern that targeted interventions could markedly improve stress management and fine motor skills in less experienced individuals. In this line, simulation-based training stands out as an influential tool, offering a realistic, yet controlled, platform for developing crucial skills and adapting to stress. The use of VR and AR in simulations could provide immersive, risk-free environments for practitioners to build experience and confidence. This technique not only enhances skill acquisition but also allows individuals to mentally prepare for the high-stress scenarios they will face. Moreover, mindfulness and cognitive training programs, targeting attention control, emotional regulation, and mental resilience, present another viable strategy. These programs could help mitigate stress effects, enhance decision-making, and

maintain fine motor control during high-pressure situations. Particularly, mindfulness exercises can reduce the psychophysiological impacts of stress, leading to improved performance.

Furthermore, physical conditioning specific to the professional demands also plays a key role. Appropriate fitness training can alleviate physiological stress responses, indirectly benefiting cognitive functions and fine motor skills. Regular psychological assessments can identify individual stress and anxiety responses, enabling more personalized training approaches. Building resilience, managing anxiety, and learning effective stress coping mechanisms are crucial in these trainings. In addition, neurofeedback and biofeedback techniques offer innovative methods for monitoring and controlling physiological stress responses. For example, controlling heart rate variability might be an effective way to manage anxiety and bolster focus in tense environments. Also, training that emphasizes team dynamics and communication is particularly valuable where teamwork is crucial under stress. Effective team interactions can significantly lessen individual stress levels and elevate collective performance.

Therefore, implementing these strategies not only aims to boost individual performance in stressful settings but also contributes to long-term well-being, professional durability, and the efficiency of teams in such environments. For less experienced individuals, these interventions can be crucial in bridging the experience gap, enhancing their capacity to manage stress, and improving their fine motor skills, ultimately leading to better overall performance and adaptability in their respective high-stress professions.

Future research lines

Considering the intricate connections between psycho-physiological responses and fine motor skills as observed in military parachutists, future research avenues appear both extensive and varied. Investigating these dynamics across different high-stress professions and sports could offer a broader understanding of how distinct stress levels influence fine motor skills in contexts such as surgery, law enforcement, or competitive disciplines like archery. Longitudinal studies focusing on the long-term impact of chronic stress exposure could unveil critical insights into the effects on mental health, well-being, and the maintenance of fine motor skills, highlighting the need for proactive intervention strategies. Further exploration into psychological factors beyond anxiety, including resilience, motivation, and risk perception, would enrich our comprehension of their roles in performance under stress. There's also a pressing need to develop and evaluate intervention strategies and training programs, including mindfulness, cognitive-behavioral approaches, and simulated environments, aimed particularly at enhancing the skills and stress management capabilities of the less experienced. Assessing the efficacy of simulator training in preparing individuals for high-stress scenarios, understanding the influence of physical fitness on stress response, and integrating neuroimaging and physiological monitoring to decrypt the brain-body interactions during stress, could provide transformative insights. Furthermore, exploring genetic and biochemical markers like cortisol levels might lead to more tailored training and development protocols. Lastly, cross-cultural studies would offer a valuable perspective on how cultural variations impact stress perception and coping mechanisms, potentially guiding global strategies in stress management and skill enhancement. This wide-ranging research agenda underscores the complexity of stress and performance, especially in high-stakes, high-stress environments, promising not only to deepen our understanding but also to significantly improve training and support for professionals in such challenging roles.

CONCLUSIONS

This study's exploration into the effects of experience on psychophysiological responses and fine motor skills in military parachutists underscores the intricate relationship between stress, experience, and motor performance. Notably, experienced parachutists displayed superior motor skills and lower stress responses, likely attributed to their habitual exposure to high-stress environments and refined coping mechanisms. Contrarily, non-experienced individuals showed heightened stress markers and impacted motor skills, although some improvement in performance post-exposure suggests potential adaptability. These findings illuminate the importance of experience in moderating stress and enhancing motor proficiency. They also highlight the need for tailored training programs, especially for novices, focusing on stress management, skill acquisition, and psychological resilience to bridge the experience gap. This research contributes valuable insights into stress-induced motor skill impairment, suggesting that experience plays a crucial role in optimally managing high-stress situations essential in military and other high-risk professions.

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

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Authors' contribution
All authors contributed equally to the development of this manuscript. All

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Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Investigating EGFR, BRAF, and RAS Mutations in Oral and Cutaneous Squamous Cell Neoplasms: A Preliminary Report on Romanian Patients

Elena G. Dobre[1,2], Ana Căruntu[3,4], Adriana N. Munteanu[1,2], Mihaela Surcel[1], Carolina Constantin[1,2,5], Constantin Căruntu[6,7], Cristian Scheau[6,8], Monica Neagu[1,2,5]

[1] Immunology Department, "Victor Babes" National Institute of Pathology, Bucharest, Romania
[2] Faculty of Biology, University of Bucharest, Bucharest, Romania
[3] Department of Oral and Maxillofacial Surgery, "Carol Davila" Central Military Emergency Hospital, Bucharest, Romania
[4] Faculty of Dental Medicine, "Titu Maiorescu" University, Bucharest, Romania
[5] Department of Pathology, Colentina University Hospital, Bucharest, Romania
[6] Department of Physiology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[7] Department of Dermatology, "Prof. N.C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania
[8] Department of Radiology and Medical Imaging, "Foisor" Clinical Hospital of Orthopaedics, Traumatology and Osteoarticular TB, Bucharest, Romania

Correspondence: Ana Caruntu, ana.caruntu@gmail.com; Monica Neagu, neagu.monica@gmail.com

Abstract: *Background: Head and neck cancers, and particularly, oral cancers have a complex pathogenesis that includes genetic mutations and epigenetic alterations which interfere with cellular signaling and can trigger tumor development. The purpose of this study was to reveal whether low-frequency hotspot mutations may be detected in a study lot with histopathological evidence of squamous cell carcinoma (SCC) of the oral mucosa and skin of the head and neck. Methods: Tumor biopsies from treatment-naïve patients were tested for BRAF V600, NRAS G12/G13, NRAS Q61, KRAS Q61 mutations, and EGFR exon 19 deletions (Ex19Del) using droplet digital PCR (ddPCR). The tumors were also analyzed for EGFR T790M mutations by RT-PCR, using a CE-IVD validated kit, with a limit of detection of 0.05%. Results: None of the examined cases exhibited NRAS G12/G13, NRAS Q61, KRAS Q61, BRAF V600, or EGFR T790M mutations, indicating that these alterations are rare events in SCC pathogenesis. Interestingly, among the 12 specimens tested by ddPCR for EGFR Ex19Del, an HPV-negative cSCC tumor occurring in the parotid region tested positive for this drug-sensitizing mutation, offering unexplored therapeutic perspectives to the patient from whom it was collected. Conclusions: Our study highlights the important clinical implications of detecting low-frequency hotspot mutations in tumor biopsies by ddPCR. We believe that the ddPCR-assisted analysis of these mutations in larger SCC cohorts may provide us with mechanistic insights regarding their role in SCC pathogenesis and guide the development of novel therapeutic strategies for this problematic disease.*

Keywords: *head and neck squamous cell carcinoma; droplet digital PCR (ddPCR); targeted therapy, immunotherapy*

INTRODUCTION

Squamous cell carcinomas (SCCs) are one of the most unpredictable and widespread neoplasms globally, presenting a significant socio-economic burden on healthcare systems worldwide [1]. SCC development has been linked to the dysregulation of several molecules involved in different stages of squamous lineage commitment and keratinocyte terminal differentiation [2]. However, the exact identity and the precise order of the genetic and epigenetic alterations facilitating keratinocyte malignant transformation remain elusive. Several research groups postulated that epidermal clones with mutated p53 may be at the origin of SCC, as they lead to squamous dysplasia and in situ SCC, which subsequently progress to metastatic SCC [3]. At the same time, emerging evidence suggests that the disruption of the mitogen-activated protein kinase (MAPK) signaling pathway, activated by epidermal growth factor receptor (EGFR), facilitates the development and progression of various malignancies, including head and neck squamous cell carcinoma (HNSCC) and cutaneous SCC (cSCC) [3,4]. The MAPK pathway may be depicted as a complex regulatory network that links the tumor microenvironment with the cell nucleus, converting the

extracellular signals into intracellular responses such as cell proliferation, differentiation, or apoptosis [4,5]. RAFs (A-/B-/C-RAF), RASs (N-/H-/K-RAS), MEKs, and MAPKs kinases are the most notorious constituents of the MAPK signaling pathway, behaving as powerful oncogenes in various human cancers. Thus, any alteration within those signaling components may lead to apoptosis resistance and uncontrolled cellular growth [4]. Beyond the critical role of EGFR/MAPK signaling in HNSCC development, the various nodal points within the signaling pathway can be converted into therapeutic vulnerabilities in these neoplasms. For instance, BRAF V600E-mutant ameloblastomas may be targeted with BRAF inhibitors such as vemurafenib and dabrafenib, whereas HRAS-mutant HNSCC can be approached with farnesyltransferase inhibitors (FTis) like tipifarnib [4,6]. Despite these recent discoveries, the main therapeutic approach for HNSCC and cSCC combines surgery with radiation therapy and systemic chemotherapy, with ancillary reconstructions depending on case complexity and structures involved [7–9]. Surgical excision of the tumor mass remains the cornerstone of treatment for resectable SCC, followed by adjuvant radiotherapy, with or without chemotherapy [10,11]. The tumoral resection may carry additional risks when considering local infiltration, anatomical variants, or surgical approach [12–14].

The stratified epithelium from which SCC originates can be found distributed on the surface of the outer integument or covering organs and cavities such as the oral cavity, sinonasal cavity, oropharynx, larynx, esophagus, lung, and anogenital region [15]. SCCs are currently classified and examined taking into account the anatomical divisions of the human body; therefore, the SCCs of the head and neck are treated differently from those of the lung [2]. Here, we will focus exclusively on SCCs of the skin and of the mucosal epithelium of the oral cavity and lips (OSCC). Although both tumor types arise from the disruption of the same differentiation programs, cSCC and OSCC are considered distinct entities with different risk factors [16,17]. The main etiological factors for OSCC include tobacco and alcohol usage, and human papillomavirus (HPV) infection [15,18,19]. For cSCC and lower lip SCC, the most prominent risk factor remains increased exposure to ultraviolet radiation (UVR), followed by a personal history of skin cancer and HPV infection [19,20].

cSCC is the second most common cancer worldwide, and its incidence is steadily increasing in the Caucasian population. As cSCC is often invasive, inaesthetic, and locally destructive, such diagnosis usually involves complex skin surgeries and facial reconstructions [21]. While the majority of cSCC patients present with a very good prognosis, 1.5-5.2% of cases may develop life-threatening metastases [22,23]. Moreover, owing to the high incidence rates, in the United States, the mortality rate associated with cSCC is comparable to that reported for more aggressive tumors such as leukemia and melanoma [22]. The number of cSCC-associated deaths is hence expected to surpass that of melanoma in the next 5 years, mainly due to the rise in HPV infections [24]. HNSCC, the sixth most common type of cancer globally, represented mainly by OSCC, also exhibits a poor overall survival (OS) rate. Despite advancements in diagnostic and treatment methods, the HNSCC 5-year OS rate remains approximately 50% [25], suggesting that more intensive scientific efforts should be devoted to understanding the complex biology of HNSCC tumors. Molecular studies to identify the key factors driving cSCC and OSCC development are ongoing, aiming to identify the most promising diagnostic biomarkers and therapeutic targets for these problematic diseases [5,26,27].

The latest advancements in omics technologies facilitated the identification of several hotspot mutations in the EGFR/MAPK signaling pathway which have been harnessed for targeted pharmacological interventions in cancer patients. For instance, the observation that 80% of HNSCCs exhibit EGFR overexpression led to the development and subsequent approval by the Food and Drug Administration (FDA) of Cetuximab, an anti-EGFR monoclonal antibody, to be used in combination with radiotherapy in locally advanced HNSCC [28]. Yet, the dissection of the mutational spectrum of the EGFR gene served as a foundation for the development and implementation of novel EGFR-targeted therapies in clinical settings. For instance, genetic alterations such as exon 19 deletions (Ex19Del) and exon 21 substitutions (e.g., L858R), representing the most common EGFR mutations in non-small cell lung cancer (NSCLC), confer sensitivity to first (gefitinib and erlotinib) and second-generation (nilotinib, dasatinib) tyrosine kinase inhibitors (TKIs) [29]. In addition, exon 20 substitutions which include EGFR T790M mutations and are generally associated with NSCLC resistance to first-generation TKIs, may be also matched with targeted therapy in cancer patients, particularly with third-generation TKIs such as osimertinib and alflutinib [29]. In HNSCC/cSCC clinical cohorts, single-target TKIs, such as gefitinib and erlotinib were shown to improve modestly patient survival rates when given as monotherapy [30]. However, promising results have been reported when small TKIs were combined with other agents such as pembrolizumab, cisplatin, or docetaxel, in recurrent and metastatic settings [31]. This suggests that EGFR mutations may be exploited for targeted therapeutic interventions in the future, once the clinicians validate the most beneficial drug combinations. Alternative strategies for tailored therapy in HNSCC include a) FTIs, such as tipifarnib, for HRAS-mutant HNSCC (recently FDA-approved); b) cetuximab for SCC presenting with KRAS germline variants; c) EGFR inhibitors for HNSCC tumors harboring MAPK1 mutations; d) BRAFi and

MEKi for BRAF V600E-mutant ameloblastomas, combined or not with immunotherapies [4].

As we described earlier, the genetic assessment of BRAF, a serine/threonine protein kinase mutated in 60% of cutaneous melanomas and more than 15% of human cancers, may also expand the anti-cancer armamentarium in cSCC/HNSCC [32]. Despite the low prevalence of BRAF mutations in SCC of the head and neck (2%), HNSCC patients carrying these alterations present with improved clinical outcomes after BRAF and MEK inhibition [33]. RAS is another key oncogene in the pathogenesis of human cancers. KRAS mutations were identified in 2.89% of HNSCC cases, most frequently in tumors with sinonasal localization, whereas NRAS alterations were reported in 2.2% of cases, especially in tumors of the nasopharynx [34]. A recent meta-analysis highlights that oncogenic KRAS may be associated with significantly enhanced immune checkpoint molecule (ICM) expression and immune evasion programs within the tumor microenvironment, making KRAS-mutant tumors promising candidates for immune checkpoint inhibition (ICI) in the clinical setting [35]. However, several recent reports highlight that the MAPK-mutant status may serve as an indicator of a favorable response to immunotherapy in HNSCC, mainly due to the increased CD8+ T cell and dendritic cell infiltrations reported within these tumors, representative of an active cytolytic CD8+T-inflamed status [36].

The present study aims to explore the role and prevalence of KRAS, NRAS, BRAF, and EGFR mutations in a pilot group of patients diagnosed with SCC of the oral mucosa and skin.

MATERIALS AND METHODS

Patients and samples

Twelve patients diagnosed with squamous cell neoplasms of the head and neck between February 2017 and October 2019 were included in the present study. Three patients presented with cSCC, whereas nine had OSCC. Archived formalin-fixed paraffin-embedded (FFPE) tissues from these 12 subjects were obtained from the Department of Oral and Maxillofacial Surgery of the Carol Davila Military Emergency Hospital in Bucharest, Romania. All participants provided written informed consent to take part in this study. Ethical approval for this retrospective analysis was obtained from the Ethics Committee of the Carol Davila Central Military Emergency Hospital. The study including all procedures and handling of patients' samples was carried out in accordance with the guidelines of the Declaration of Helsinki and national as well as institutional guidelines for research ethics. The clinical and histopathological characteristics of the SCC tumors were analyzed following the latest version of the international standard American Joint Committee on Cancer (AJCC) tumor/node/metastasis (TNM) system for the classification and staging of tumors. Presence of the EGFR T790M mutation was analyzed with the gb ONCO EGFR (T790M) Real-Time PCR assay (PN: 3245-048, Generi Biotech, Czech Republic); additional mutations in the EGFR-RAS-RAF mutations were tested using the droplet digital PCR (ddPCR) Screening assays for BRAF V600 (PN: 12001037), NRAS G12/G13 (PN: 12001627), NRAS Q61 (PN: 12001006), KRAS Q61 (PN: 12001626) mutations and EGFR exon 19 deletions (PN: 12002392) (all BioRad, Hercules, USA); details of these techniques are outlined below.

DNA extraction

The total genomic DNA was extracted from FFPE tissues (3 x 15-µM-thick sections) using the ReliaPrep™ FFPE gDNA Miniprep kit (PN: A2352, Promega). The protocol includes a proprietary deparaffinization method that does not rely on the use of xylene or any other hazardous solvents, ensuring at the same time the extraction of high-purity nucleic acids from FFPE samples. Following the deparaffinization of the FFPE tissue sections, the extraction protocol involves the lysis of the tissues. Nucleic acids released from the cells are further bound on the surface of a spin filter membrane and eluted in 50 µL of elution buffer after several rounds of washing. The purified genomic DNA was kept on ice and quantified using a Nanodrop spectrophotometer.

Droplet digital PCR

Before carrying out the ddPCR reaction, the genomic DNA samples were subjected to uracil-DNA-glycosylase treatment (UDG; PN: 1B1634, VWR) following the manufacturer's instructions. This step aimed to eliminate potential C>T transitions that could arise from tissue fixation, therefore reducing the risk of false-positive ddPCR reactions. All gDNA samples were analyzed in triplicate.

The ddPCR mixture requires the addition of 10 µL of 2x ddPCR Supermix for Probes (no dUTP) per reaction. The 2x ddPCR Supermix is a proprietary reagent containing an optimized reaction mix for primers and probes. Additionally, 1 µL of 20x BRAF (NRAS/KRAS/EGFR) primer and 3 µL of UDG-treated gDNA (7 ng/µL) were included in each ddPCR reaction, and the final reaction volume was adjusted to 20 µL by adding an appropriate volume of RNase/DNase-free water. Each analytical run included negative (wild-type control, WT) and positive controls at the same concentration as the analyzed gDNA samples. The positive controls were provided by Horizon Discovery (PN: HD701) and contained recurrent mutations in BRAF, KRAS, NRAS, cKIT, EGFR, and PIK3CA genes, at allelic frequencies ranging from 1% to 24.5%. The negative controls were WT genomic DNA samples acquired from Promega

Corporation (PN: G1471).

In the droplet generation step, individual reaction mixtures were loaded into disposable DG8 cartridges (Bio-Rad) along with 70 µL of droplet generation oil and passed through the microfluidic channels of a droplet generator. The resulting droplets from each well were transferred into a 96-well PCR plate, heat-sealed, and subjected to the following PCR conditions: 95°C for 10 min, followed by 40 cycles of 94°C for 30 s, 60°C for 1 min, and 98°C for 10 min. Post-PCR, the droplets from each well were analyzed using a QX200 droplet reader (Bio-Rad) and quantified based on target DNA fluorescence. The obtained data were analyzed using the QuantaSoftTM Analysis Pro software, version 1.0.596 (Bio-Rad). Additional details were reviewed recently [37].

Real-Time PCR

The EGFR T790M mutational status was evaluated using the CE-IVD validated gb ONCO EGFR (T790M) Real-Time (RT-) PCR kit (PN: 3245-048, Generi Biotech, Czech Republic). All reagents included in the kit were prepared for use according to the manufacturer's instructions. For each sample, we set two separate RT-PCR reactions. One reaction contained the Assay qPCR EGFR Control T790M and the other, the Assay qPCR EGFR MUT T790M, both in a total volume of 20 µL. 5 µL of the same gDNA sample (diluted to a final concentration of 50 ng/µL) was added to each of the two reactions. Thermal cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 50 cycles with denaturation at 95°C for 10 s, and annealing/elongation at 60°C for 20 s. Fluorescence acquisition was set on the FAM/SYBR channel. All gDNA samples were analyzed in triplicate for the detection of EGFR T790M mutation. Each run contained two types of positive controls according to manufacturer instructions. We included one EGFR wild-type (WT) control and one EGFR T790M positive control, with 5 µL of the provided kit standards pipetted to each. Finally, to check the purity and quality of the PCR reagents, we introduced a third assay control, the so-called 'negative control'. In this case, RNase/DNase-free water was used to replace the corresponding volume of gDNA. The qRT-PCR assays were run on the Rotor-Gene Q 5plex HRM equipment (Qiagen, Germany) and the results were analyzed using the Rotor-Gene Q Series Software (version 2.3.1) (Qiagen, Germany). For each sample, the assessment of the mutational status was based on calculating fold change (FC) values from the Ct values obtained in the two separate reactions, as per the manufacturer's specifications.

Table 1: Demographic and clinicopathological data of SCC patients

Patient #	Gender	Age (years)	Area	Tobacco usage	Alcohol use	The primary site of the tumor	HPV infection	TNM	T stage	Lymph node metastases	Outcome
1	M	80	rural	no	no	lower lip	no	I	1	0	alive
2	M	67	urban	yes	no	tongue	no	IV A	3	2	dead due to disease progression
3	M	50	urban	yes	yes	tongue	no	IV A	2	3	alive
4	M	65	rural	no	yes	lower lip	no	I	1	0	alive
5	M	45	urban	yes	no	lower lip	no	III	3	1	alive
6	F	54	urban	yes	no	tongue	no	II	2	0	alive
7	M	60	rural	no	no	retromolar trigone	no	IV A	4	5	dead due to disease progression
8	M	69	urban	yes	yes	buccal mucosa	no	IV A	3	1	alive
9	F	85	rural	no	no	lower lip	no	II	2	0	alive
10	M	88	urban	no	no	cSCC of the parotid region	no	III	3	1	alive
11	M	51	rural	yes	yes	cSCC of the nasal region	no	IV A	4	2	dead due to disease progression
12	F	86	urban	no	no	cSCC of the genian region	no	I	1	0	alive

The limit of detection (LoD) declared for this assay is 5 copies per 25 µL reaction. This corresponds to a minimum detectable mutant allelic fraction of 0.05% in a background of 100,000 WT copies.

Statistical analysis

Data analysis was performed using The GraphPad Prism software (Boston, Massachusetts USA, www.graphpad.com) version 10.0.0. Patients' demographic and clinicopathological data were analyzed by descriptive statistics.

RESULTS

Patients' general characteristics

Table 1 describes the demographic and clinicopathological characteristics of the SCC patients. The clinical cohort consisted of 12 patients diagnosed with SCC of the head and neck region, encompassing 9 men (75%) and 3 women (25%). Among them, nine patients presented with OSCC (Patients #1-#9), while three subjects had cSCC (Patients #10-#12). The median age of the patients was 66 years, ranging from 45 to 88 years.

Fifty-nine percent (n=7) of the analyzed patients are from an urban environment, and 50% (n=6) of them are active smokers. Additionally, 33% (n=4) of the patients declared that they frequently consume alcohol, and 58% (n=7) presented with pathologically confirmed lymph node metastasis. Five patients were diagnosed with TNM stage IVA disease, three with stage I, and two patients each with stages II and III. All patients tested negative for the presence of high-risk HPV strains.

DNA extraction

The average concentration of DNA extracted from the archived FFPE tissues was 255.7 ng/µL (ranging from 70.2 to 484.3 ng/µL), and the average A260/A280 value was 1.83.

The A260/A280 values obtained for gDNA from FFPE tissues are within the optimal range (~1.8), indicating that the extracted gDNA is free from protein contamination. In addition, all A260/A230 values fall within the recommended range (2.0-2.2), indicating that gDNA has minimal contamination by polysaccharides. These observations confirm the obtained gDNA's suitability for subsequent molecular manipulations.

ddPCR results

The ddPCR analysis demonstrated that BRAF V600, KRAS Q61, NRAS G12/G13, and NRAS Q61 mutations were not present in the examined samples, emphasizing the rarity of these genetic alterations in the pathogenesis of squamous cell cancers. Figure 1 illustrates the absolute quantification of the NRAS Q61 mutation in FFPE samples using the ddPCR system. In addition, by exploiting the ultrahigh sensitivity of ddPCR technology, we successfully identified the presence of the EGFR Ex19Del mutation in a cSCC tumor developing in an 88-year-old male diagnosed with stage III disease (Figure 2). None of the remaining 11 SCC patients within the clinical cohort exhibited this particular mutation.

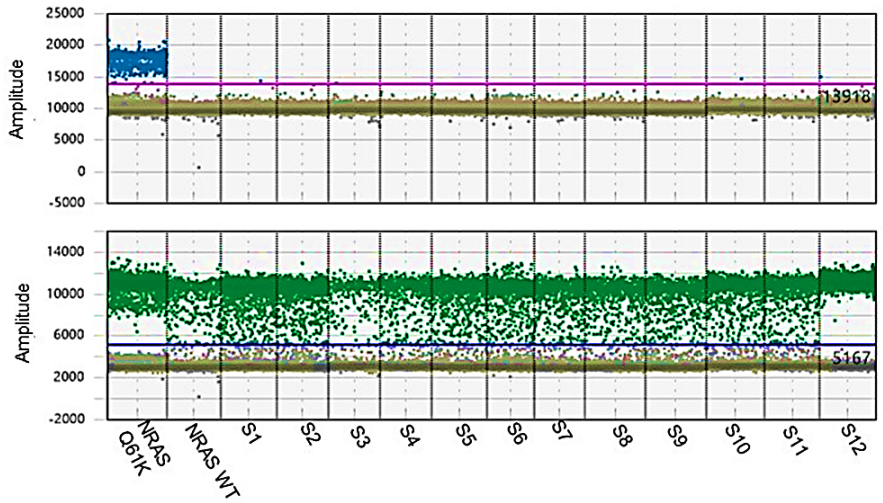

Figure 1: One-dimensional (1D) droplet fluorescence plot for NRAS Q61 mutation analysis. Blue dots represent the FAM-labeled droplets containing the NRAS Q61 -mutant allele, while green dots depict the HEX-labeled fluorescence probes associated with the NRAS WT allele. The pink line represents the threshold between positive and negative signals of the droplets. This determination enables precise NRAS Q61 mutation quantification in FFPE samples (S1-S12- SCC samples; NRAS Q61K- positive control; NRAS WT- negative control).

Real-Time PCR results

The 12 SCC samples were additionally subjected to qRT-PCR analysis to detect the EGFR T790M mutation. All SCC samples included in the study tested negative for the presence of the EGFR T790M mutation.

According to the manufacturer's recommendations, each analytical run included a positive control (containing the investigated mutation at an allelic frequency of 1%), a WT control (where only the WT allele was amplified), and a negative control (without gDNA), to investigate the purity and the quality of the reagents used. Figure 3 illustrates the

positive control of the EGFR T790M RT-PCR assay, the negative control along with a sample negative for this mutation (EGFR wild-type; in blue).

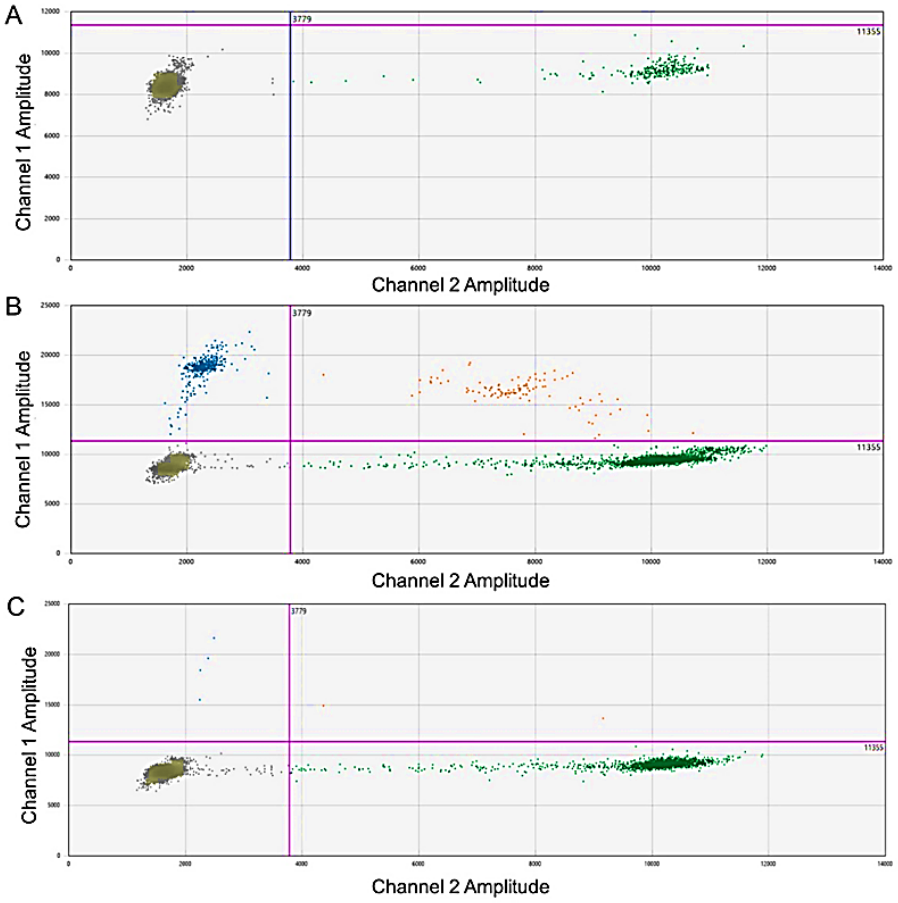

Figure 2: Two-dimensional (2D) ddPCR plots of EGFR Ex19Del mutation detection in FFPE samples. (A) ddPCR results for an EGFR wild-type DNA sample (negative control). The 2D plot illustrates that no droplets are positive for EGFR Ex19Del mutant alleles on channel 1 (FAM labeled, in blue) while EGFR Ex19 wild-type alleles are abundantly detected on channel 2 (HEX labeled, in green). Empty droplets (in grey) do not contain the EGFR Ex19Del target amplicon. (B) ddPCR results for the positive control (presenting the EGFR Ex19Del mutation). Mutant alleles are detectable on channel 1 (FAM labeled, blue). Double-positive droplets (orange) contain both mutant and wild-type EGFR Ex19 alleles. (C) ddPCR results for the cSCC tumor tested positive for EGFR Ex19Del.

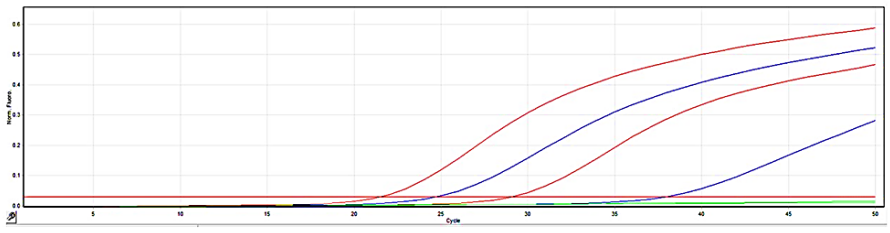

Figure 3: Determination of the EGFR T790M mutational status by RT-PCR. The positive control of the qRT-PCR analysis is marked in red, whereas the negative control is in green. The amplification curve of the EGFR WT SCC sample is depicted in blue. The threshold for the entire run was set at 0.03. A sample can be considered mutated when its FC value is lower than the cut-off FC value and the difference between the CtM of a sample and the CtC of the WT standard is lower than 13. If the latter condition is not met, or the FC value of a sample is higher than the cut-off FC, the sample is evaluated as WT.

DISCUSSION

SCC neoplasms of the head and neck are one of the most heterogeneous and aggressive tumors ever described [10]. Despite recent advances in SCC diagnosis and therapy, the prognosis of advanced patients remains poor, highlighting the need for a more comprehensive evaluation of these tumors [38–40]. From the beginning of modern medicine until now, SCCs have always been approached separately, depending on the tissue from which they originate (e.g., cutaneous SCC, oral SCC) or the anatomical region where they occur. However, in the present study, we wanted to deliver a unifying approach regarding cSCCs and HNSCCs, by analyzing the shared molecular mechanisms involved in their pathogenesis. Our point of view is also supported by the fact that the cSCC and HNSCC pathogenesis converge toward the same risk factors and genetic alterations [2,41]. Additionally, we believe this approach may facilitate a comparative examination of intrinsic tumor cell heterogeneity, stromal composition, and therapeutic efficacy across SCCs occurring in different anatomical sites, an idea that is attracting considerable research interest from the international community as well [2].

Here, we embarked on a preliminary analysis of the frequency and role of hotspot mutations in the EGFR-RAS-RAF signaling components within a clinical cohort encompassing 12 Romanian patients diagnosed with SCCs (3 cSCCs and 9 OSCC cases). We employed the third-generation PCR technology, also known as ddPCR, to screen for mutations such as BRAF V600, NRAS G12/G13, NRAS Q61K, KRAS Q61, and EGFR Ex19Del, while the mutational status of EGFR T790M was assessed using RT-PCR. None of the examined tumor specimens exhibited mutations in the BRAF, KRAS, and NRAS genes. Our findings are consistent with previous observations in the field, supporting the assertion that these genes are rarely mutated in cSCCs and HNSCCs [34,42].

The analysis of the demographic data of the subjects enrolled in the study revealed that 75% of them are men (n=9) and half (n=6) are smokers. We also observed that 58% of the subjects (n-=7) have metastases with lymph node involvement and all SCC tumors are HPV-negative (n=12).

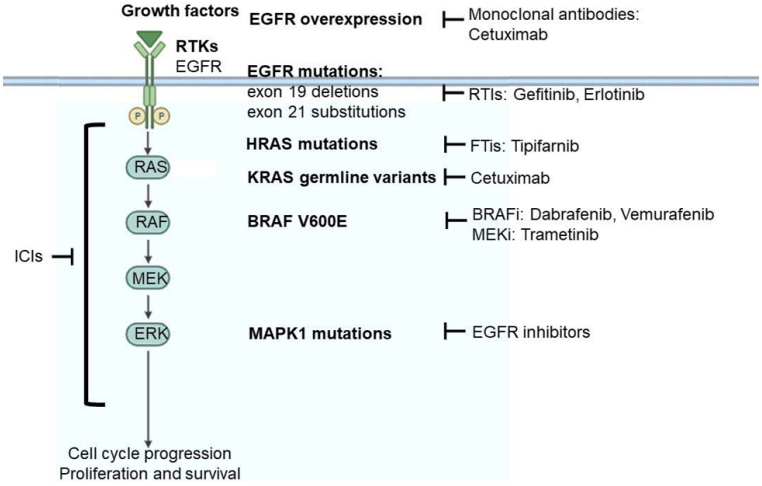

Figure 4: Precision treatment strategies for EGFR/MAPK pathway-mutated head and neck squamous cell neoplasms. ICIs- Immune checkpoint inhibitors; RTKs- Receptor tyrosine kinases; RTIs- Receptor tyrosine kinase inhibitors; FTIs- Farnesyl transferase inhibitors; BRAFi- BRAF inhibitors; MEKi- MEK inhibitors

However, among the 12 FFPE samples analyzed for EGFR Ex19Del, one HPV-negative cSCC tumor identified in the parotid region of a male tested positive for this mutation. Interestingly, the patient presenting with this tumor is an 88-year-old man from an urban environment who neither smokes nor drinks alcohol. Nevertheless, current literature emphasizes the role of HPV infection status as a valuable prognostic indicator for HNSCC/cSCC patients. Usually, HPV-negative SCCs display alterations in the tumor suppressors such as p53 and cell cycle regulators and present with an unfavorable prognosis compared to HPV-positive tumors, which show less genomic complexity [43]. Several research groups have examined the prevalence of EGFR Ex19Del mutations in head and neck SCCs, reporting a 1% occurrence in the Caucasian population [44] and 7.3% in the Asian population [45]. Despite providing tumor cells with different adaptive advantages via downstream hyperactivated pathways (ERK/MAPK, PI3K/Akt, and JAK/STAT), these mutations also confer sensitivity to TKIs [3]. Several ongoing clinical trials are investigating TKIs' clinical safety and efficacy in cSCC/HNSCC. Single-target TKIs gefitinib and erlotinib given as monotherapy have not demonstrated clinical efficacy in

HNSCC and cSCCs [46,47]. However, remarkable benefits have been reported when small-molecule TKIs were administered in conjunction with other therapies such as cisplatin, docetaxel, or immunotherapy [31,48]. Therefore, these mutations might be exploited for targeted pharmacological interventions in the future, once the most effective drug combinations are identified.

The evaluation of the mutational status of the MAPK signaling pathway is another hot topic in the field of SCC bio-medical research. Several recent studies potentiated that mutations in different components of the MAPK signaling pathway, such as BRAF and RAS, may serve not only as important therapeutic targets but also as valuable indicators of prognosis in SCC, indicating those patients who are most likely to respond to immunotherapy (Figure 4) [36].

This is why we focused our research on investigating these mutations in a small group of patients diagnosed with SCC of the skin and oral mucosa, the present study being also the first one of this type conducted in Romania. Recent studies have shown that even in p53-mutated HNSCC tumors, notorious for their unpredictable evolution, MAPK pathway mutations may help identify those patients with superior outcomes [36]. It is also worth mentioning that the positive prognostic value of these mutations in HNSCC has been reported independently of HPV status [36].

Bioinformatic analysis of HNSCC patient data retrieved from The Cancer Genome Atlas (TCGA) revealed that MAPK-mutant HNSCC tumors present with an increased infiltration of CD8+ T cells and dendritic cells and immune-active cytolytic and interferon-gamma signatures in situ, data that were also reported by our group [36,38,49].

Those features are not observable in HNSCC tumors with mutations in the PI3K, WNT, NOTCH, JAK/STAT, NF-kB, or TGF-beta signaling pathways [36]. Moreover, independent of tumor mutational burden (TMB), MAPK pathway mutations alone predicted those HNSCC patients who benefited the most from clinical anti-PD1/PD-L1 immunotherapy [36].

Similar prognostic roles of MAPK pathway mutations were reported in pan-cancer immunotherapy studies, suggesting that the mutational analysis of this signaling pathway might be essential to improve SCC patient risk stratification.

CONCLUSIONS

Oral and cutaneous squamous cell neoplasms (SCCs) remain a public health challenge and a topic of current scientific interest. Genetic analysis is essential to understand the biology and the clinical behavior of SCCs, unraveling potential biomarkers and therapeutic targets with practical applications in this challenging disease. Therefore, to align with the precision medicine initiatives in the field, we initiated a clinical study to explore the prevalence and significance of hotspot mutations in the EGFR-RAS-RAF signaling pathway within a small clinical cohort of Romanian patients diagnosed with squamous cell neoplasms of the head and neck. Taking into account the shortcomings reported with traditional chemotherapies, administered on a 'one size fits all' basis and resulting in poor efficacy, limited selectivity, and increased toxicity in HNSCCs/cSCCs, we are actively supporting the transition towards the adoption of more individualized anticancer agents in SCC clinical practice.

The present retrospective study emphasizes the absence of mutations in the NRAS, BRAF, and KRAS genes within a small clinical cohort of cSCC and HNSCC Romanian patients, marking the first study of this type conducted in Romania. In addition, our research underlines the remarkable sensitivity of the third-generation PCR technology, the so-called 'droplet digital PCR' (ddPCR), which may be instrumental for detecting low-frequency actionable mutations in cSCC/OSCC tissue biopsies.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research was funded by grants from the Ministry of Research, Innovation and Digitization, CCCDI-UEFISCDI, project number [PN-III-P4-PCE-2021-0549] and [PN-III-P2-2.1-PED-2021-2243], and within PNCDI III by the Core Program, implemented with the support of NASR, project [PN 23.16.01.03].

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Authors' contribution

Conceptualization, M.N., and EG.D.; methodology, EG.D.; software, A.C, and C.Constantin.; validation, A.N.M., C.Constantin., M.S. and C.S.; formal analysis, C.Caruntu.; investigation, A.C.; resources, M.N. and C.Carruntu.; data curation, EG.D.; writing—original draft preparation, EG.D., A.C., A.N.M., M.S., C.Constantin, C.Caruntu, C.S., M.N.; writing—review and editing, M.N. and C.Caruntu.; funding acquisition, M.N., and C.Caruntu. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee from Carol Davila Central Military Emergency Hospital, Bucharest (No. 333/28.03.2019).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Should Serum Levels of Creatine kinase be Evaluated in Patients with Acne Vulgaris Treated with Systemic Isotretinoin?

Funda Tamer[1], Laman Farajsoylu[1], Esra Adisen[1]

[1] Gazi University School of Medicine Department of Dermatology, Ankara, Turkey

Correspondence: Funda Tamer, fundatmr@yahoo.com

Abstract: *The laboratory tests that should be performed and their frequency during isotretinoin treatment are controversial. We wanted to investigate muscle and joint pain and laboratory tests in acne patients who received isotretinoin. Between April 2019 and February 2023, serum CK, AST, ALT, and GGT levels were retrospectively evaluated in acne patients before and three months after systemic isotretinoin treatment. This study included 410 patients. The median serum AST, ALT, and GGT levels significantly increased, whereas serum CK levels were similar in all patients three months after treatment. 23 (5.6%) patients revealed muscle or joint pain after treatment. No significant difference was detected in patients with or without pain in the median serum CK, AST, ALT, and GGT levels after treatment. Isotretinoin treatment at a dose of 10 mg/day was more common in patients with pain than in those without pain, whereas none of the patients who received isotretinoin 40 mg/day developed muscle or joint pain. We suggest that routine evaluation of serum CK levels is unnecessary in acne patients treated with isotretinoin. However, muscle and joint pain may develop even at doses as low as 10 mg/day. Furthermore, monitoring AST, ALT, and GGT levels may be beneficial in acne patients treated with isotretinoin.*

Keywords: *acne vulgaris; creatine kinase; isotretinoin; joint pain; muscle pain; treatment*

INTRODUCTION

Acne vulgaris is a common and chronic inflammatory skin disease that usually occurs during adolescence. Acne vulgaris may also affect adults or even initiate in adulthood. The etiopathogenesis of acne includes seborrhea, follicular hyperkeratinization, Cutibacterium acnes, and inflammation. Lesions mainly appear on the face and may lead to scarring and pigmentation changes. Therefore, acne has been associated with psychological complications such as depression and anxiety. Prompt and appropriate treatment is crucial both to improve lesions and to prevent the development of hyperpigmentation and scars [1].

Oral isotretinoin is a systemic retinoid used to treat patients with moderate to severe acne. Oral isotretinoin inhibits the function of sebaceous glands and shows anti-inflammatory effects [2]. Cheilitis is the most frequent dose-dependent side effect of isotretinoin, observed in approximately 90% of patients [3]. However, various side effects such as xerosis, pruritus, hair loss and photosensitivity have been reported after systemic isotretinoin treatment. In addition, musculoskeletal system disorders such as tendinitis and muscle and joint pain can also develop [2,3].

It has been suggested that about 50% of patients under isotretinoin treatment might develop increased serum creatine kinase (CK) levels, which indicate muscle damage [4]. Slight elevations in creatine kinase are usually reversible, while significant elevations may show rhabdomyolysis. Half of the patients with rhabdomyolysis are asymptomatic, and a minority of patients may develop fatigue and myalgia. Therefore, it has been proposed that it might be useful to evaluate serum CK levels before the initiation of isotretinoin treatment and regularly or when necessary during treatment [5].

In addition, patients with acne are usually monitored monthly, performing laboratory tests for potential side effects such as deterioration of the lipid profile and liver enzymes or complete blood count abnormalities such as leukopenia [6]. However, no consensus exists on the tests that should be performed in

patients treated with isotretinoin. For instance, routine evaluation of complete blood count has not been recommended by Hansen et al. [6,7]. Moreover, it is controversial whether frequent laboratory testing is necessary during systemic isotretinoin treatment. It has been reported that laboratory tests might be checked before and two months after treatment, and in case the results are within normal limits, there is no need for further evaluation [6,8].

Herein, we investigated muscle and joint pain and serum levels of CK, aspartate aminotransferase (AST), alanine amino-transferase (ALT), and gamma-glutamyl transferase (GGT) in patients with acne vulgaris treated with systemic isotretinoin.

MATERIALS AND METHODS

Gazi University Ethics Committee approval was obtained before this study (approval number: 2023-210). Between April 2019 and February 2023, medical records of patients with acne vulgaris treated with systemic isotretinoin were reviewed retrospectively. Age, sex, past medical history, chronic medication use, duration of acne, the age of onset of acne, and the severity of acne were evaluated. The severity of acne was classified as papulopustular and nodular acne. Isotretinoin dose, muscle or joint pain developed after isotretinoin treatment, localization of the pain, and time to onset of pain after treatment were also noted. In addition, serum levels of CK, AST, ALT, and GGT before and three months after the initiation of systemic isotretinoin treatment were evaluated by the biochemistry panel. The reference range was (46-171 U/L) for CK, (<34 U/L) for AST, (<49 U/L) for ALT and (<73 U/L) for GGT. Exclusion criteria were having liver and kidney diseases, using medications that may affect liver or muscle enzymes and intense exercise.

Statistical analysis was performed using SPSS version 25.0. Quantitative variables were stated as mean ± standard deviation or median (interquartile range). Categorical variables were stated as counts and percentages. The normality of the distribution of the data was evaluated by Kolmogorov-Smirnov test. Wilcoxon signed ranks test, chi-square test, and Mann-Whitney U test were used to determine the differences between the groups. P value less than 0.05 was considered statistically significant.

RESULTS

This study included 410 patients, 324 (79%) females and 86 (21%) males. The median age of the patients was 21 (6) years (range: 14-34 years). The past medical history was unremarkable in 391 (95.4%) patients. The characteristics of the patients are stated in Table 1. None of the patients reported regular use of a medication or excessive alcohol consumption. The patients were treated with oral isotretinoin at a dose of 10 to 40 mg daily. Among females, 220 (67.9%) had papulopustular, 104 (32.1%) had nodular acne, whereas among males, 44 (51.2%) had papulopustular and 42 (48.8%) had nodular acne (p=0.005). The median acne duration was 5 (5) years, (range: 1-20 years) in patients with papulopustular acne and 5 (4) years, (range: 1-20 years) in nodular acne (p=0.174). The median age of onset of disease was 15 (4) years, in patients with both papulopustular and nodular acne (p=0.693).

Table 1: Characteristics of patients with acne vulgaris

	Patients with acne vulgaris (n = 410)
Sex, n (%)	Females: 324 (79) Males: 86 (21)
Age Median (IQR), years Range, years	 21 (6) 14-34
Medical history, n (%)	Unremarkable, 391 (95.4) Seborrheic dermatitis, 3 (0.7) Asthma, 3 (0.7) Depression, 2 (0.5) Hashimoto's thyroiditis, 2 (0.5) Insulin resistance, 2 (0.5) Thalassaemia carrier, 2 (0.5) Allergic rhinitis, 1 (0.2) Urticaria, 1 (0.2) Familial Mediterranean fever, 1 (0.2) ITP, 1 (0.2) Mitral valve insufficiency, 1 (0.2)
Acne duration Median (IQR), years Range, years	 5 (5) 1-20
Age of onset of acne Median (IQR), years Range, years	 15 (4) 10-22
Severity of acne, n (%)	Papulopustular, 264 (64.4) Nodular, 146 (35.6)

IQR: Interquartile range. ITP: Idiopathic thrombocytopenic purpura. This study included 324 females and 86 males with acne between the ages of 14 and 34 years

The median serum levels of AST (p<0.001), ALT (p=0.024), and GGT (p<0.001) statistically significantly increased in all patients three months after systemic isotretinoin treatment. However, no statistically significant difference was ob-served in serum CK levels in all patients three months after systemic isotretinoin treatment (p=0.991) (Table 2). On the other hand, high serum GGT levels were significantly more frequent after isotretinoin treatment than those before treatment (p=0.012) (Table 3).

23 (5.6%) patients revealed muscle or joint pain during systemic isotretinoin treatment, while 387 (94.4%) patients did not have any musculoskeletal complaints. Low back pain was the most common and detected in 16 (3.9%) patients. In

addition, widespread pain was observed in 5 (1.2%) patients and leg pain in 2 (0.5%) patients (Table 4).

Table 2: Laboratory test results of patients with acne vulgaris before and three months after isotretinoin treatment

	Before treatment	After treatment	P value
CK, median (IQR) U/L Range: U/L	74 (43) 30-756	75 (40) 32-717	0.991
AST, median (IQR) U/L Range: U/L	18 (5) 9-215	21 (6) 8-853	<0.001
ALT, median (IQR) U/L Range: U/L	13 (9) 3-95	14 (9) 4-250	0.024
GGT, median (IQR) U/L Range: U/L	13 (6) 5-60	16 (7) 6-110	<0.001

ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CK: Creatine kinase. GGT: Gamma-glutamyl transferase. IQR: Interquartile range. A statistically significant increase was observed in serum levels of AST, ALT, and GGT, whereas no significant difference was observed in serum CK levels three months after systemic isotretinoin treatment.

Table 3: The frequency of high enzyme levels in patients with acne vulgaris before and three months after isotretinoin treatment

	Before treatment n (%)	After treatment n (%)	P value
CK	Normal: 379 (92.4) High: 31 (7.6)	Normal: 377 (92) High: 33 (8)	0.739
AST	Normal: 403 (98.3) High: 7 (1.7)	Normal: 398 (97.1) High: 12 (2.9)	0.251
ALT	Normal: 398 (97.1) High: 12 (2.9)	Normal: 395 (96.3) High: 15 (3.7)	0.532
GGT	Normal: 404 (98.5) High: 6 (1.5)	Normal: 393 (95.9) High: 17 (4.1)	0.012

ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CK: Creatine kinase. GGT: Gamma-glutamyl transferase. High serum GGT levels were significantly more frequent after isotretinoin treatment

Table 4: Patients with acne vulgaris who revealed muscle or joint pain after systemic isotretinoin treatment

	Patients n (%)
Muscle or joint pain	Yes: 23 (5.6) No: 387 (94.4)
Localization of the pain	Low back: 16 (3.9) Widespread: 5 (1.2) Leg: 2 (0.5)
Time to onset of pain after treatment	Within the first month: 5 (1.2) Within the second month: 9 (2.2) Within the third month: 9 (2.2)
Isotretinoin dose	10 mg/day: 6 (26.1) 20 mg/day: 10 (43.5) 30 mg/day: 7 (30.4)

Muscle or joint pain was detected in 23 (5.6%) patients with acne vulgaris after systemic isotretinoin treatment. Among them, low back pain was the most common

When patients were evaluated according to whether muscle and joint pain developed or not, no difference was observed in age (p=0.640), gender (p=0.436), duration of acne (p=0.919), the age of onset of acne (p=0.898) and the severity of acne (p=0.660). Nevertheless, among patients who developed muscle or joint pain, 6 (26.1%) received oral isotretinoin 10 mg/day, 10 (43.5%) received 20 mg/day, and 7 (30.4%) received 30 mg/day, whereas, among patients without pain, 13 (3.4%) received oral isotretinoin 10 mg/day, 177 (45.7%) received 20 mg/day, 174 (45%) received 30 mg/day and 23 (5.9%) received 40 mg/day (p=0.001). Furthermore, no statistically significant difference was detected in patients with or without pain in the median serum levels of CK, AST, ALT and GGT before and after systemic isotretinoin treatment (Table 5).

DISCUSSION

Musculoskeletal symptoms have been reported in about 25% to 87% of patients under isotretinoin treatment [9,10]. Various adverse effects have been described in patients with acne following systemic isotretinoin treatment such as myalgia, arthralgia, gout, spondyloarthritis, low back pain, muscle weakness, muscle stiffness, and myopathy [9,11,12]. Among them, temporary arthralgia or myalgia is the most common [13]. Musculoskeletal symptoms have been described even with low-dose regimens [9]. Drozd et al. revealed that low back pain was the most frequent musculoskeletal complaint in patients with skin diseases who received isotretinoin and that symptoms initiated within the first month of the treatment in 32.9% of patients, whereas between the second and the sixth

month in 64.7% of patients. A dose reduction or temporary discontinuation of isotretinoin was recommended in patients with muscle pain or high CK levels [10].

On the contrary, De Francesco et al. stated that arthritis did not have a dose-dependent effect that it was unnecessary to discontinue the drug or reduce the dose, and that isotretinoin treatment could be continued at the same amount without complications [13]. Furthermore, Feily reported patients with acne aged 18 to 29 years who developed musculoskeletal symptoms such as back and leg pain within two months after the initiation of oral isotretinoin treatment and responded to folic acid and vitamin B12 supply [14].

Table 5: Characteristics and laboratory test results of patients with and without muscle or joint pain after systemic isotretinoin treatment

	Patients with pain	Patients without pain	P value
Sex, n (%)	Female: 20 (87) Male: 3 (13)	Female: 304 (78.6) Male: 83 (21.4)	0.436
Age, median (IQR) years Range, years	21 (4) 16-31	21 (6) 14-34	0.640
Acne duration, median (IQR) years Range, years	5 (2) 1-14	5 (5) 1-20	0.919
Age of onset of acne, median (IQR) years Range, years	15 (3) 10-22	15 (4) 10-22	0.898
Acne severity, n (%)	Papulopustular: 16 (69.6) Nodular: 7 (30.4)	Papulopustular: 248 (64.1) Nodular: 139 (35.9)	0.660
Patients received different isotretinoin doses, n (%)	10 mg/day: 6 (26.1) 20 mg/day: 10 (43.5) 30 mg/day: 7 (30.4)	10 mg/day: 13 (3.4) 20 mg/day: 177 (45.7) 30 mg/day: 174 (45) 40 mg/day: 23 (5.9)	0.001
Before treatment CK, median (IQR) U/L	70 (29)	75 (45)	0.494
n (%)	Normal: 22 (95.7) High: 1 (4.3)	Normal: 357 (92.2) High: 30 (7.8)	0.549
AST, median (IQR) U/L	17 (3)	18 (5)	0.389
n (%)	Normal: 22 (95.7) High: 1 (4.3)	Normal: 381 (98.4) High: 6 (1.6)	0.334
ALT, median (IQR) U/L	14 (7)	13 (9)	0.620
n (%)	Normal: 23 (100) High: 0	Normal: 375 (96.9) High: 12 (3.1)	0.392
GGT, median (IQR) U/L	13 (5)	13 (6)	0.299
n (%)	Normal: 23 (100) High: 0	Normal: 381 (98.4) High: 6 (1.6)	0.548
After treatment CK, median (IQR) U/L	69 (39)	75 (41)	0.147
n (%)	Normal: 22 (95.7) High: 1 (4.3)	Normal: 355 (91.7) High: 32 (8.3)	0.502
AST, median (IQR) U/L	21 (8)	21 (5)	0.649
n (%)	Normal: 23 (100) High: 0	Normal: 375 (96.9) High: 12 (3.1)	0.392
ALT, median (IQR) U/L	15 (14)	14 (9)	0.832
n (%)	Normal: 21 (91.3) High: 2 (8.7)	Normal: 374 (96.6) High: 13 (3.4)	0.203
GGT, median (IQR) U/L	16 (9)	16 (7)	0.285
n (%)	Normal: 23 (100) High: 0	Normal: 370 (95.6) High: 17 (4.4)	0.613

Muscle damage due to isotretinoin treatment is generally mild and presents with well-tolerated pain or tenderness of muscles and increased serum CK levels [15,16]. However, isotretinoin can lead to severe muscle damage such as rhabdomyolysis which may result in renal insufficiency and even mortality [5,16]. Evaluation of CK has been recommended in patients treated with oral isotretinoin, especially in those who exercise regularly [5,15]. Furthermore, during follow-up, muscle and joint pain should be questioned [16]. Besides CK, laboratory tests performed to evaluate possible side effects in patients treated with systemic isotretinoin include serum levels of AST, ALT, lipid panel, and complete blood count [17]. The American Academy of Dermatology guidelines of care for the management of acne vulgaris recommend monitoring liver function tests and serum lipids until treatment response is achieved [18]. Zane et al. stated that among patients who used isotretinoin, 11% developed increased serum levels of AST or ALT which was usually mild and reversible, and that they principally evaluated ALT levels since ALT was more specifically indicated liver damage than AST [19]. On the other hand, Webster et al. stated that although AST and ALT were used to detect liver damage, they were not specific to the liver, they were also found in muscles, whereas GGT was a liver-specific and CK was a muscle-specific enzyme. In addition, Webster et al. suggested that monitoring serum GGT level was more beneficial than AST and ALT to detect liver damage in patients treated with isotretinoin for acne and that the increase in AST originated from the muscle rather than the liver [17].

Within this study, the median serum levels of CK, AST, ALT, and GGT were increased three months after oral isotretinoin treatment, however, a statistically significant increase was detected in serum AST ($p<0.001$), ALT ($p=0.024$) and GGT ($p<0.001$) levels. The number of patients with high serum CK, AST, ALT, and GGT levels increased after treatment. Nevertheless, a statistically significant increase was observed only in the number of patients with high GGT levels ($p=0.012$).

Muscle or joint pain occurred in 23 (5.6%) patients, among them, low back pain was the most common. There was no significant difference in age, gender, acne severity, acne duration, age of onset of acne, and serum CK, AST, ALT, and GGT levels between patients who developed and did not develop pain after isotretinoin treatment. On the other hand, isotretinoin treatment dose differed significantly in patients with and without pain ($p=0.001$). Treatment with isotretinoin at a dose of 10 mg/day was more common in patients with muscle or joint pain than in those without pain, whereas none of the patients who received isotretinoin 40 mg/day developed muscle or joint pain.

CONCLUSIONS

In conclusion, since no significant change was detected in serum CK levels with isotretinoin treatment, as well as no association was observed between serum CK levels and muscle or joint pain, we suggest that routine evaluation of serum CK levels is not necessary in patients with acne vulgar-is treated with isotretinoin. Although muscle or joint pain was observed in 5.6% of patients with acne vulgaris used isotretinoin, it should be kept in mind that muscle and joint pain may develop regardless of the isotretinoin dose, even at doses as low as 10 mg daily. Our results revealed a significant increase in serum AST, ALT, and GGT levels due to isotretinoin which indicated that monitoring serum AST, ALT, and GGT levels might be beneficial in patients with acne vulgaris treated with systemic isotretinoin.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, F.T., L.F., E.A.; methodology, F.T., L.F.; software, F.T.; validation, F.T., L.F., E.A.; formal analysis, F.T.; investigation, F.T., L.F.; resources, F.T., L.F., E.A.; data curation, F.T., L.F., E.A.; writing-original draft preparation, F.T.; writing-review and editing, E.A.; visualization, F.T.; supervision, E.A.; project administration, F.T., L.F., E.A. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and local ethics committee approval for this study was obtained from Gazi University (approval number: 2023-210).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

A Comprehensive Review of the Factors Affecting the Redefinition of Therapeutic Use in Metropolitan Cities during the Emerging and Re-emerging Pandemics

Hamidreza Asgari[1], Zohra Daudpour[1], Vahid Bigdali[1], Bahare M. Kermani[2]

[1] Department of Urban Planning, Qazvin Branch, Islamic Azad University, Qazvin, Iran
[2] Department of Urban Planning, Karaj Branch, Islamic Azad University, Karaj, Iran

Correspondence: Zohra Daudpour, zdavoudpour@yahoo.com

Abstract: *Resilience refers to the means and ability to deal with adverse conditions. Resilience is defined as the ability of the system to recover easily after disturbances and changes in the system and the ability of the organization to respond quickly to them. The resilient system can withstand environmental pressures so that it can provide optimal performance in critical situations. Resilience in the use of healthcare services is related to reducing vulnerability to shocks caused by natural disasters and increasing adaptive capacity due to improved measures and opportunities. Considering the importance of evaluation, monitoring, and planning to improve user resilience against accidents, spatial evaluation and redefinition of the use of health and medical services to prepare a comprehensive model for measuring the resilience of hospitals against accidents and calamities Naturally, at the top of which is the coronavirus epidemic, it is necessary. The aim of the current study is a comprehensive review of the factors influencing the redefinition of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a pandemic disease with a resilience approach. It is expected that the managers and decision-makers of the country's health field can use the results of the present research for better planning.*

Keywords: *Resilience, Economic factors, Metropolis, Healthcare services, Coronavirus*

INTRODUCTION

The accelerated and unbalanced development of physical space in some countries in the last few decades has brought adverse social, economic, and physical effects and consequences [1].

The exorbitant costs of urban transportation and services, energy waste, wasting material and social capital in the city, intensifying social segregation, environmental destruction, lack of beauty and cohesion of the city environment, social identitylessness, and instability are among the most important problems of cities in the discussion [2]. Undesirable development of physical space and low density of cities are considered. We are now living in the era of urbanization and urban development, where the ecology and morphology of cities have been increasingly affected by human and natural activities, with the development of urbanization and the desire of people to live in cities, as well as the occurrence of such diseases [3]. In recent years, the existing healthcare spaces in big cities have been seriously challenged. So during the peak of the epidemic, it reaches its peak time and the lack of these uses (hospitals and other medical centers) is felt in urban communities. For this reason, in recent years, many cities have implemented measures to create such spaces (sanitary-therapeutic) during the crisis of pandemic diseases [4].

In Iran, especially since the 1950s, the need to compile different levels of the health network cycle of the country has been felt and based on specific studies of culture, geographical environment, the population of each region, and various types of treatment facilities, from health centers to hospitals. This important thing has been specified and defined. Of course, the country's goal of these divisions is to create the possibility of greater efficiency of financial, equipment, construction, human resources, etc. [5]. In the shortest possible time and with the highest quality of treatment and health, especially when pandemic diseases (coronavirus) occur. But the format of city managers looks for other things beyond these necessities [6]. Improving access to medical services as an indicator of quality of life and social welfare plays an important

role. Equal access to medical services for different sections of society in a fair and balanced distribution of these services is one of the important foundations of social welfare [7]. Treatment spaces can be designed in 2 formats: 1) Health spaces and 2) Health-treatment spaces The basis of work in the health management system of countries is the discussion of the care and survival system of health centers and the chain of health spaces. Along with and parallel to the completion of this chain, the treatment system is proposed and then the treatment spaces are defined [8]. Although in most countries, the health care system is necessarily more complete than the treatment chain, but the possibility of full adaptation of these two chains to provide health services in cities is one of the ideals of every country. Today, the growth and development of urbanization have led to the creation of many facilities, but it should be kept in mind that with the growth of urbanization, the factors that because crises have also increased [9]. The issue of access to urban services is one of the important issues that affect the city from various dimensions. In some cases, the lack of proper access to some uses puts people's health at risk. And suitable for it is necessary for all members of the society to access services from two dimensions: the access of the target space is not the space that can be examined [10]. Access is one of the key components affecting the important quality of the urban environment, among the important ideals in this field is homogeneous access with the highest degree related to urban facilities [11]. The sustainability of cities and even megacities when pandemic diseases start, depends on the function and ability and more optimal use of health and treatment spaces, especially urban hospitals, therefore, based on the announced policies of the urban planning and treatment system of each country, it is effective for the life of that city [12]. It will be useful, however, when such crises (pandemic disease) occur, in such a way that it shows the best reaction and response in the shortest possible time during the accident. This will require the review, definition, and re-review of the uses of health and treatment spaces during pandemic crises (after the start of the coronavirus) [13]. Natural and man-made disasters inflict various types of damages, injuries, and death on the affected communities. Such disasters can disrupt various infrastructures and facilities, including hospitals, schools, transportation systems, and emergency services [14]. Non-attendance or lack of preparation in the continuity of services in emergencies caused by accidents has destructive and unfavorable effects on the responsiveness of hospitals. Recent disasters around the world have resulted in irreparable damage to hospitals and disruption of medical services [15]. One of the calamities that has spread all over the world today and has affected all the countries of the world is the coronavirus. In December 2019, the first case of coronavirus was reported in Wuhan, China, and due to the high death rate caused by it, it caused global panic. The rapid transmission of this virus has led to the diagnosis of more than a few million patients over the past two years [16]. A significant increase in the number of infected patients has made the work of medical and health centers difficult in dealing with this disease. Disruption and inconsistency in the provision of medical and health services can lead to irreparable losses and damages [17]. Therefore, it is very important to encourage researchers and decision-makers in order to prepare strategies for improving resilience in health and treatment centers [18]. The aim of the current study is a comprehensive review of the factors influencing the redefinition of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a pandemic disease with a resilience approach.

METHODS

This research is a scoping review that has been conducted by searching for systematic studies to identify the factors influencing the redefining of the spatial pattern of therapeutic uses in big cities at the time of the emergence of a new and re-emerging pandemic disease with a resilience approach using the Cochrane protocol in 1402. accepted. The study inclusion criteria were defined as follows:

Publication date: Due to the comprehensiveness and breadth of both keywords of the research, preparedness against emerging and re-emerging pandemic diseases and the spatial pattern of therapeutic uses of megacities were searched with a resilience approach. In terms of time, only researches that were published in the last decade, from 2010 to 2023 AD and from 1390 to 1402 AD, were examined.

Language: Due to time, financial, and manpower limitations, only the research published in English or Farsi was examined. On the other hand, to evaluate the quality of the studies and use them in the review, only the researchers whose texts are accessible to the researcher have been used.

Place of publication: Relevant sources published in reputable scientific journals, conferences, and theses were used.

Type of studies: Quantitative, qualitative, mixed, or review studies were examined.

The extracted sources were examined by two researchers independently. In this way, the mentioned keywords were first searched in reliable Persian and English scientific databases, including Direct Science, Scopus, PubMed, Google Scholar, and SID in the period from 2010 to 2023. The reason for choosing these databases was their validity and comprehensiveness including a large number of authoritative English-language publications in the field of health and environmental studies,

as well as the availability of access to most of the articles and books of these databases for the researcher. In the next step, articles whose full text could not be accessed were excluded from the study. Then, the articles that appeared more than once in the search results were removed using Endnote software, and according to the title of the article, articles not related to the topic were also removed. In the next step, the abstracts of these articles were studied more deeply, and according to the proposed limitations, at this stage, the articles that did not meet the mentioned criteria were removed. Finally, to evaluate the quality of the articles, the standard evaluation checklist (Critical Appraisal Skills Program: CASP) of qualitative studies was used, and the studies that received a score of 25 or higher were included in the study. Due to the inconsistency of included studies and the impossibility of meta-analysis, the meta-synthesis method was used to analyze the data. Out of 2583 reviewed articles, after considering the entry and exit criteria, 37 articles were included in the final review.

RESULTS AND DISCUSSION

The topic of social justice was first introduced to the science of geography by David Harvey in the book Social Justice and the City in 1976. In this book, Harvey has examined social justice with a radical Marxist perspective. In general, many articles and theses have been presented in the discussion of social justice in Iran, the most important of which are mentioned in the following. English et al. (2021) in the research they conducted in the city of Seoul, South Korea, have concluded that in a city, despite having enough services per capita (such as a park), due to improper distribution, they may be available to everyone.

Therefore, the residents have evaluated the distribution of parks concerning the population and other uses of the city and the proposed density of the areas. The results of their findings show that some of the residential areas in Seoul are deprived of access to city parks [19]. Esser et al. (2019) conducted research titled Capacity in Education: A Step Towards Social Justice, which was carried out from a 3-year collaborative activity of 8 primary schools in Darussalam and the coastal region of Powani in Tanzania, and concluded that there are countless obstacles to capacity (Education) In the world, communities and school levels exist, and in Tanzania some progress has been made in terms of (education) capacity, but equity and quality of education still have a long way to go [20].

In their article, Yasobant et al. (2019) express the idea of locating hospital centers in cities, in general, there is no long history in the field of locating medical and health service centers, and it goes back to the 1970s, since 1979, the department Social security health in England paid attention to the strategic development of medical and health service centers in cities, and it was followed in Austria in 1980 and 1982 [21].

Manenti et al. (2020) in an article on urban health addressed the evidence, challenges and guidelines. As a result, we note that although in this review we highlight challenges, this is due to the potential power of urban health research and the importance of studying health. Although the study of urban health encompasses considerable complexity, research with specific research questions and appropriate study designs can improve our understanding of the relationship between specific characteristics of urban nature and health in both specific cities and as generalizable to National cities and recent methodological advances, especially the widespread adoption of multilevel methods in public health research, have made it possible to test hypotheses about urban characteristics and their association with specific health outcomes. Finally, newer methods may contribute to a better understanding of competing influences on the health of urban populations over time. Such research can inform local interventions and policies in urban areas. We hope that efforts such as this review aim to build Our thinking about cities and health will help stimulate both empirical and theoretical developments that can lead to improved health in cities around the world [22].

Schäfer et al. (2021) in research have examined the spatial access of primary care, including methods, challenges, and concepts, and finally concluded that almost all healthcare centers to date, whether based on simple or complex measures, are limited to social inequality in access to health services and to investigate how much primary care centers affect people's health [23]. Sevá et al. (2017) have conducted a research regarding the access and use of health services in Ghana, in which the aim is to investigating the impact of access and socioeconomic factors on the use of health services in developing countries (specifically Ghana). Utilization of health services in Ghana distribution shows a significant difference between rural and urban areas in all variables, except health status and age. Even though the metropolis uses relatively more health services than the villages, the difference in the health status (need) of the two districts is not significant. The coefficient between the two districts is insignificant. Therefore, there is a difference between the need and use between the districts. Success is part of a local understanding of the dimensions and determinants of access to health services, as well as concrete efforts to improve services for the poor. Part of the innovations in health care financing and regulation of care promises to improve access for the poor in any case.

The challenge remains to find ways to ensure that vulnerable populations have a say in how strategies are developed,

implemented, and formulated, and to ensure that information and incentives are channeled in ways that can lead to improvements in access for the poor. It is expressed whether or not it can be given [24].

Morris et al. (2021) researched regarding the regulation of access and use of health services. The results showed, first, descriptively, how the countries are using different doctors to refer to a specialist. Second, a multilevel analysis shows how regulatory indicators affect each quantity of care consumed in a country, and finally, they examine how institutional structure affects differences in service utilization among groups with different incomes, education levels, and Health statuses [25].

Lingam et al. (2021) studied the determinants and pattern of health service consumption among graduate students and the result of this research showed that the healthcare system in Pakistan includes public and private health centers. In Pakistan, the majority of doctors are in the regions. They live in the city and attract the educated classes than the private sector. The use of public healthcare facilities is not the main priority of the educated class of the country, and government officials should pay more attention to making it more common for the people [26].

Gatto et al. (2022) studied poverty and access to health care in developing countries. The result of this research showed that despite the progress in access to health care in developing countries, a significant number of their population has limited access. Poor countries have a higher burden of disease but usually have less access to health care. Whether based on geographical access, accessibility, physiological access, acceptance or quality of care. However, recent studies show that this exodus is inevitable [27].

Peng (2020) discussed the optimal location for the construction of a hospital. In this study, the AHP method was used as one of the methods of multi-criteria analysis and GIS. The result of the study showed the use of the hierarchical analysis process in location. The hospital has been very efficient in the study area [28].

Lewis et al. (2021) investigated the improvement of access to health services in urban and regional areas for indigenous people. The results of this literature study provide information about barriers to access to health and mental health services for local people living in urban and regional communities. These barriers can be classified as barriers of accessibility (physical accessibility), affordability, appropriateness, and cultural acceptance [29].

Sousa et al. (2021) analyzed the equity and use of health services in Uganda in a research that focused on several factors (supply and demand) that affect the access and use of health services in Uganda, such as Education, age, household expenses, use of the outpatient department, hospital capacity and the distance from medical services, this study has analyzed a number of measures that are used in the implementation of policies, especially in the main fields. Key policies such as malaria, tuberculosis, and HIV/AIDS have been created. The analysis shows, however, that despite efforts to improve health by improving health outcomes for people, mountainous challenges still remain. For example, living near essential health services, although they work as an incentive to use care, does not necessarily ensure that health services are actually used. Secondly, the elimination of the usage fees of all government centers will increase the utilization of new healthcare facilities, especially fixed and fee-based service providers with catastrophic costs [30].

Joshi et al. (2022) in a study investigated the access and use of health care among rural households in Nigeria. The results showed that the average age of the respondents was 46 years and the average household was 8 people. The majority of the respondents, 43.5%, do not have formal education and the main occupations are agricultural officials. 58% of the respondents have access to medical services, while only 42.5% use these services. Most of the respondents, 40.5%, are 5-9 kilometers away from accessing medical centers. Accessibility indicators show unequal access to modern health facilities in the study area, so this study recommends that rural development policies should create an organizational environment to increase participation and access to justice in the provision of modern health care throughout rural areas. strengthen in the country [31].

McKenzie 2017, in an article on the access and use of health services in Massachusetts, the United States, conducted a qualitative study of the views and experiences of immigrant women in Brazil. The findings of the research show that illegal immigrant women in Brazil are more likely to access health services in countries that have health services. They are satisfied with the global level. Health care is critical to quality of life, but many immigrants and ethnic minorities often lack adequate access to health care, increasing health risks. This study adds to the current literature on healthcare access and use by immigrants living in the United States and also provides new information on Brazilian immigrant women's perspectives and experiences of healthcare access and use in Massachusetts, USA to give While identifying the opportunities to improve the services and health of the entire immigrant population [32].

Allam et al. (2022) examined the relationship between transportation networks and healthcare services and their impact on urban sprawl. The findings of this study show that there are more dynamic relationships between the content of

these urban components and access to health care [33].

Das et al. (2021) investigated the design of decision support systems for access to space services. The results of the research showed that improving access to health care can help reduce health costs, increase healthcare equity, and improve health outcomes. Through our research, we have designed a new application-based framework for measuring access to health care and a practical example of SDSS that is rooted in the theoretical concepts of BM and existing FCA methods. Our unique method to obtain the weight of the population characteristics is provided by the use of predictive analysis using real data to evaluate the need to integrate non-spatial factors into existing algorithms in the creation of SPAI. The findings should encourage future research to further examine how demographic characteristics may shape the demand for health care gives the hardness by considering the actual manufacturer's needs [34].

Cotta et al., 2020, reviewed the approaches to measuring potential spatial access to urban health services in a research where they examined the types of distances and their related issues [35].

Berendes et al. (2018) examined the urban status and health competence of critical issues and exercises, and it was concluded that if the global community is serious about the sustainable development goals of SDGs, more attention should be paid to understanding and taking action to improve urban conditions to have a life and social and economic conditions that can promote health competence. The result of the research showed that healthy and fair urban places are very important to achieve health for all the population, however, strict emphasis is placed on built environments or risk factors. A single person does not address the numerous and often cumulative social and environmental obstacles that exist in cities today and does not help the inequalities that burden poor urban communities. To collaborate or help the urban poor to analyze and solve problems. We realize that this will not be easy and the evidence base for how to do this effectively is still emerging. Our challenge is great, but in an urban planet public health Environment is urban health and health justice should be our goal [36].

Pak et al. (2018) investigated network and spatial analysis to evaluate and guide decision-making about equal access and health services in the general patient care system in Ederdamora (Spain). The simultaneous use of access and coverage maps to show the relative distribution of resources in a population, while showing the coverage levels according to the time of access to physical and professional facilities with and without administrative restrictions, can help decision-makers to improve the optimal level of stocks in a region. Large and low-density geography is very valuable [37].

Coccia et al. 2020 studied the issue of access to health care by urban refugees and the host population around Uganda. The results of this research showed that health services were available every five kilometers for both refugees and the host population. Refugees are generally unable to pay for access to health services, including transportation, research, essential drugs, and other informal costs. Refugees' perception of public health services is weaker compared to general citizens. Primarily due to long-term expectations of a small number of inadequate essential drug services, perceived discrimination and the existence of unofficial fees [38].

Paris et al. (2020) in a study to measure the resilience of traditional societies against natural disasters have concluded that traditional societies face natural disasters using four dimensions: nature of risk, level of resilience, cultural structure and policies. Managers can show resilience in the face of disasters [39].

Finn (2022) conducted a research as a place-based model to understand the resilience of local communities against natural disasters. This study presents a new framework of the place of urban resilience in order to improve the method of assessing resilience against disasters at the local and regional level, and a set of variables have been considered in this study as the first step in the target research. The final output of this research is presented in the form of environmental, social, economic, institutional, infrastructure, and competence of society [40].

Park et al (2022) in an article titled "Towards resilient regions: regional development policy" use a descriptive-analytical method and emphasizes the economic dimension of resilience and analyzing indicators of employment rates, large-scale economic centers, income and production gross and knowledge mobility introduced the process of making the regions resilient [41].

Plastina et al (2019) worked on an article entitled Regional planning and the future of resilience in the Ning ladder coral coastal region of Australia in 2019, which was the indicator of resilience and the amount of tourist attraction, which finally reached the correlation between resilience and tourist attraction [42].

Teng et al (2022) in an article titled "Tissue-based identification of old urban neighborhoods in Hyderabad, India using remote sensing data with a renovation approach, concluded that the location map of worn-out tissue is an efficient tool in identifying areas." The densely populated city can be used as a reliable source for vulnerability and resilience assessment in the next step. The presented method allows for quick analysis and comparison of multi-temporal data and can

be used in many densely developing cities around the world [43].

Acharya et al. (2017) collected the articles and opinions of different people in one collection and investigated the reconstruction of built environments after disasters in order to increase resilience and found that resilience should be considered among the necessities of reconstruction [44].

Hasson et al. (2022) categorized urban resilience indicators from a landscape ecologist perspective and the result of this article introduces five urban design and planning indicators to create urban resilience, which include redundancy, multi-functional (biodiversity) Being an urban system, urban ecological networks and connection with connections, adaptive design or adapting to changes, he still emphasizes the combination of ecological principles and urban planning to achieve urban resilience [45].

Ferdowsi and Arkhodi (2016) in an article entitled "Evaluation of health and medical resilience of Semnan province cities against natural disasters" stated that due to the high importance of health and medical resources in order to improve the resilience of settlements against natural disasters Naturally, managers and planners should have a special approach to providing healthcare facilities and infrastructure. The purpose of this research is to evaluate the cities of Semnan province based on health-medical indicators effective in resilience. In this research, the desired indicators have been evaluated using the Vikor model. According to the results, the cities of Shahrood, Damghan, Garmsar, Aradan and Sorkheh have a more efficient performance in providing services during the occurrence of natural disasters by having proper medical facilities and infrastructures, and in other words, they have favorable health-medical resilience in the face of natural disasters. They will have accidents. On the other hand, the cities of Mahdishahr, Miami and Semnan, due to the lack of appropriate proportion between the population and medical facilities and infrastructure, will have low resilience when faced with accidents compared to other parts of the province. In general, by evaluating and analyzing this process in Semnan province and resilience strategies, managers and planners can lead the cities of the province to strengthen resilience against natural hazards, considering the existing capacities and competitive advantages [46].

Saffari Darberazi et al. (2019) in an article entitled "Designing a Comprehensive Hospital Resilience Model in the Face of Corona Disease" stated that healthcare centers should be able to quickly adapt to catastrophic events such as natural and human disasters. One of the ways to deal with various disasters in healthcare centers is to increase resilience.

The current research aimed to design a comprehensive hospital resilience model by identifying the factors affecting hospital resilience, and the way these factors are related in the face of the corona disease. At first, by using the background study of the research, the effective factors on hospital resilience were identified, and then by using the interpretive structural modeling technique, the communication model between the identified factors was obtained. The conceptual model obtained from interpretive structural modeling was fitted using Smart PLS software version three. For this purpose, the questionnaire containing 33 questions was provided to 80 managers, experts, and employees of Yazd teaching hospitals. The research results indicate the identification of eight general factors affecting hospital resilience. The eight factors identified in the present study were structured in four general levels by interpretive structural modeling. The starting level of the model is the factors of "sustainability" and "information technology and communication system". Also, the model-fitting results confirm the relationships formed by interpretive structural modeling. Based on the results of the present research, the country's health and treatment managers can use to make the country's hospitals resilient in the face of natural disasters and unforeseen events [47].

Ebrahimzadeh et al (2009) in an article entitled "Spatial-spatial planning and organization of health and medical services using GIS case: Zanjan city" found that in most cities there is a lack of appropriate allocation of space and optimal placement of service and physical elements. The city, especially the medical centers (hospitals) and the number of effective factors in the location of these centers, has followed and continues to increase the problems of the city and its citizens. Therefore, this has revealed the necessity of using effective methods and computer systems in the optimal location of these centers. The current location of most of the medical centers and hospitals in Zanjan city does not match the scientific criteria of the needs of this land use, at the same time, the findings of this analysis show that the current need of medical centers in Zanjan city is at least 7 hospitals according to the city's per capita, which the city officials They can use 11 points extracted from gis to solve this deficiency [48].

Rahnama and Amirfakhrian (2010) analyzed spatial access to healthcare services in the city of Mashhad. They stated that access planning, as a method of integrated planning of users and transportation networks, has two spatial and non-spatial dimensions. The availability and availability of services is one of the dimensions of spatial access, and this study examines medical services in Mashhad. The study method is of descriptive-analytical type, based on which, after forming a spatial data bank of therapeutic spaces in ARC-GIS software (including 6 major uses with 4528 frequency items) and using accessibility models. two-stage floating basin", the condition

of Mashhad's districts' access index to each of the treatment spaces was determined. The advantage of using this method, compared to other methods, is the simultaneous use of indicators such as distance, functional radius, and number of facilities. The result of this analysis shows the mismatch between the spatial distribution of the population and the distribution of medical services. In other words, the central areas of the city have the best access and the peripheral areas have the weakest access to medical services. In the following, while using SPSS software and the "two-stage cluster analysis" model, 1266 population zones of Mashhad city were classified in terms of access to health care services. In this section, the results of the findings showed that the city of Mashhad can be classified into two clusters, including the first cluster with an average accessibility index of 3.24, including 2.3% of urban areas (areas with excellent access conditions) and the cluster The second one with an average accessibility index of 0.061 includes 97.7% of the domains, which does not show a good situation compared to the first cluster. The graphs prepared from the evaluation of this model showed that the most important indicator regarding the difference between the first cluster and the second cluster is the index of access to pharmacies. On the other hand, the index of access to emergency bases shows the least role in this classification [49].

Shojaiyan and Rahimpour (2017) in an article entitled "Evaluation of land suitability of health-treatment centers using CF analysis (case study: regions one and eight of Ahvaz city)" stated that the assessment of urban land suitability is the basis of urban planning. Especially in the field of proper use of land. This important issue directs the existing uses in the city in such a way as to prevent incorrect and incompatible adjacencies and ultimately lead to the establishment of land suitability. The present research was carried out with a descriptive-analytical approach in order to evaluate the land suitability of health-therapeutic centers in areas one and eight of Ahvaz city. ArcGIS software and CF relationship were used to achieve the mentioned goal and analyze the obtained information. Finally, the spatial distribution of land and the suitability percentage of each of the selected uses were determined with health-therapeutic use. The results of the research showed that among the uses studied, industrial use with 16.17%, residential use with 8.17%, and then fire stations with 7.53% had the highest incompatibility, and main roads with 0.71% had the lowest incompatibility. Is. In the end, 41.09% of the total area of the studied area is occupied by incompatible areas, 18.66% by neutral areas, and 13.02% by compatible areas. According to the conducted studies, the suitability of the land and the proximity of the selected uses in relation to the health-therapeutic use in the studied area have not been proportionately observed [50].

Motamedi (2019) in a research article entitled "Investigation and locating the access of the citizens of Shirvan city" to therapeutic uses. In this article, one of the basic and important tasks of urban and regional planners is to allocate land to various urban uses while paying attention to the role and function of the city, the economy of the city, and also the effect of mutual factors of uses on each other. The results showed that in terms of location, the studied clinics are concentrated in the eastern side of the city, and the central and western parts of the city are limited in terms of access to the clinics, and only 28,136 people out of the city's population of 83,000 have access to the standard radius range of the clinics; In other words, it can be said that about 75% of the city's population is outside the standard radius of the clinics, and the access radius of the clinics is not suitable for the citizens of the city, and there is a need for a well-equipped hospital in the west of Shirvan [51].

Hataminejad et al (2017) in a research entitled "Analysis of access to urban services in Esfrain" which was conducted using a questionnaire and unbalanced Morris index, concluded that the upper socio-economic classes in the city of Esfrain, in terms of having better financial conditions, they have more favorable uses, and the distribution pattern of service uses is in favor of more prosperous groups so that it is not consistent with the concepts of social justice [52].

Rostami (2010) in the research "Investigation and analysis of urban public service distribution from the perspective of spatial justice, case study: Yasouj city" the degree of access and need of residents to public services using the access network analysis model, hierarchical analysis model and analysis model local spatial autocorrelation has been investigated and analyzed. He also used the Gini coefficient to analyze the inequality of access to urban services and the Moran coefficient to analyze the pattern of distribution of injustices among urban blocks and finally concluded that urban public services are based on accessibility, public benefit, efficiency Population distribution and residents' needs for public services have not been fair and facilities are concentrated in certain areas [53].

Rahnama and Zabihi (2011) analyzed the distribution of public facilities in line with spatial justice with the integrated model of access in Mashhad in 2013 and in this research they concluded that the integrated access analysis of services with the help of geographic information system and spatial autocorrelation technique This indicates that the spatial distribution of public facilities based on Moran's index is clustered and based on the general index, it is a cluster with low concentration. The final result is that the spatial distribution of public facilities is not fair based on the integrated model [54].

CONCLUSIONS

The range of topics and topics of strategic urban research is very wide. For example, studying sports, recreational, therapeutic, educational spaces, police stations, fire stations, etc. All of them are topics that the researcher can investigate. To scientifically and accurately examine urban issues, it is necessary to carefully examine the components and influencing factors according to their type and degree of importance. In addition, due to the rapid growth and expansion of cities, the existence of a multitude of variables affecting them, as well as the incredible amount of information, the use of traditional methods in the analysis of problems is not very reasonable and expressive, and it is misleading. Factors such as population growth, migration and its consequences, and the fair and optimal distribution of services in the city environment and especially the metropolises are associated with obstacles and problems, therefore, adopting efficient and appropriate policies in relation to the provision of services and optimal allocation of space for the population living in different sectors of the city (outskirts, center, etc.) is one of the important goals of urban planning for quick and low-cost access to citizens. In this chapter, theories and models such as spatial allocation models, access models, attraction models, therapeutic approaches, sustainable development theory, and spatial theories were discussed.

Therefore, considering the problems stated above and the large spatial scale and analysis of a large amount of mechano-descriptive and statistical information, the researcher was motivated to solve various problems by having analytical functions (especially network analysis facilities, spatial allocation models and modeling in general) including analysis of accesses, determination of sphere of influence, location, and allocation of resources, sustainable development, development of social justice and so on.

With the help of these tools and statistical analysis, reach scientific results. Biological accidents and threats, re-emerging, and emerging diseases are increasing, and in these diseases, accidents, and threats, the first place to get involved is the Ministry of Health, especially hospitals. Hospitals must have the necessary preparation to face these incidents and threats so that in case of such incidents, they have the least vulnerability reduce casualties, and can provide the best and safest care to clients. One of the ways to create readiness is to conduct exercises and maneuvers, therefore, for this purpose, hospitals must write and practice several scenarios regarding various types of accidents and biological threats.

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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REVIEW

Immunomodulatory Effects of Statins in the Treatment and Prophylaxis of Influenza Viruses

Mojtaba Sharti[1], Hadi E.G. Ghaleh[2], Amir Vahedian-Azimi[3], Reza Ranjbar[4], Morteza Izadi[5], Ali Razei[1]

[1] Chemical Injuries Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran
[2] Applied Virology Research Center, Baqiyatallah University of Medical Science, Tehran, Iran
[3] Trauma Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[4] Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
[5] Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Correspondence: Ali Razei, ali.razei@gmail.com

Abstract: *Background and aim: Influenza, commonly referred to as "the flu," is an infectious illness caused by influenza viruses (IV). Despite the availability of efficient antiviral drugs, the IV still significantly increases mortality. Antiviral drugs swiftly remove these viruses from pulmonary secretions, suggesting that a prolonged inflammatory response may be to blame for the poor result. Therefore, using immunomodulatory medications looks advantageous. Numerous anti-inflammatory effects are produced by statins. Numerous retrospective studies recommended that statins be considered for IV therapy. This study set out to comprehensively examine the immunomodulatory potential of statins in IV prophylaxis and therapy. Methods: From the beginning until 25 March 2022, a number of electronic databases (Scopus, EMBASE, PubMed, Web of Science, ProQuest, OVID, EBSCO, and CINAHL) were searched for cohort studies and randomized trials assessing the association between outcomes or risk of infections and statin therapy. Data were gathered on the investigated characteristics, measuring statin usage, quality evaluation, and results (set for potential confounders). Results: Finding additional therapeutic agents for influenza prevention and management is crucial since there are uncertain public health issues regarding the clinical efficacy of conventional medications. Pro-inflammatory cytokines are released as a result of influenza. According to the results, by using anti-inflammatory medications, severe influenza infections can be prevented along with pleiotropic effects. As a result, statins, which are cardioprotective medications with immunomodulatory and anti-inflammatory properties, may be beneficial for individuals with IV. Conclusion: Based on the positive immunomodulatory effects of statin administration, our systematic review's findings suggested that IVs may be prevented and treated.*

Keywords: *immunomodulation, antiviral, anti-inflammation, statin, prophylaxis, Influenza viruses*

INTRODUCTION

Despite the Influenza virus (IV) being recognized as a serious pathogen during the past century, influenza continues to cause a sizable number of ailments. According to estimates, only in the US in 2017–2018. (Table 1) [1].

Universally, The worldwide WHO estimates that each year, influenza epidemics cause 3,000,000–5,000,000 serious illnesses, with 290,000–650,000 recorded fatalities due to the severity of IV [2]. Although yearly influenza vaccinations are advised and antiviral drugs are available, both have a number of limitations. Following influenza infection, immunological dysregulation, which is determined to release the pro-inflammatory cytokines generating major risk factors for mortality and morbidity, is the cause of the severity of the disease and clinical presentations [3]. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are the most significant implicated cytokines, generating innate immune responses and inflammation [4]. The antigenic changes over time (requiring a precise strain match annually), vaccine strain egg-adapted changes, slow manufacturing processing, the absence of cross-reactivity, brief duration of protection, and poor immunogenicity in certain populations (for example, the elderly) all play a significant role in the effectiveness of the seasonal influenza vaccine [5,6]. If given at the right moment during the illness, antiviral medications such as neuraminidase inhibitors might have a profound effect; otherwise, they have

only a subtle impact on clinical symptoms [7]. Prophylaxis is defined as a preventative measure against disease.

A preventative measure has immediate effects against response-triggering and can reduce the systemic reaction to physiological stress or illness. This development might greatly reduce the detrimental disease-related circumstances [8]. The most often given medications in the world are statins, which decrease cholesterol. Statins block the enzyme 3-hydroxy-3-methyl glutaryl coenzyme-A reductase, which catalyzes the rate-restrictive step of cholesterol production. Statins have been shown to have positive cardiovascular benefits (Figure 2). [9].

Table 1: It was extremely well-established how viral respiratory tract illnesses compare epidemiologically

Disease	Flu	COVID-19	SARS	MERS
Disease Causing Pathogen	Influenza virus	SARS-CoV-2	SARS-CoV	MERS-CoV
R_0 Basic Reproductive Number	1.3	2.0 - 2.5 *	3	0.3 - 0.8
CFR Case Fatality Rate	0.05 - 0.1%	~3.4% *	9.6 - 11%	34.4%
Incubation Time	1 - 4 days	4 - 14 days *	2 - 7 days	6 days
Hospitalization Rate	2%	~19% *	Most cases	Most cases
Community Attack Rate	10 - 20%	30 - 40% *	10 - 60%	4 - 13%
Annual Infected (global)	~ 1 billion	N/A (ongoing)	8098 (in 2003)	420
Annual Infected (US)	10 - 45 million	N/A (ongoing)	8 (in 2003)	2 (in 2014)
Annual Deaths (US)	10,000 - 61,000	N/A (ongoing)	None	None

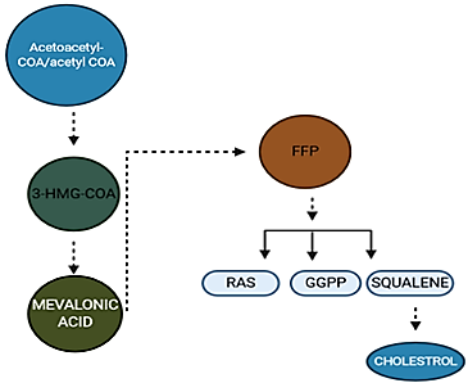

Figure 1: Statins' advantageous cardiovascular effects

Regardless of its effects on cholesterol levels, statins have been shown in clinical and experimental studies to decrease inflammation, improve endothelial function, and stabilize atherosclerotic plaques through a direct mechanism [10]. Statins can also alter cell shape, which is necessary for viral propagation [11]. Despite conflicting scientific findings, several retrospective observational analyses identified a link between statin use and decreased IV morbidity effects in people. However, these evaluations are constrained by the significant variation in medication delivery timing and duration across contributors or the participation of pneumonia morbidity in the absence of laboratory-approved IV infection [12]. A systematic review of statins' possible immunomodulatory properties in the treatment and prevention of IVs was the goal of this assessment.

METHODS

From the beginning until 25 March 2020, a number of electronic databases (Scopus, PubMed, EMBASE, Web of Science, ProQuest, OVID, EBSCO, and CINAHL) were searched for cohort studies and randomized trials exploring the connection between statin prescription and the result or risk of infections. Data on trial characteristics, statin consumption measurement, quality analysis, and outcomes were retrieved (set for potential confounders).

Influenza and the immune system

IVs are single-stranded RNAs that belong to the Orthomyxoviridae family and are divided into 4 categories based on their negative sense, segmented, and ssRNA genomes [12]. (classes of A, B, C, and D). In the meantime, many animal species might contract the influenza A virus (IAV) [13]. The IAV genome is almost 13,500 bases long and has 8 ribonucleoproteins (RNP) units, which together may encode at least 17 different proteins, including the recently identified

NS3, M42, PA-N155, and PA-N182 [14].

The genetic characteristics and molecular makeup of the neuraminidase (NA) and hemagglutinin (HA) proteins allow for further classification of IAVs. However, 9 NA and 16 HA subtypes were shown to be present in both human and animal bodies [15]. There have been reports linking many important IAV-encoded proteins to the host immune response and virus pathogenesis to the viral infection. According to research, changes in viral proteins' amino acid composition are linked to greater immune evasion and illness severity in humans and birds caused by IAVs [16]. In order for B cells to start producing neutralizing antibodies, HA contains epitopes. Therefore, the primary factors influencing recombination processes and viral mutation are the HA epitopes [17]. An innate immune response is the initial line of defense for the body against viral infection. IAV replicates itself by concentrating on respiratory tract epithelial cells. Understanding the innate immune systems defending these cells against IAV infection is crucial. Pathogen-associated molecular patterns (PAMPs), which are present in airway epithelial cells, are identified by receptors such Toll-like receptor (TLR) 3, melanoma differentiation-associated protein 5 (MDA5), and retinoic acid-inducible gene I (RIGI) [18]. Different autocrine signaling pathways can trigger the production of class 1 and 3 interferons (IFNs) and other cytokines instead of locating PAMP [19]. IFN lambda receptor 1 (IFNLR1-IL10R2) and IFN alpha/beta receptor (IFNAR1-IFNAR2), which are the receptors for class 1 and class 3 IFNs, respectively, can result in the paracrine response, which in turn activates a number of ISGs with antiviral activity and, as a result, induces the defensive activity of neighboring cells against viral infection [20]. The majority of adaptive immunity against IAV infection comes from B and T cells. T lymphocytes are mostly recognized as CD8+ and CD4+. In order to create cytokines and other powerful chemicals that can stop viral replication and kill virus-infected cells, CD8+ T cells are developed into cytotoxic T lymphocytes (CTLs). T cells are therefore essential to limit viral infection [21]. The naive CD8+ T cells are stimulated by the DCs to go from the lungs to the draining lymph nodes' T-cell zone during IAV infection, where they differentiate and multiply into CTLs [22]. Additionally, IL-12, IL-2, class 1 IFN-, and IFNs help to differentiate CD8+ T cells into CTLs [23]. It has also been demonstrated that IFN- increases T-cell proliferation during IV [24]. The workings of CTL have been extensively studied. The CTLs create cytotoxic granules that contain chemicals like granzymes and perforin while the virus targets the cells [25]. Additionally, CTLs can activate death receptors in IAV-infected cells by secreting cytokines including TNF, TRAIL, and FASL [26]. Following infection, lymphoid organs, blood, and the infection site are circulated with virus-specific DCs and CTLs [27]. The differentiation and activation process during the first infection increases the memory CTL cells' effectiveness and proficiency in secondary infection, and the memory CTL cells respond swiftly to subsequent IAV infection [28]. Although neutralizing antibodies are protected from subsequent infection with the same IAV serotype, CTLs are epitope-specific in conserved IAV proteins including NP, PA, and M1. As a result, the CTL response is heterosubtypic [29]. CD4+ T cells are a crucial subset of immune cells that are involved in adaptive immunity against IAV infection. In mouse models, CD4+ T lymphocytes may promote MHC type 2 expression in epithelial cells and target IAV-infected epithelial cells [20]. The CD40 ligand (CD40L) is notable among the several co-stimulatory ligands produced by CD4+ T cells that are important in antibody production and B cell activation [21]. TNF, IL-2, and IFN- are examples of antiviral cytokines generated by Th1 effector CD4+ T cells that activate alveolar macrophages [22]. IFN- and IL-2 released by Th1 cells control CD8+ T-cell differentiation to clear viral infection [23]. Additionally, CD4+ T cells can develop into killer, Th17, Th2, follicular helper, regulatory, and rarely Th2 cells [24]. Antigen-offering cells attach to MHC type II-related peptides produced from viruses, and Th2 cells produce IL-13 and IL-4 primarily to encourage B cell responses [25]. It was discovered that the control of cellular immunity against IAV infection includes the Treg and Th17 cells.

Immunopathogenesis of Influenza

The primary targets of IV for infection are respiratory epithelial cells, which are also amplified cytokines by the choreographers [26]. Following first exposure, the offspring viruses that have multiplied inside of these cells can spread to other cell types, including alveolar macrophages. The inflammatory reactions start when infected cells die through necrosis or apoptosis [27]; acute inflammation is thought to be the body's main defense mechanism against damaging stimuli. In order for leukocytes and plasma to reach the site of extra-vascular injury, blood flow must be increased. Acute inflammation is identified by an increase in local temperatures and discomfort [3]. Acute inflammation is indicated by the activation of pro-inflammatory chemokines or cytokines [28]. Inflammatory cell recruitment may happen as a result of these pro-inflammatory chemokines or cytokines. Then, there is extensive tissue damage, immune cell infiltration, and a gradual expression of antiviral, inflammatory, and apoptotic genes [29]. Both the repair of the damage and the regeneration processes begin at the same time. Most of the time, this repair technique can fully restore the function [30].

The formation of hyaline membranes, diffuse alveolar damage, fibrotic healing, and fibrin exudate (Figure 3), which are thought to be signs of severe capillary damage, persistent organ dysfunction, and immunopathologic injury, are among the more pathologically severe alterations found for severe

inflammation related to cytokine storm. Additionally, the release of strong inflammatory chemokines and cytokines into the bloodstream can result in systemic cytokine storms and multiple organ failure [32].

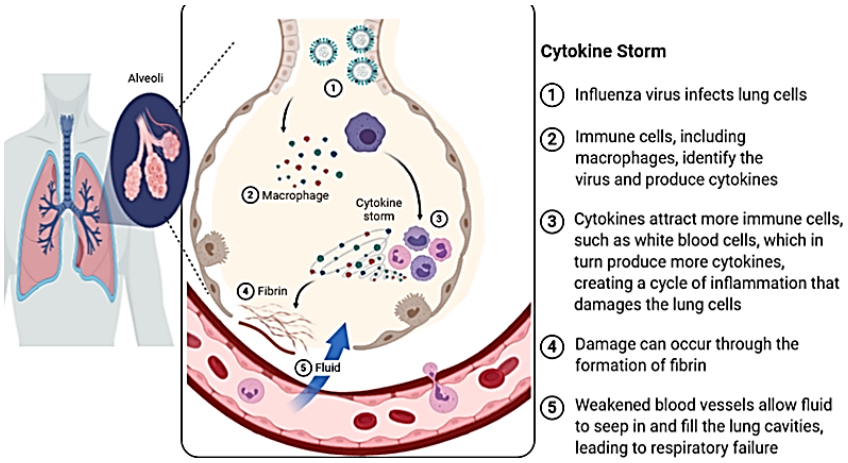

Figure 2: Severe inflammation related to cytokine storm

Because viral virulence and host resistance both have a role in the severity of influenza outcomes, it is imperative to use immunomodulatory treatment in addition to traditional antiviral medication. Indeed, several studies have shown that administering immunomodulatory medicines, with or without antiviral medications, can reduce organ damage and death from severe influenza. Additionally, the majority of these medications are not costly and are made simply, making them potentially useful in large-scale influenza pandemics. Here, the characteristics of statins and some instances of these were discussed.

Statins as immunomodulatory agents

The enzyme hydroxyl methylglutaryl-coenzyme A (HMG-CoA) reductase is inhibited by statins. By catalyzing the reduction of HMG-CoA for mevalonate during cholesterol synthesis, this enzyme can gauge the pace of the early step of the cholesterol biosynthesis pathway [33]. In addition to their ability to lower cholesterol levels, statins are pleiotropic cardioprotective medications that are gradually becoming recognized as direct cellular effect mediators. Statins inhibit downstream molecules, which are essential for viral contagiousness [34]. They reduce or prevent the production of downstream isoprenoid intermediates such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGPP) (FPP). These isoprenoid intermediates interact with membranes when post-translational proteins are modified. Statins regulate GDP and GTP-bound states by controlling their isoprenylation and preventing them from switching from the cytosol to the membrane when they are inactive [35].

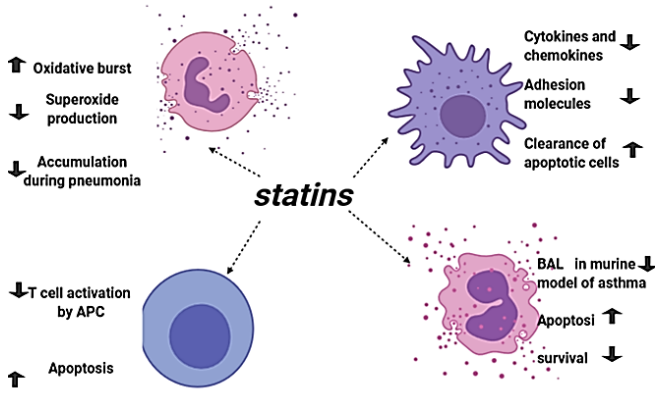

Figure 3: Some functions of statins

As a result, statins stop the activity and membrane translocation of isoprenoid intermediates and reduce their production. It is thought that lowering the isoprenylation of several proteins mediates the pleiotropic effects of statins.

This is best shown by the Rho GTPase family, which includes RhoA, Cdc42, and Rac, and which performs distinct roles dependent on the shape, secretion, motility, control, and proliferation of gene expression in cells [36]. To apply their activities and membrane interactions, members of the Ras family must add a farnesyl group to processes such as platelet activation, cell division, differentiation, intracellular vesicle transport, and the production of oxygen radicals [37]. These effects of statins include immunomodulatory actions such as the reduction of T-cell activation, the inhibition of inflammatory responses in neutrophils and macrophages, and the inhibition of a number of pro-inflammatory cytokines like TNF, IL6, and IL8. Furthermore, it has been demonstrated that blocking MAPK pathways such p38 MAPK, JNK, and ERK1/2 prevented the expression of RANKL, the production of cytokines and MMPs, and the degeneration of cartilage in different cells from RA patients (Figure 3). [38-42]

Use of immunomodulatory features of statins on IV treatment

According to three categories, including in vitro, human, and animal investigations, the effects of statin-related immune-modulation were examined to treat influenza in a number of studies. The following sections were used to review particular research from each category.

In vitro study

In the US Vaccine Effectiveness Network trial across 6 influenza courses (2011-2012 to 2016-2017), Havers et al. evaluated the influenza VE in statin users and nonusers with age higher than 45. All individuals with acute respiratory illnesses were screened for influenza at outpatient clinics. According to the data, out of 11692 eligible contributors, 3359 (30%) used statins, and 2806 (24%) tested positive for IV illness. Meanwhile, only 60% of nonusers and 78% of statin users obtained influenza vaccination. Setting for possible confounders came in at 39 percent (95 percent CI, 32 percent -45 percent) among nonusers and 22 percent (95 percent confidence interval [CI], 22 percent -47 percent) among statin users, with influenza VE coming in at 36 percent. They discovered no appreciable VE alteration as a result of statin use. Between nonusers and statin users, VE against influenza A(H3N2), A(H1N1) pdm09, and B viruses were comparable. When they analyzed the data by type and subtype, they discovered that the use of statin administration did not change the effect of vaccination on influenza [43].

According to research by Gower et al., lovastatin can stop virally induced cell-to-cell fusion and RSV replication. The experimental research showed that isoprenylation inhibition is a potential new antiviral strategy for treating or preventing severe RSV infection [44]. Statins, a widely used and affordable pharmacological medication, may be effective in preventing current and future influenza epidemics, particularly in less developed and underdeveloped nations, according to Mehrbod et al. By lowering cytokine over-expression and modulating strong inflammatory responses, statin therapy may protect the body from inflammatory reactions in the pulmonary system and other inflammatory reactions connected to severe influenza sickness. The availability of statins, which might be used as preventative or supplemental drugs to lessen the severity of the disease, and possible preventive medical therapy for unanticipated pandemics, may help to diminish the inflammatory repercussions of influenza that are connected with it [45]. Simvastatin and H1N1 elevated membrane localization and LC3 protein lipidation, according to Mehrbod et al. Simvastatin, however, has a direct influence on LC3 lipidation through a reduction in cholesterol, while H1N1's contribution to autophagy is more convoluted due to its effects on the activation of several autophagy-related genes to boost the autophagic flux. Their results were in line with those of an earlier study on the primary human lung mesenchymal cells, highlighting the relationship between the relative amount of LC3-II and the dynamic turnover of LC3-II through lysosome activity [46].

Animal study

Despite the ambiguous results, several animal studies showed that alternative statin medications may be protective against acute lung damage. In C57BL/6 mice infected by IAV, Haidari et al. observed that atorvastatin (40 mg/kg/day) prescription decreased the lung viral titers and mortality. Statins work directly against influenza by inhibiting the Rho/Rho-kinase pathway. The statins' protection against influenza might be viewed as a component of their cardiovascular event prevention benefits [47]. According to Lee et al., the treatment of simvastatin plus the neutralizing RANTES antibody effectively inhibited the growth of neutrophils caused by poly I:C in the lungs. Additionally, they said that simvastatin inhibits neutrophilia in poly I:C-induced pneumonia by inhibiting STAT3-mediated RANTES production. Understanding the etiology of viral pneumonia is crucial in order to develop effective anti-inflammatory or antiviral medications for certain inflammatory conditions. Simvastatin should be considered as a potential therapeutic option for viral pneumonia, particularly in neutrophilic inflammation due to a viral component, they further said. Simvastatin can reduce airway inflammation, but its exact mechanism of action has to be understood [48]. Simvastatin's efficacy against influenza A/PR/8/34 virus infection in the mouse model was evaluated by Glück et al. The first trial used oral administration of simvastatin. In a subsequent investigation, intraperitoneal administration was used to achieve high plasma levels. Bodyweight loss, survival

rate, histologic lung damage, and viral titers in the lungs and trachea were evaluated. Surprisingly, simvastatin treatment caused the mice to lose more body mass and have lower survival rates than the virus-infected control animals. Simvastatin also did not affect histological lesions, tracheas, or lung viral loads. In general, they demonstrated that simvastatin did not shield the mice from IV infection [49]. According to Radigan et al., rosuvastatin treatment did not successfully treat influenza A illness. Inflammation of the lungs, lung damage, and the degree of weight loss were comparable in rosuvastatin- and control-treated mice. Rosuvastatin treatment had no impact on mortality in influenza A-infected mice (A/WSN/33) Statins did not affect influenza A replication in vitro or its removal from the lung in vivo. Statins did not affect influenza A-related mortality and did not lessen the severity of the lung damage caused by the virus. According to them, there is no connection between HMG CoA reductase inhibitors and improved outcomes for patients with sepsis and pneumonia related to their effects on influenza A infection [50].

Human study

In critically ill patients with severe sepsis, Kruger et al. investigated the clinical and biochemical effects of atorvastatin treatment. Before using statins, the site stratified a phase II, prospective, multicenter, double-blind, randomized, and placebo-controlled experiment. A matching placebo or atorvastatin was given to 250 individuals in a cohort trial who had severe sepsis and acute infection (127 received placebos, 123 received statins) (20 mg daily). The results showed no interactions between treatment groups, showing that there were distinct behaviors across the groups throughout time (P = 0.26), and no variations in IL-6 concentrations (initial endpoint) between the placebo and atorvastatin groups (P = 0.76). The baseline IL-6 plasma titer was decreased (129 [87-191] vs. 244 [187-317] pg/ml; P = 0.01) in those who had previously taken statins. There was no difference between the two groups in terms of the length of hospital stays, hospital discharges at 28 or 90 days (15% vs. 19%), changes in Sequential Organ Failure Assessment scores, death at intensive care unit release, or negative effects. Patients on atorvastatin had reduced cholesterol (2.6 [0.06] vs. 2.4 [0.07] mmol/L; P = 0.006). Greater than 28-day mortality was detected for those randomly assigned to the placebo (28 percent vs. 5 percent; P = 0.01) than for those getting atorvastatin in the selected group of 77 past statin users. At 90 days, there were no statistically significant differences (11 percent vs. 28 percent; P = 0.06). A therapy with atorvastatin for severe sepsis did not appear to have an impact on IL-6 levels. A lower baseline IL-6 concentration in this population was associated with improved survival, while prior statin usage was associated with the continuation of atorvastatin [51].

According to Papazian et al., simvastatin (60 mg) prescriptions for supplementary VAP therapy did not lower day-28 mortality. Simvastatin did not substantially worsen any side effects. Although the superiority of a placebo to simvastatin was not tested in our investigation, it is important to note that day-28 mortality in the simvastatin group increased by approximately 6% absolute and by almost 8% absolute in the statin-naive category. It would be pertinent to look at the potential supremacy of a placebo over a statin as normal practice does not involve de novo statin administration in ICU patients with infection. They thus investigated the claim that simvastatin is preferable to a placebo. The results did not support the use of supplementary statin medication in ICU patients with VAP. Therefore, this result may be extended to ICU patients who have any type of nosocomial infection [52].

With the aid of connected administrative databases, Kwong et al. conducted a population-based cohort analysis across 10 influenza seasons (1996–2006) in Ontario, Canada. Before the start of the influenza season, they identified all persons over the age of 65 who had had an influenza vaccination, and they also separated those who had received prescription statins (23 percent) from those who had not (77 percent). A final cohort of 2,240,638 patients—exactly half of those taking statins—was obtained by matching based on propensity, which took into consideration each patient's likelihood of receiving a statin. Statins had poor protective effects against pneumonia hospitalization (odds ratio [OR] 0.92; 95 percent confidence interval [CI] 0.89-0.95), all-cause mortality (0.87; 95 percent CI 0.84-0.89), and pneumonia mortality within 30 days (0.84; 95 percent CI 0.77-0.91). The significantly decreased with time, varied between risk groups and propensity score quintiles, and remained unaltered across post-influenza season periods after multivariate regulation and while omitting multiple clarifications for each individual. They suggested that statin use could have a statistically significant, but little, protective effect on influenza morbidity, which was merely attributable to public health authorities and residual confounding. Additionally, doctors must focus on minimizing mortality and morbidity from the ensuing influenza epidemic [53].

CONCLUSIONS

The immunomodulatory effects of statins are mediated by a number of mechanisms, including direct activation of heme oxygenase and inhibition of major histocompatibility type II complexes, which interfere with the interaction between leukocytes and endothelium. The most crucial mechanism is intracellular inflammatory signaling, however. GTP-binding proteins serve as molecular on/off switches for various protein kinases, which makes them essential for intracellular

inflammatory signaling. Both the cycle's inactive and active stages require isoprenylation for the route to be activated. By blocking 3-hydroxy-3-methylglutaryl-CoA reductase, statins lessen but do not completely abolish protein isoprenylation. The response strength of the impacted signaling pathway is decreased by this drop in prenylated protein concentration. As a result, it could balance out the "cytokine storm" associated with IVs. Based on the aforementioned effects, statins were found to have direct antiviral effects in some cases when used at physiologically appropriate concentrations. This suggests that they may prove to be an effective adjuvant treatment in the fight against pandemic and seasonal influenza, particularly in health systems that cannot afford expensive vaccines or antiviral medications. Despite conflicting results from several research, statins are affordable and easily available medicines in underdeveloped nations. By controlling immune system cytokine overexpression and modulating severe inflammatory reactions, they may be used as preventative medication or therapy for influenza sickness.

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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ORIGINAL ARTICLE

Pyloric-preserving Pancreaticoduodenectomy with Duct to Mucosa Pancreatico-gastrostomy Reconstruction: Long-term Follow-up in a Tertiary Center

Shahriar Najafizadeh-Sari[1], Jamal A. Moghadam[2], Hamed Gholizadeh[2], Alireza Ranaei-Kenarsari[3], Mohammad Heiat[4], Mehdi Raei[5], Shahram Manoochehry[2]

[1] Baqiyatallah University of Medical Sciences, Tehran, Iran
[2] Department of General Surgery, Trauma Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[3] Department of Emergency Medicine, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
[4] Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran
[5] Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Correspondence: Shahram Manoochehry, shahram.manoochehry@yahoo.com

Abstract: *Background: Duct to mucosa Pancreatico-gastrostomy (DMPG) is a method for reconstruction of pancreatic mass following pancreaticoduodenectomy (PD). There are still controversies about the benefits of this surgical technique compared to the old ones. Objectives: This study aimed to evaluate the long-term outcomes of DMPG reconstruction following PD operation. Method: Through a prospective observational setting, 164 patients, undergoing PD surgery, with diagnosed pancreatic head or peri-ampullary cancer, were enrolled. A pylorus-preserving PD and DMPG were done for all patients to reconstruct the pancreatic mass. Patients were followed up to a mean of 21.06±14.12 months. Demographic data, risk factors, tumoral staging, and postoperative follow-up data were collected for the analytic study. Results: The mean survival of the patients was 21.06±14.23 months after surgery. DGE (delayed gastric emptying) (34.75%), pancreatic fistula (21.34%), bile duct fistula (7.31%), and hemorrhage (5.5%) were recorded as the most important complication in patients. Some post-operative complications were significantly observed in patients with diabetes mellitus (pancreatic fistula, increased amylase, and DGE), Smokers (bile duct fistula, hemorrhage, and increased Amylase), and the elders (bile duct fistula) (p-value <0.05). Conclusions: Although our results indicate that DMPG reconstruction is a safe technique, our findings did not confirm the priority of this method over the older techniques.*

Keywords: *pancreatic neoplasms, gastrostomy, jejunostomy, pancreaticoduodenectomy*

INTRODUCTION

Pancreaticoduodenectomy (PD) is the main surgical treatment option for ampullary and peri-ampullary cancers, and some modifications have been described for this surgical operation. Therefore, these surgical modifications, complications, and outcomes need to be evaluated.

Despite the surgical techniques and management improvements, there are still many concerns about its high incidence of post-operative complications and morbidity rate (30-50%) [1,2]. Pancreatic fistula is considered the most common postoperative complication followed by bile duct fistula, thromboembolism, intra-abdominal hemorrhage, intra-abdominal abscess, wound infection, and gastro-intestinal bleeding as other important complications [2].

Reconstruction with pancreaticojejunostomy (PJ) is the preferred anastomosis technique following PD surgery. While this method has been commonly used for several decades, its high prevalence of complications (40-50%) prompts surgeons to come up with new approaches [3-5].

Duct to mucosa pancreaticogastrostomy (DMPG) is a method of reconstruction following PD surgery. There are still controversies about its benefits compared to previous ones. Few studies are available evaluating DMPG's outcomes, and complications [6-8]. This study aimed to evaluate the long-

term outcomes of DMPG reconstruction following PD operation.

MATERIALS AND METHODS

Patients

In this prospective observational study, patients with the diagnosis of pancreatic head or peri-ampullary tumors who underwent PD surgery at Baqiyatallah Hospital from January 2007 to February 2016 were enrolled. Informed consent was taken from patients. All operations were performed by the same surgeon. Patients were excluded from the study if failed to follow or their data were incomplete or inaccessible. Altogether 191 patients were included. During the study, 27 patients were excluded, (12, due to changes in their address, and 15, because of incomplete data and/or failure to follow). The study was approved by the ethics committee of Baqiyatallah University of Medical Sciences (national approval number: IR.BMSU.REC.1393.87).

Surgical Procedure

In all operations, a right subcostal incision was made and a pylorus-preserving PD and DMPG (using 4-0 polydioxanone sutures) were performed for the reconstruction. The Head of the pancreas was transected with at least a 2 cm macroscopic surgical margin from the mass, usually at the margin of the head and neck of the pancreas. The proximal duodenum was transected about 1.5-2 cm distal to the pylorus.

During the DMPG reconstruction, a small piece of the feeding tube was inserted into the DMPG anastomosis, like a stent, to secure the patency of the anastomosis within the first few days. The tube was not fixed with sutures. An end-to-side duodeno-jejunostomy hand-sewn anastomosis was made using 3-0 vicryl sutures. The coledoco-jejunostomy anastomosis was created about 50 cm distal to duodeno-jejunostomy using 3-0 Vicryl sutures. Tumor Lymphadenectomy was performed in all patients with palpable regional lymph nodes. A Penrose drain was inserted in the bed of the surgical site, and a closed-suction drain was placed in the Morison pouch.

Data Collection

Demographic information including age, sex, risk factors for the disease and surgery, history and laboratory, data essential to calculate the American Society of Anesthesiology scoring (ASA) class, and clinical staging of the tumor were collected and recorded into a checklist before surgery.

Duration of the operation, length of stay (LOS) in the hospital, post-operative complications, short- and long-term outcomes, pathologic result of the sample, morbidity, and mortality were recorded into the checklist during close follow-up. The patients were followed up to a maximum of 11 years or up to the date of death. The post-operative complications include pancreatic fistula, bile fistula, thromboembolic events, intra-abdominal fluid collection such as hemorrhage and abscess, wound infection, gastrointestinal bleeding, and gastroparesis. International Study Group on Pancreatic Fistulas (ISGPF) criteria was used to estimate pancreatic fistula [9]. Delayed gastric emptying (DGE) was graded according to the validated criteria of the ISGPF classification [10]. Pre-operative ASA classification was also assessed according to the ASA classification of physical status [11].

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows (version 21, IBM Corp, NY). Averages were expressed as mean ± SD. Kolmogorov-Smirnov test was used to assess the normal distribution of data. Possible statistical relationships between the risk factors and complications after the surgery were calculated using student t-test, ANOVA, and Chi-Square tests. The p-values equal to or less than 0.05 were defined as statistical significance levels.

RESULTS

In this study, a total of 164 patients with a mean age of 62.21± 9.34 years (including 93 males and 71 females) were included. The demographic information, risk factors, and also the number of cases in each ASA class are demonstrated in Table 1. Most of the patients were in class II of ASA classification (45.73%). According to the exclusion criteria, the patients with class V of ASA classification were excluded from the study.

Table 1: Demographic information, risk factors, and ASA class of patients

Variable (N=164)		
Age, (Mean± SD)	62.21 ± 9.34	
Sex, n (%)	Male	93 (56.70)
	Female	71 (43.29)
Smoking, n (%)	58 (35.36)	
DM, n (%)	48 (29.24)	
IHD, n (%)	42 (25.60)	
ASA Class n, (%)	I	15 (9.14)
	II	75 (45.73)
	III	64 (39.02)
	IV	10 (6.09)
	V	0 (0.00)

DM: Diabetes Mellitus, IHD: Ischemic Heart Disease, ASA: American Society of Anesthesiology scoring

Adenocarcinoma was the most prevalent tumoral type in this study (41.46%). Less common pathologies were cholangio-

carcinoma, ampullary carcinoma, high-grade dysplasia, neuro-endocrine carcinoma, and intraductal papillary mucinous neoplasm (IPMN). The mean pancreatic duct size was 4±1 millimeters. According to the TNM tumoral staging, 55 (33.5%), 70 (42.7%), and 39 (23.8%) of tumors were classified as T1, T2, and T3, respectively.

Post-operative data such as complications, mortality rate, and their relationship with the risk factors are illustrated in Table 2. In brief, DGE (delayed gastric emptying) (34.75%), pancreatic fistula (21.34%), bile duct fistula (7.31%), and hemorrhage (5.5%) were recorded as the most important post-operative complications in patients. Some post-operative complications were significantly observed in patients with diabetes mellitus [pancreatic fistula (p-value 0.038), increased amylase (p-value 0.010) and DGE (p-value 0.024)], smokers [bile duct fistula (p-value 0.027), hemorrhage (p-value 0.026) and increased amylase (p-value 0.045)] and the elders [bile duct fistula (p-value 0.006)]. No patient reported a history of alcohol consumption as a risk factor for pancreatic cancers.

Table 2: Post-operative data; and their relationship with the risk factors

Complications		Descriptive, n(%)	Age (P)	Sex (P)	Smoking (P)	DM (P)	IHD (P)	HTN (P)
Pancreatic fistula	0	129 (78.65)	0.690	0.607	0.293	0.038*	0.777	0.919
	A	6 (3.65)						
	B	17 (10.36)						
	C	12 (7.31)						
Bile duct fistula		12 (7.31)	0.006*	0.626	0.027*	0.99	0.506	0.134
PGIB		9 (5.48)	0.075	1.00	0.722	0.722	1.00	0.733
IAAC		23 (14.02)	0.174	0.176	0.062	0.272	0.9701	0.120
TE		18 (10.97)	0.503	0.266	0.740	0.134	1.00	0.375
SSC		23 (14.02)	0.117	0.664	0.950	0.392	0.955	0.493
Amylase[a]		34 (20.7)	0.943	0.619	0.045*	0.010*	0.755	0.170
DGE		57 (34.75)	0.611	0.791	0.117	0.024*	0.418	0.949
Hemorrhage		9 (5.5)	0.409	0.459	0.026*	0.199	0.838	0.896
Reoperation		18 (10.97)	0.332	0.366	0.169	0.213	0.567	0.908
R-resection[b]		30 (18.29)	0.503	0.223	0.869	0.429	0.883	0.909
Survival (month)		21.06±14.23	0.282	0.915	0.665	0.924	0.616	0.291
LOS (day)		13.12±7.15	0.115	0.880	0.800	0.580	0.700	0.241

PGIB=Post-operative-Gastrointestinal Bleeding, IAAC= Intra-abdominal abscess collection, TE= Thromboembolism, SSC= Surgical Site Complication, DGE= Delayed gastric emptying, LOS= Long of stay, P= p-value

[a]: Significant increasing amylase = triple time level for more than 3 days

[b]: Surgical resection margins were not restricted to malignant tumors, and the surgeon had to conduct more resection to reach a free margin

*The character * in the above P values indicates that there is a significant statistical relationship between the two methods of surgery*

The mean follow-up time of the patients was about 21.06±14.12 months. The mean survival of the patients was 21.06±14.23 months after surgery. There were 5 early post-operative mortality; one case due to massive lung embolism, three because of post-operative intra-abdominal hemorrhage, and one patient due to myocardial infarction. The survival rate of patients who were diagnosed with adenocarcinoma was 19.93±15.02 months. Survival rates for other groups are shown in Table 3. Also, the mean survival years in each pathologic type of tumor are shown in Figure 1. The longest survival is seen in non-malignant masses and the shortest survival is seen in PMN tumors.

DISCUSSION

Pancreaticoduodenectomy is an accepted surgical treatment for pancreatic head and peri-ampullary cancers. However, its complications have made surgeons find a better surgical technique in recent years [12].

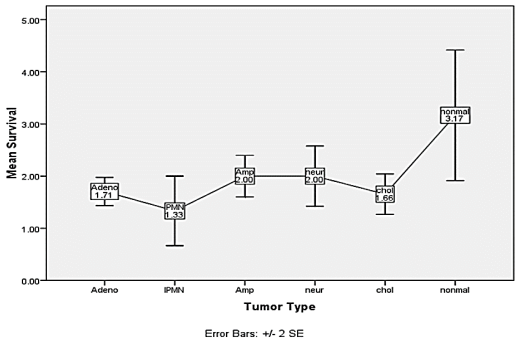

Figure 1: Mean survival year of the patients for each tumor type (*Adeno: Adenocarcinoma, IPMN: Intraductal papillary mucinous neoplasm, Amp: Ampullary Carcinomacarcinoma, Neur: Neuroendocrine tumor, Chol: Cholangiocarcinoma*)

PJ reconstruction is one of the accepted and common methods

of anastomosis after the excision of the pancreatic head and duodenum. However, the current method has many challenges such as a high rate of postoperative pancreatic fistula (POPF) and abdominal collections that are reported to be decreased in PG reconstruction in comparison to PJ reconstructions in different studies [13].

Table 3: The patients' survival rate (±SE); according to the pathologic type of tumor

Tumor / Survival	Ade (%)	IPMN (%)	Amp-Car (%)	Neur (%)	Chol (%)	Non-mal (%)	Total (%)
1-year	94±3	83±17	97±4	100	90±6	100	93.9±1.8
2-year	51±5	50±22	65±10	67±17	48±10	75±13	54.8±3.8
3-year	15±4	0	67±17	33±17	24±8	50±15	23.1±3.3
4-year	4±2	0	33±17	0	3±3	33±14	4.8±1.6
5-year	1±1	0	0	0	0	25±13	2.4±1.2
6-year	1±1	0	0	0	0	17±11	1.8±1.0
7-year	1±1	0	0	0	0	17±11	1.8±1.0
8-year	1±1	0	0	0	0	0	0.6±0.6
9-year	1±1	0	0	0	0	0	0.6±0.6
10-year	1±1	0	0	0	0	0	0.6±0.6

Ade: Adenocarcinoma, IPMN: Intra-ductal papillary mucinous neoplasm, Amp-Car: Ampullary Carcinomacarcinoma, Neur: Neuroendocrine tumor, Chol: Cholangiocarcinoma

Table 4: The Difference of Overall POPF Complication Rate in Recent Studies between PG and PJ Anastomosis after PD Surgery

Study	Year of Publication	PG		PJ		P value*
		N (%)	Total	N (%)	Total	
Wang et al. (DMPG) [7]	2014	2 (2.7)	75	-	-	-
Figueras et al. [4]	2013	10 (15.38)	65	20 (34.48)	58	0.006*
Topal et al. [14]	2013	13 (8.02)	162	33 (19.76)	167	0.002*
Wellner et al. (grade B/C) [20]	2012	6 (10.16)	59	7 (12.28)	57	0.775
Current study (DMPG)	2018	35 (21.32)	164	-	-	-

PG= Pancreatico-gastrostomy, PJ= Pancreatico-jejunostomy, DMPG= Duct to mucosa Pancreatico-gastrostomy
*The character of * in the above P values indicates that there is a significant statistical relationship between the two methods of surgery*

Table 5: Comparison of the various complication rates after PG Anastomosis in the previous studies

Complications/Study (Year, N)	Wang et al. (2014, 75) [7]	Figueras et al. (2013, 65) [19]	Topal et al. (2012, 162) [14]	Wellner et al. (2012, 59) [20]	Current Study (2018, 164)
Bile duct Fistula, n (%)	1 (1.3)	1 (2)	-	-	12 (7.31)
IAAC, n (%)	3 (4.0)	5 (8)	9 (6)	7 (12)	23 (14.02)
SSC, n (%)	3 (4.0)	-	65 (40)	_	23 (14.02)
DGE, N (%)	13 (17.3)	19 (29)	25 (15)	14 (27)	57 (34.75)
Hemorrhage, n (%)	2 (2.7)	13 (20)	21 (13)	6 (10)	9 (5.5)
Reoperation, n (%)	1 (1.3)	4 (6)	14 (9)	7 (12)	18 (10.97)
R-resection[a], n (%)	-	-	22 (10)	-	30 (18.29)
LOS (day, min-max)	19 (12-39)	12 (1-52)	19 (14-25)	15 (7-135)	14 (7-40)

PGIB=Post-GI Bleeding, IAAC= Intra-abdominal abscess collection, SSC= Surgical Site Complication, DGE= Delayed gastric emptying, LOS= Long of stay
a: Surgical resection margins were not restricted to malignant tumors, and the surgeon had to conduct more resection to reach a free margin

Duct to mucosa pancreatico-gastrostomy anastomosis is a method of anastomosis following PD surgery. There are few studies to show the distribution of complication rates after the DMPG surgical technique [7].

There are many accepted risk factors for POPF. Many risk factors are related to the pancreas itself and are different among patients; these include the size of the duct, histologic features, and the blood supply of the tail. However, many risk factors are related to the techniques of surgery such as anastomosis type after PD, stenting of the anastomosis, and blood loss during surgery [5,7,14].

In this study, there was also a significant relationship between the history of diabetes mellitus and the development of POPF (P=0.038). In a previous study, the risk of POPF was significantly higher in patients with diabetes mellitus (P=0.03). There was no significant relationship between age and POPF (P=0.69). Our finding in this regard is different from other studies [15]. Amylase levels and delayed gastric emptying (DGE) in diabetic patients were higher than in other patients.

In most studies, the male gender has a strong risk factor for pancreatic cancers. In our study, 56.7% of patients were male. However, gender was not an important risk factor for complications after surgery, the rate of bile duct fistula was higher in the elders significantly (P=0.006).

Smoking is another major risk factor for pancreatic cancer. The actual mechanism of this process is unknown. However, cigarettes contain thousands of carcinogenic compounds that can cause cancers [16]. Smoking is also an important risk factor for complications after surgery as seen in our study smokers developed higher rates of bile duct fistula and hemorrhage after the surgery. In these patients, the increase in amylase level was also higher than in other patients (Table 2).

In the current study, the prevalence of POPF was higher than the similar study on DMPG (21.32% vs. 2.7%), and also higher compared with PG (pancreatico-gastrostomy) reconstruction [17]. Although have no justification for that, it seems that DMPG doesn't have any priority in this regard. There are few reports on the comparison between POPF rates following DMPG and PJ anastomosis (21.32% vs. 12.28 and 34.48%) [18, 19]. In other studies, some complication rates such as hemorrhage, bile duct fistula, DGE, and also LOS are higher in PG in comparison to PJ anastomosis [17,19].

In this study, the prevalence of DGE was 34.75% which is higher compared to another study [5]. It might be because of the pylorus-preserving surgery done for the patient; however, this finding is not in favor of the priority of DMPG (Table 4). The prevalence of hemorrhage as a life-threatening post-operation complication was lower in this study (5.5%). On the other hand, the prevalence of bile duct fistula after PG was higher than in other studies. (7.31%, Table 5).

We calculated the DMPG survival rate for the first time according to a recommendation for future researchers by Topal et al [17]. The Survival rate after a mean follow-up of 21.06±12 months was 21.06±14.23 months in our study [17]. Survival rates for all pathologic types were similar and ranged from 1.33-2 years except for non-malignant tumors which resulted in a higher mean survival of 3.17 years (Figure 1).

Although our results indicate that DMPG reconstruction is a safe technique following PD, the claimed lower rate of post-operative complications and lower hospital stay is still in question and needs further investigation. Compared to other studies and the prevalence of this condition, the number of patients in this study is acceptable, however, it had some limitations. We did not perform subgroup analysis based on duct diameter. Also, pancreatic remnant consistency was not documented after our operations. Results might also be influenced by the surgical technique used for PG here. Also, we did not compare outcomes after DMPG with PJ anastomosis as a control group.

CONCLUSIONS

This study with a rather large number of patients and long-term follow-up after DMPG showed a lower rate of many post-operative life-threatening complications such as hemorrhage. On the other hand, the prevalence of bile duct fistula after PG was higher than in other studies. Though our results indicate that

Duct-to-mucosa pancreatico-gastrostomy reconstruction is a safe technique following PD however, does not confirm the priority of this method over older techniques. However, this contradiction calls for more studies including clinical trials and metaanalysis to clear all obscure dimensions.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Acknowledgments

None

Authors' contribution

SNS, JAM, HG, ARK, MH, MR, SM were responsible for the study concept and design. SNS, JAM, HG, ARK, MH, MR, SM led data collection. SNS, JAM, HG, ARK, MH, MR, SM were responsible for the analysis and interpretation of data. SNS, JAM, HG, ARK, MH, MR, SM wrote the draft.

Ethics approval and consent to participate

This study was approved by the ethics committee of Baqiyatallah University of Medical Sciences. Throughout the study, researchers adhered to the Declaration of Helsinki. Written informed consent was obtained from the participants in this research.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Primary Headaches and Their Most Important Imagistic Features in Clinical Practice

Răzvan I. Dumitru[1], Andreea F. Niculae[2], Florina A. Antochi[3], Adina M. Roceanu[3], Octavian Vasiliu[4], Anca M. Sirbu[5], Carmen A. Sirbu[6,7], Cristina A. Panea[6,8]

[1] Department of Radiology, Dr. Carol Davila Central Military Emergency University Hospital, Bucharest, Romania
[2] Department of Haematology, Colțea Clinical Hospital, Bucharest, Romania
[3] Neurology Department, University Emergency Hospital of Bucharest, Romania
[4] Department of Psychiatry, Dr. Carol Davila University Central Emergency Military Hospital, Bucharest, Romania
[5] National Institute of Medical Expertise and Work Capacity Recovery, Bucharest, Romania
[6] Clinical Neuroscience Department, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[7] Academy of Romanian Scientists, Bucharest, Romania
[8] Department of Neurology, Elias Emergency University Hospital, Bucharest, Romania

Correspondence: Florina Anca Antochi, flrant@yahoo.com

Abstract: *Background. Primary headaches are comprised of three big categories: migraines (with or without aura), the third most common and the 6th most disabling disease worldwide, tension-headaches, and trigeminal autonomic cephalalgias, each with different, complex, and not completely understood pathophysiological, clinical, and neuroimagistic characteristics. Structures like the brain stem, hippocampus, or hypothalamus have been depicted as being the main triggers of these types of headaches, which have a significant impact on the quality of life. Methods. We performed a broad literature review on primary headaches from January 2016 to June 2023, analyzing the latest scientific updates and emphasizing the pathophysiological and neuroimagistic characteristics. Results. We provide a concise review of the main neuroimaging studies that help in differential diagnosis between several types of primary headaches. Conclusions. It is difficult to approach the pathophysiology of these three primary headache categories. Keeping in mind that neuroimagistic methods are rapidly developing (especially in the MRI sphere), the most important endpoint of future studies will be to establish a reliable distinction system for all these types of cephalalgias, thus updating the protocols and guidelines. Currently, imaging studies are indicated only in certain circumstances.*

Keywords: *migraine; tension-type headache; pathophysiology; trigeminal autonomic cephalalgias; imaging studies; cluster headache; SUNCT; MRI; fMRI; SUNA*

INTRODUCTION

Headache is a recurrent and upsetting symptom in the neurological sphere, also being one of the most frequent ones, patients often present to the physician for it. Most of the studies suggested that more than 90% of the population has suffered from headaches at least once in their lives [1]. The diagnostic standard for headaches of any cause has been offered over the past two decades by ICHD since 2004 and today reaches the updated ICHD-3 [2]. This accounts for approximately 3% of emergency department admissions, but some authors have identified percentages as high as 8% [3,4]. It is important to emphasize that the repeated presentation of a patient to ED for the same symptoms significantly increases the costs associated with medical care. These recurrent presentations occur in approximately 10% of headache patients [3]. When a severe headache begins, patients present to the ED frightened by the thought of a serious condition. However, one study showed that of 3026 imaging studies of patients presenting to the emergency department, pathology was objectified by imaging in less than 3% of them [5].

Given the high prevalence of this type of neurologic symptomatology, clinicians must carefully consider the indication for imaging studies because they are expensive and usually do not provide the desired information to guide subsequent treatment. In addition, headaches can lead to absenteeism from school or work, increasing the indirect costs associated with pathology. In a recent study, workers with

headaches were observed to have low productivity or, more rarely, to be absent from work for up to 7.5 days/month [6].

Headaches of a primary cause are set apart by the presence of headache without any underlying pathology. These are far more common than secondary headaches. For example, the prevalence of TTH is as high as 70% during a person's lifetime [7]. In the past, categorization of these pathologies has been difficult because symptoms vary from person to person, but also in the same individual over time [8]. Standardization of primary cause headaches has been achieved by the ICHD-3 classification, which divides primary headaches into migraine, both with aura or without it, tension-type headache (TTH), and cephalalgias which belong to the trigeminal autonomic sphere [2,9]. The mechanism of action of these pathologies is not yet known, but research is ongoing. In this paper, we propose to present the situations in which imaging studies are indicated.

MATERIALS AND METHODS

We reviewed the literature related to primary cause headaches by searching the Google Scholar and Pubmed English databases between 2000 and 2023 with keywords such as "headache", "cephalalgia", "imaging", "MRI", "primary", "migraine", "tension-type", "cluster", "trigeminal", "autonomic", or any logical combination of these terms. In the first stage, the retrieved studies were filtered by a title check to exclude duplicates and assess their relevance. After this first step, the outstanding studies were distributed evenly and randomly to the authors of this study, in the first phase by reviewing the abstract to determine relevance and later by studying the entire text. We have extracted relevant, basic information from these articles, but also important news that may change the paradigm of primary headaches.

RESULTS

Migraine

Migraine is a disabling disease, being the most prevalent type of primary headache, with 12-15% of the general population suffering from it [10,11]. Prevalence is different between males and females, and also it varies by the age group: interestingly, in children, puberty, this pathology is more common in boys. In young adults, it differs, being more usual in females, probably due to the hormonal changes they undergo [12]. Throughout life, the prevalence is 12-17% for women and 4-6% for men [13]. It manifests as moderate to severe headache episodes, usually unilateral, with mild to moderate cognitive deficit and cortical hyper-excitability manifestations such as phonophobia or photophobia, nausea, and vomiting. Two phases of migraine are described: the ictal phase, which occurs during a migraine attack, and the interictal phase, which occurs between two migraine attacks.

Up to 30% of patients with headaches accuse migraine attacks, and their frequency also influences cognitive deficits [14,15]. The ratio of men to women is 1:3. According to the ICHD-3, the main types of migraine are those without aura, those with aura, chronic-type migraine, complications of migraine, probable migraine, and episodic syndromes associated with migraine [16]. Migraine appears as a result of electrical abnormalities occurring in the brainstem, and vascular abnormalities in the cerebrum, and when we talk about migraine with aura, the result of cortical spreading depolarization [17].

The aura manifests most often as a visual aura (80-90% of cases), characterized by flashes of light in the simplest situations to scotomas or complex hallucinations [18].

Four phases of migraine have been described: the prodromal phase, in which the first warning symptoms appear (depression, fatigue, hunger, food cravings), which are most likely associated with dopaminergic activation and precede the headache by 2-48 hours [19]; the aura (which occurs in one-third of migraine patients) represents reversible neurological symptomatology that usually lasts about one hour and it usually manifests as sensory, verbal or motor symptoms. However, the visual ones are the most common. These changes come before the start of the cephalalgia [16,20]; headache is commonly on only one side of the head, having a progressively aggravating pattern at the beginning, being linked with autonomic symptoms like photophobia or phonophobia, or with symptoms like nausea, sickness-like condition or vomiting [20]; the postdromal phase, which is associated with a localized headache that is aggravated by movement, fatigue, or dizziness.

Migraine can be clinically assessed by structural MR imaging. For example, we now know that both aura and headache are mainly associated with vasoconstrictive changes in cerebral arteries and veins. Hypersensitivity in the region innervated primarily by cranial nerve V, but also in extracranial regions, causes allodynia when a normally painless stimulus becomes painful, such as wearing glasses or a light touch to the face.

MRI examinations are not routinely recommended in migraine patients without aura when preventive treatment is not necessary, and some authors recommend imaging studies when three lines of preventive treatment fail [21]. Another recent study emphasizes that imaging examinations are not needed in migraine patients without red flags [22]. In migraine patients with aura, no examinations are recommended in typical cases, and in the remaining ones, MRI or MRA of the brain is recommended to rule out vascular anomalies [21]. Cerebral MRI is also recommended for vascular comorbidities

or headache onset after 50 years of age. MRI is not recommended for chronic migraine, especially if the history shows progressive worsening of headache with typical symptoms [21]. In patients with persistent aura, hospitalization, and diffusion-type imaging are mandatory to rule out cerebral ischemia.

Migraineurs are sensitive to olfactory stimuli. Up to 40% of them complain of headache exacerbation when exposed to strong, irritating, or sensitizing odors, even in the interictal phase, with studies demonstrating abnormal activation of the amygdala and insular cortex in the case of certain crises caused by odor stimuli [23,24]. On the other hand, other studies determining thermal stimulation showed unequal activation of certain areas between migraine patients and the control population, such as in the lentiform nucleus, parahippocampal gyrus, subthalamic nucleus, prefrontal cortex, or temporal lobe [25,26]. 40% of migraineurs report that their migraines are activated by strong light stimuli in the interictal phase, and 90% of them report that they suffer from photophobia in the ictal phase. Some studies demonstrate visual-nociceptive integration at the brainstem level [27].

Usually, imaging studies are not required in patients with a normal clinical examination who present with typical migraine symptoms according to the ICHD-3 definition [20,28]. To distinguish between patients who do not require additional imaging studies and patients with warning signs who do require imaging studies, the SNOOP algorithm is used successfully, as follows: S for systemic disease (e.g., infections, HIV), N for neurologic assessment (confusion, seizures, focal neurologic signs), O for onset (sudden onset, first episode after age of 50), O from other associated features (medications, toxins, trauma, association of headache with Valsalva maneuver), and P from previous episodes (or headache progression). The American College of Emergency Physicians 2019 (ACEP 2019) and American Academy of Neurology (AAN) guidelines recommend imaging studies only for warning signs that are not indicative of a common headache [29,30]. The American College of Radiology (ACR) emphasizes that clinical physicians should not perform repeated imaging studies when the headache is not complicated [31].

However, in migraine, deep white matter lesions (Figure 1A, T2, and FLAIR hyperintensities) are seen in 20-30% of MRI examinations, regardless of age or periventricular white matter lesions. Their exact pathophysiology is not known, but two theories have been mainly discussed: the theory of ischemia due to circulatory disturbances in the small perforating vessels together with inflammatory changes with consequent hypoperfusion, evidenced in MRI imaging by the appearance of small focal ischemic spots, mainly at the level of the frontal lobe (Figure 1B), but also in the corpus callosum, and the theory of neuroinflammation [32,33]. The latter emerged after the CAMERA study, which highlighted in females that deep WML takes place without a connection with ischemic lesions [32]. It is noteworthy that numerous subsequent studies have highlighted the fact that in migraine, the deep supratentorial white matter lesions are important but these do not correlate with the development of the disease, whereas the infratentorial lesions do not affect migraine attacks [30]. In addition, migraine patients appear to have a higher risk for cerebrovascular disease, but also doubling the risk of ischemic stroke [34,35].

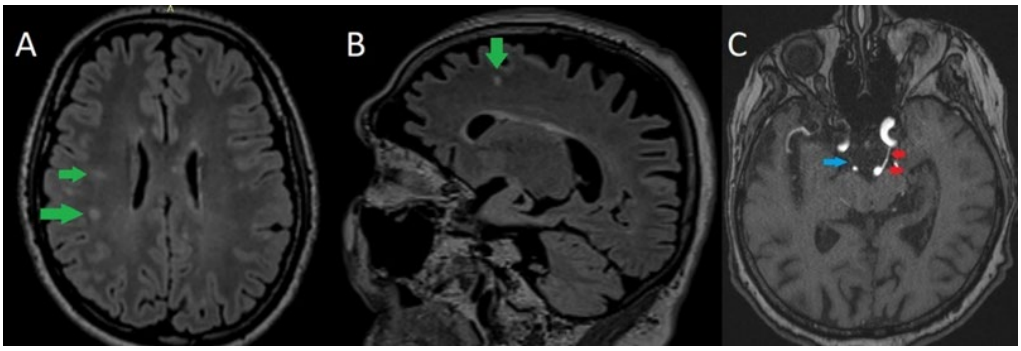

Figure 1: A: T2-weighted and FLAIR hyperintense lesions, in the centrum semiovale on the right side and deep white matter in a patient accusing repeated migraine attacks (green arrows). **B**: T2-weighted and FLAIR hyperintense lesion in the superior frontal gyrus (green arrow), the usual location for demyelinating lesions in migraine patients. **C**: asymmetry of the circle of Willis, showing a filiform right posterior communicating artery (blue arrow) compared to the contralateral one that shows significant blood flow (red arrows).

In migraine with aura, there may be transient restricted diffusion in the affected areas during the aura, in many studies taking place, especially in the corpus callosum [36].

Following some recent studies using MRI angiography (MRA), a new theory arises, advocating that there are asymmetries pointed out in the circle of Willis in a bigger population of

migraine patients, particularly with aura (Figure 1C) [37,38].

In summary, in migraine patients without aura, imaging studies are not recommended for usual symptomatology that does not require treatment. For treatment-emergent migraine episodes, some authors recommend cerebral MRI or MRA when three lines of therapy have failed. In migraine patients with aura, an MRI of the brain is almost always recommended, or MRA if posterior fossa symptoms are present. In chronic migraine patients, additional imaging studies are not recommended as long as the symptomatology is not progressing, as long as the symptoms do not progress, but fundoscopy should be an integral part of the clinical examination in all patients with headaches [2,21].

Tension-type headache (TTH)

Tension-type headache represents a mild to moderate cephalalgia with a pressure-like character and usually occurs bilaterally, sometimes with anterior or posterior topography and sometimes it is longitudinal, lasting from minutes to days or even weeks. In contrast to migraine, it does not go along with autonomic manifestations or nausea and vomiting, and the presence of the above features precludes the diagnosis of tension-type headache. ICHD-3 classified TTH into four broad categories: infrequent epi-sodic TTH lasting < 1 day/month, frequent episodic TTH lasting 1-14 days/month throughout> 3 months, chronic TTH with a duration of > 14 days over > 3 months, and probable TTH [16]. The best diagnostic tool for tension-type headaches is the creation of a diary in which the patient records the frequency of painful episodes, their intensity, and their characteristics [39]. Considering the lower pain intensity, a lower proportion of patients with TTH consult a physician compared with migraine patients. One study has shown that the number of physician visits for TTH is 3 times lower than for migraine patients [40].

TTH is the second leading cause of chronic disease worldwide and is a socioeconomic burden, with 820 days missed from work or school per 1000 patients [41,42]. The most common differential diagnosis is migraine, as migraine patients often overlap with tension-type headaches.

The reported prevalence is 70% of the population, although 59% of patients report only episodic TTH [7,43]. Prevalence peaks in the 30-to-39-year-old age group and then decreases slightly [44]. Several studies have observed that sleep deprivation in combination with a disorganized lifestyle contributes to the occurrence of TTH [42,45].

Central factors are particularly important in tension-type headaches. While the pain threshold is normal in patients with eTTH, this threshold is somehow lower in tension-type headache patients with a chronic form [46,47]. Negative states, like depression or anxiety, and also chronic stress in TTH patients can increase the risk of headaches by lowering the nociceptive threshold [48,49].

In a study from Taiwan, it was suggested that the risk factors for TTH were more than 7 days of headache and female gender [50].

To date, although we use more and more advanced imaging techniques, the TTH pathophysiology is still not completely elucidated. Myofascial factors are incriminated to have a crucial role in episodic TTH along with pericranial muscle contractures, whereas sensitization of nociceptive sensory pathways by repetitive headaches leads to chronic TTH [51-53].

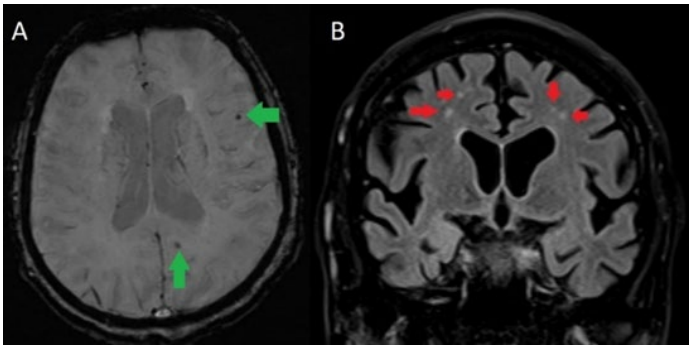

Figure 2: A. Magnetic susceptibility sequence in the axial plane. Changes in the magnetic susceptibility sequence with a suggestive appearance for microhemorrhages in the deep white matter and cortico-subcortical junction. **B**. Lesions in T2 and FLAIR hypersig-nal at the level of the bilateral corona radiata, in TTH patients without being specifically associated with some deep localizations such as in migraineurs.

As we already found out in the migraines chapter, imaging studies should not be carried out with typical-ly-described symptoms. More specifically, in patients with infrequent tension-type headaches, imaging is not recommended. In patients with frequent or chronic TTH, similar to migraine, some authors recommend an imaging examination when three lines of preventive treatment have failed [21]. Patients with chronic tension-type headaches should undergo one

single imaging examination, either MR or CT, at the initial admission in the ED to exclude other differential diagnosis pathologies. The same applies to the TTH patients. The latter ones will generally have small foci of hemorrhage defined as hypodensities on susceptibility-weighted sequences deep in the white matter (Figure 2A) and deep WML, having the same physiopathological substrate as the migraineurs, increasing the risk of dementia or stroke later in life [54-56]. TTH and migraine patients generally have a similar number and amplitude of deep WML, but contrary to migraineurs, the TTH-associated WML does not show a preferred topography (Figure 2B). However, studies have shown that imaging examinations are not recommended because they do not improve the diagnosis and treatment, but they can cause and increase anxiety related to health status [57,58]. Acetaminophen and aspirin are thought to be efficient in cutting down acute symptomatology of hypertensive headaches. Opioids or triptans should be avoided. Ibuprofen and ketoprofen are the most effective NSAIDs for pain relief. Most patients benefit from lifestyle changes that promote a healthy lifestyle by quitting smoking and eating foods rich in antioxidants, and nonpharmacologic and behavioral therapies such as relaxation, biofeedback, or acupuncture can be helpful in most cases.

Trigeminal autonomic cephalalgias (TAC)

Cluster headache

Cluster headache is not a common headache, but is the most prevalent one in the group of TAC cephalalgias, having a prevalence of about 0,1% in the general population, affecting males and females differently, in a 6:1 manner. Many of the patients affected are older than 20 years of age [14,59]. Its most characteristics are severe pain with spontaneous remission, recurrent (usually circadian), unilateral, lasting 15-180 minutes, associated with autonomic ipsilateral cranial clinical signs such as lacrimation, conjunctival injection, or nasal congestion, the latter often leading to the misdiagnosis of sinus pain, with a completely different treatment from cluster headache [16,60]. In rare cases, cluster headaches may occur without autonomic symptoms [61].

80-90% of patients have episodic cluster headaches that occur apart from circadian development related to season (especially spring and autumn), number of daily light hours, and sleep stage, being more frequent during REM sleep [62]. Moreover, the relationship between sleep and cluster headache is very close. Many studies have shown that approximately 80% of patients who have manifestations of cluster headache will present cephalalgia attacks in the middle of the night, disturbing their sleep, but only 30% of them will show cephalalgias when they are napping during the day [63]. However, most of the patients complain of disturbed sleep quality, whether it occurs during or outside the headache period, and as a compensatory measure, some patients forget nightly sleep to avoid developing cephalalgic episodes [64]. An association between cluster headaches and sleep apnea has also been noted [63]. 10-20% of patients suffer from chronic cluster headaches, which have a very short period of remission, generally of the order of days.

The physiopathologic mechanism of this pain is not fully known. Both neurovascular causes and central mechanisms are suspected [65,66]. Given the predominant development with circadian recurrences, it has been suggested that the hypothalamus is of central importance for the development of pain, and also the trigeminovascular system and the parasympathetic systems being incriminated for starting and maintaining the symptomatology, those being involved in the autonomic trigeminal reflex [67]. Even though the V1 division of the 5th cranial nerve plays an important role in the development of algic symptoms, the autonomic ones are developed by the parasympathetic fibers of the facial nerve [68]. Since the 1990s, but also in more recent research, a genetic substrate is suspected, as there is a familial predisposition [69-71].

An association between cluster headaches and smoking has been confirmed. A recent study showed that 88% of the participating patients were active smokers or had smoked in the past [72]. When partial Horner syndrome occurs, cerebral MRI scans and targeted imaging studies at the level of the lung apex must be performed to identify lesions of the recurrent laryngeal nerve. Polysomnography may also be performed in cluster headache patients, as it is often associated with restless legs syndrome and helps to identify and treat this pathology [21].

In all types of TAC, cerebral MRI with high-resolution sequences of the cranial nerves is required at baseline to evaluate the parasellar structures. Similar to migraine, MRA is recommended if the first three lines of preventive treatment fail [21]. Changes in some brain structures were observed on imaging, particularly at the level of the thalamus, nucleus cinguli, prefrontal, parietal, and insular cortex [73,74]. Some older studies showed structural changes in the hypothalamus by MRI scans when headache was active, whereas other studies did not find these changes [75-77]. Some authors suggested that a decrease in grey matter volume in the visual cortex can occur, but it is non-specific [78,79].

Treatment of cluster headaches is divided into acute episode treatment and prophylactic treatment [80]. It includes drug treatment as well as patient education and lifestyle modification. As lifestyle changes, we can mention the

avoidance of alcohol consumption, daytime sleep, or excessive physical activity. Treatment of the acute episode is initially by administering 100% oxygen by mask, up to 10 L/minute, for 15-20 minutes, which can relieve the cephalalgic episode in up to 15 minutes. Other therapeutic options include sumatriptan, a serotonin receptor agonist known to be the most effective therapeutic option and effective within 15 minutes when administered intravenously; dihydroergotamine, effective within 10 minutes when administered intravenously; zolmitriptan, effective within 30 minutes when administered orally; and local anesthetics [81]. Prophylactic treatment consists mainly of prednisone, dexamethasone, and ergotamine derivatives.

SUNCT and SUNA

SUNCT and SUNA are the other two types of TAC. Together, they are referred to as SUNHA [82]. The etiology and pathophysiology are not fully known. The association between these pathologies and posterior fossa lesions is well known, and recently the association between pituitary adenomas and these headaches has been discussed [83]. The pain generally occurs in the trigeminal area, more specifically in the V1 division and there is a high difficulty in differentiating between SUNHA and trigeminal neuralgia. These headaches have a sharp or burning character, last for a minimum of 5 seconds to a maximum of 10 minutes, and have a "sawtooth" development. A minimum of 20 pain attacks correlating with autonomic symptoms is required for clinical diagnosis, criteria defined by ICHD-3 [16,84]. The incidence is 0.1 per 10,000 population and the prevalence is 0.6 per 10,000 population [85].

The physiopathologic mechanism incriminated is represented by the activation of the trigeminal reflex and, similar to cluster headache, by triggering the hypothalamus [86]. Some recent studies have shown that trigeminal neurovascular conflict is important in the etiology of SUNCT and probably SUNA, with neuroimaging highlighting the similarity of changes in both pathologies, even assuming that the distinction between these two entities can be discarded [87,88]. High-resolution MRI imaging revealed neurovascular conflicts in more than 85% of patients with SUNCT or SUNA [87]. In addition, a recent study demonstrated ischemic changes in the left ventricular septal defect in approximately 15% of patients, leukoaraiosis in 5% of patients, and brainstem infarction in 5%. 88% of all patients had a vasculonervous conflict between the trigeminal nerve and certain vascular branches [85].

Therapeutically, the administration of many drugs used in other primary headaches has been tried, but with rather poor results. In this respect, SUNCT and SUNA differ from the other primary headaches. They respond mainly to the treatments used to treat neuropathic pain. Lamotrigine is effective in a large percentage of patients with the first pathology and less effective in the latter and is administrated at a dose of 100 to 300 mg daily. Topiramate isn't as effective as lamotrigine, and a smaller percentage of patients report symptomatology improvement. Carbamazepine is extensively studied and given in the treatment of these two pathologies, although with some poor results for the improvement of symptomatology in the SUNA group of patients. It is generally administrated in the standard dose of 200-1600 mg. Lidocaine is administered in an intravenous infusion of 1-4 mg/kg/hour to relieve severe headache attacks. Attempts may also be made to damage the trigeminal nerve or Gasser's ganglion by stereotactic surgery, radiofrequency stimulation, glycerol gangliolysis, or mechanical compression [89]. It is important to note that SUNCT and SUNA may also occur secondary to lesions at the level of the posterior fossa: damage to the cerebellum or brainstem by ischemic, tumoral, or vascular mechanisms. It is important to note that high-resolution MRI imaging studies can provide details about neurovascular compression, but these are not essential [2,21].

Hemicrania continua (HC)

Hemicrania continua is another primary headache that presents as a unilateral headache of moderate intensity with development over several months and belongs to the group of TACs according to ICHD-3 [2]. As the name implies, the pain is continuous and constant, but sometimes exacerbations associated with autonomic symptoms may occur. The response to indomethacin is pathognomonic [90]. It is estimated that the incidence of pathology is 1.0% and that continuous hemicrania accounts for approximately 1.5% of all primary headache cases [90]. Cases of continuous bilateral hemicrania were rarely presented [91]. According to ICHD-3, a clinical diagnosis requires a unilateral headache with pain duration of more than 3 months, ipsilateral autonomic symptoms, restlessness associated with the symptoms, and response to indomethacin treatment. Exacerbations may occur as a result of stress, alcohol consumption, or, in women, menstruation [90]. If HC is clinically suspected, cerebral MRI is recommended in all patients, and MRA or CT angiography of the head and neck should be indicated if an aneurysm or vascular pathology is suspected. Response to treatment with indomethacin is pathognomonic. Start with a dose of 25 mg three times daily, and if the patient does not respond to this treatment within 2-3 days, the dose is gradually increased up to a maximum of 300 mg per day [92].

Other primary headaches

Primary cough headache (PCH) represents a rare disorder in which bilateral cephalgia starts suddenly during coughing and

may last up to two hours. According to ICHD3, the clinical diagnosis of primary-type headache is made by at least two episodes of pain that occur only after coughing or the Valsalva maneuver and last between one second and two hours, with no other associated intracranial pathology. However, up to 50% of patients hospitalized for this type of pain may have underlying lesions, such as Chiari malformation type 1, obstructive hydrocephalus, or tumors. Therefore, it is strongly recommended that imaging studies of the brain be performed, such as MRI or MRA [2,21,93]. Indomethacin is the treatment of choice for this type of headache [2].

Primary exercise headache (PEH) is a rare pathology similar to PCH, characterized by a bilateral pulsating headache that occurs only in the context of prolonged physical activity, lasting 45 minutes or less [2]. According to ICHD-3, when the patient first presents to the hospital with symptoms, it is mandatory to perform imaging studies to rule out "subarachnoid hemorrhage, arterial dissection, or vasoconstriction syndrome." Indomethacin is the treatment of choice for this pathology, in a dose of 25-150 mg before starting physical activity [94].

Primary headache associated with sexual activity is a diffuse bilateral headache that begins during sexual activity and lasts from 1 minute to 24 hours, accompanied by nausea or vomiting but without autonomic symptoms. These may present as progressive or sudden headaches, most often beginning at the moment of climax. Although it is generally not a serious medical problem, brain imaging is recommended to rule out other pathologies, such as subarachnoid hemorrhage or aneurysm [95]. It is difficult to quantify the epidemiology of this pathology because patients are reluctant to go to the doctor to be examined due to shame or ethical reasons. Some authors recommend prophylaxis with beta-blockers and symptomatic treatment with indomethacin [96,101].

Because of the rarity of these pathologies, there are no large, homogeneous studies quantifying the imagistic changes specific to each of these three pathologies, but rather small studies and case reports. For this reason, it is important to undergo a CT scan, especially in cases of sudden onset headache, to rule out secondary causes such as rupture of an aneurysm or subarachnoid hemorrhage, and subsequent vascular studies such as CT angiography or MRA are recommended in specific cases.

DISCUSSION

Despite the use of increasingly advanced imaging examination methods that have improved the understanding of the pathophysiology and mechanisms of development of primary headaches, the medical community still cannot fully understand and fully, optimally treat primary headaches. Structural MR imaging has demonstrated the presence of T2 and FLAIR hypersignal lesions that can be correlated with different pathologies that mimic the primary types of headache, although those appear especially with aura migraine. MR Perfusion imaging has shown several abnormalities in blood circulation and neuronal activity during cephalalgia exacerbations in every type of primary headache, demonstrating a lowering of pain threshold and, compared with the general population, deficient activation of many different areas in the brain responsible for interpreting pain, and in the case of migraineurs in particular, there is both a supernumerary activation of the areas as well as a more important or intense activation of them. Many authors have demonstrated that the autonomic nervous system takes part in the mechanism of headache development, due to age-related development and the decrease in headache amplitude in the case of noninvasive stimulation of the vagal endings for cluster headache prevention. These suspicions, especially in migraine, are not confirmed by imaging studies, either structural or functional. The use of imaging studies, regardless of their type, to diagnose migraine or tension-type headaches is not recommended because of the lack of specificity of the studies. Because these are debilitating diseases that affect both individual lives and society, sufferers search for answers and go from one physician to another in search of them. Repeated imaging studies in these cases represent a significant waste of resources, and each pseudonormal imaging result leads to even greater concern and anxiety about health status. The correlation of the new, recently discovered data with the clinical evolution of the patient can improve the selection and treatment of patients at risk for primary headache development, sometimes even from childhood. Today we know that primary headaches begin around the age of 5 years and the highest prevalence is found in the age group between 20 and 40 years, decreasing with age. The phenotypes and treatments of each pathology are different, even if they belong to the same class of pathologies. Currently, we can summarize that there are ischemic lesions, circulatory disturbances, vascular conflicts, 5th cranial nerve activation, and a lower pain threshold, all having their role in the development of these pathologies. Current studies include far too small and inhomogeneous groups of patients to be able to present convincing results regarding structural changes in the brain parenchyma or the various neuronal circuits.

CONCLUSIONS

In recent years, significant progress has been made in understanding the pathophysiologic mechanisms, both central and peripheral, of the primary cause of headaches, by using highly advanced technologies like structural high-resolution

MR, both of these imaging studies of cerebral structures allow the detection of cerebral parenchyma lesions with greater precision.

Pharmacologic interventions may help alleviate headaches, and recently several minimally invasive interventions have been tried, mainly targeting the physiopathologic mechanism that occurs via the cranial V nerve, obtaining promising results. However, there are still physiopathologic and imaging ambiguities related to these primary pathologies.

There is still an unmet need for studies in all fields, in large groups of homogeneous patients, to answer these questions.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, RID, FAA, AMR, and CAS; methodology, AFN, FAA, AMS, and CAP; software, RID, AFN, OV, and AMR; validation, AMR, OV, CAS, and CAP; formal analysis, RID, AFN, AMR and FAA; investigation, RID, AMR, OV, AMS, and CAS; resources, AFN, FAA, and OV; data curation, RID, AMR, AMS, CAS, and CAP; writing—original draft preparation, RID, AMR and CAP; writing—review and editing, AFN and AMS; visualization, RID; FAA, AMR, and CAS; project administration, FAA. RID and AMR contributed equally to this paper. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

E-cigarettes and Associated Medical Burden: For Better and For Worse

Claudiu Nistor[1,2], Anca P. Cucu[2,3], Oana C. Sima[3,4], Adrian Ciuche[1,2], Alexandra I. Trandafir[3,4], Ana M. Gheorghe[3,4], Mara Carsote[4,5]

[1] Thoracic Surgery Department, Dr. Carol Davila Central Military Emergency University Hospital, Bucharest, Romania
[2] Department 4 - Cardio-Thoracic Pathology, Thoracic Surgery II Discipline, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[3] Doctoral School, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[4] Department of Clinical Endocrinology V, C.I. Parhon National Institute of Endocrinology, Bucharest, Romania
[5] Department of Endocrinology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Correspondence: Anca-Pati Cucu, ancutapati@gmail.com

Abstract: *The harmful effects of smoking cigarettes on human health are well documented; thus various smoking cessation methods have been assessed and new options are currently under development to provide a "better' alternative to the "worse" one, namely smoking. However, no ideal method of ending or replacing conventional smoking has been developed so far, and actually, none of them has been proven entirely effective or safe. The laxity, or rather the absence of regulations in the first decade of existence of electronic nicotine delivery systems (ENDS) allowed their use with prohibited substances instead of nicotine or other substances with detrimental effect, with the emergence of a severe syndrome - e-cigarette and vaping-associated lung injury (EVALI), whose treatment required even lung transplantation in young people without any prior underlying lung disease. This narrative review aims to provide a brief overview of concerns about medical issues associated with e-cigarette use, particularly cardiovascular and respiratory panels. We organized the data in several micro-sections varying from practical aspects of understanding ENDS to clinical issues. To combat tobacco addiction, electronic cigarettes are increasingly widely accepted. A global regulatory framework is required to prevent the emergence of an illicit e-cigarette business with detrimental impacts on health.*

Keywords: *e-cigarettes, EVALI, smoking, lung, respiratory, cardiovascular, pulmonary, electronic cigarette, ENDS*

INTRODUCTION

The harmful effects of smoking cigarettes on human health are well documented – from a simple addiction to a significant risk factor for lung cancer. Recent data places smoking as the leading risk factor for lung cancer, the most common type of cancer in the world, which is responsible for more deaths annually. The detrimental effects on cardiovascular, pulmonary, and reproductive systems are also considerable. Therefore, various smoking cessation methods have been assessed – behavioral, pharmacological, and combined methods and new options are currently under development to provide a "better' alternative to the "worse" one, namely smoking. However, no ideal method of ending or replacing conventional smoking has been developed so far, and actually, none of them has been proven entirely effective or safe [1-4].

Therefore, that's why there has been a continuous struggle to find smoking methods that do not involve burning nicotine, with heat-not-burn devices being the best, safest alternative, and an efficient smoking cessation tool. However, in the last decade, considerable evidence has been presented showing an induction of addiction in adolescents and smoking initiation in those who have never smoked both adolescents and adults. On the other hand, the laxity, or rather the absence of regulations in the first decade of existence of electronic nicotine delivery systems (ENDS) allowed their use with prohibited substances instead of nicotine or other substances with detrimental effects, with the emergence of a severe syndrome – e-cigarette and vaping-associated lung injury (EVALI), whose treatment required even lung transplantation in young people without any underlying lung disease. After the emergence of EVALI, which unfortunately has been overshadowed by the coronavirus disease-19 (COVID-19)

pandemic, an increasing number of countries around the world have imposed regulations on the use of ENDS, which are now considered medical devices in some countries and are banned in others. Since it has been determined that the numerous flavors available to users have led to an increase in their use by adolescents, current legislation in most countries that have regulations limits the number and type of flavors available [5-8].

Aim

We aim to provide a brief overview of concern for medical issues associated with e-cigarette use.

METHODS

This is a narrative review of literature with no timeline restriction of research. We organized the data in several micro-sections varying from practical aspects of understanding ENDS to clinical issues.

ENDS – electronic nicotine delivery systems

ENDS include various devices, such as electronic cigarettes, e-hookahs, advanced personal vaporizers, and cig-a-likes. Currently, there are two main types of heat-not-burn products – tobacco heating devices and electronic cigarettes (four generations). From its first release in China in 2003 until today, the e-cigarette has undergone numerous improvements, with the current generation allowing a complex control of the device with significantly improved user safety. Electronic cigarettes are composed of an energy source (a rechargeable battery available from the second generation), a heating device - an atomizer, responsible for the generation of aerosols, a flow generator, a refillable (available from second generation) cartridge for e-liquid (water, propylene glycol, nicotine, flavors, and glycerol) and a mouthpiece. The third generation of e-cigarettes allows resistance and power adjustment while the fourth generation offers the possibility of temperature control (generally temperatures range from 40-65 Celsius), allowing for the modulation of the oropharyngeal experience [9-13].

It has been observed that by increasing the heating temperature, the aerosol generation rate also increases, and the aerosol particle size is modified as well (staying in the 100-600 nm range), with a potential impact on aerosol toxicity. Tobacco heating devices consist of a holder, an accumulator, and a nicotine heating unit (blade or induction heat technology) with aerosol generation. The differences between tobacco heat devices and e-cigarettes consist of user experience, the first one having a different operating time (6 minutes and a maximum of 14 puffs) and the temperature at which it operates (conventional smoking requires 200-600 Celsius). Nicotine is present in the tobacco that is used in the device. Also, not all e-cigarette devices use e-liquid with nicotine [14-19].

ENDS chemical compounds

E-liquid compounds include solvents (humectants or stabilizing agents), nicotine, cannabinoids, metals (in both the nicotine extract and the device constituents – nickel, manganese, copper, iron, arsenic, and lead), and flavors. The harmful effects of smoking are due to the inhalation of toxic products in cigarette smoke, generated by the combustion and pyrolysis of nicotine. Smoke from regular cigarettes contains more than 7000 different substances, including at least 70 carcinogens [20-24].

Unfiltered cigarette smoke is an aerosol with more than 400-500 gaseous components (nitrogen, oxygen, carbon monoxide and carbon dioxide, water, hydrogen, ammonia, argon, nitric oxide, hydrogen cyanide, hydrogen sulfide, methane, volatile alkenes, volatile alkenes, aromatic hydrocarbons - benzene, toluene, styrene, acids - formic acid, acetic acid, propionic acid, aldehyde - formaldehyde, acetaldehyde, acrolein, acetone, methanol, furan, pyridine, methylamine, etc.) as well as over 3500 other particulate constituents (nicotine, nornicotine, anatabine, anabazine, non-volatile hydrocarbons, carcinogenic polynuclear aromatic hydrocarbons, phenols, phthalates, polyphenols, acids – palmitic, stearic, oleic, linoleic, linolenic, lactic, indoles, quinolones, aromatic amines, nitrosamines and glycerol). In addition to these, various flavorings, pesticides, and metals are also present. Cigarette smoke contains 72 carcinogens and at least six compounds that harm reproduction [25-29].

Because they inhale both mainstream and sidestream smoke, passive smokers are more exposed to these dangerous substances than regular smokers. The amount of nicotine delivered by the e-cig is the result of several factors – smoking pattern (experienced versus inexperienced), aerosol characteristics (pH, particle size), pharmacokinetic and pharmacodynamics features, the site of absorption (oral or pulmonary), and of course the nicotine level in the e-liquid. The new generations of e-cigarettes deliver more aerosols and consequently higher amounts of nicotine, as demonstrated by their serum levels. The amount of nicotine in traditional cigarettes ranges between 10-15 mg nicotine/cigarette, of which 1-2 mg nicotine/cigarette is systemically absorbed, compared to 0-36 mg/mL liquid in electronic cigarettes [30-35].

E-liquid includes propylene glycol, vegetable glycerine, nicotine, and flavors. The e-cig structure includes absorbent materials in the atomizer (silicon, organic cotton, rayon fibers, stainless steel mesh, bamboo yar), as well as heating coils

manufactured from different iron, chrome, aluminum, or nickel alloys. The usual heating temperature in early generation ENDS ranged from 45 to 60 degrees, but in new generations that allow the user to adjust the temperature, it can reach over 200 Celsius, resulting in the release of potentially harmful carbonyl products, such as acrolein in increased quantities at voltages above 3.3 V [36-39].

The most commonly encountered flavorings in e-cigarettes are diacetyl, acetoin (buttery flavor), benzaldehyde (almond/ cherry aroma), cinnamaldehyde (cinnamon aroma), cresol (leather aroma), eugenol (clove aroma) or isoamyl acetate (banana flavor). The products resulting from the conversion of liquid into aerosol can vary, the temperature obtained by heating in e-cigs not being uniform and depending on several factors – the volatilization rate, the effectiveness of aerosol absorption, product design, and battery voltage. Thus, by converting solvents, formaldehyde, acetaldehyde, acrolein, methyl glyoxal, and glyoxal are obtained, and from vitamin E, ketene, benzene, and alkene are generated [33-39].

Cardiovascular effects

As a result of nicotine absorption, there is a release of catecholamines with a secondary increase in heart rate, blood pressure, and heart contractility, concomitant with reduced coronary vascular flow and increased musculoskeletal flow. The decreased risk of cardiovascular disease has been demonstrated in both in vitro and in vivo studies. It has been shown a reduction in monocyte cell adhesion to endothelial cells, a reduction in platelet activation markers (thromboxane B2), oxidative stress markers (prostaglandin F2 alpha), and C-reactive protein and cholesterol [37-40].

The currently available studies have assessed both short-term and medium to long-term effects (years). Heart rate and blood pressure abnormalities have been shown to vary according to the smoking status of e-cigarette or former conventional tobacco smokers. Thus, in short-term use of e-cigarettes with or without nicotine, an increase in heart rate and blood pressure was observed, whereas in chronic smokers the data are not conclusive. In e-cig smokers, an increase in arterial pressure was found only for short-term users. In contrast to conventional cigarette smokers converted to e-cigarettes, the switch to ENDS reportedly promotes a reduction in cardiovascular risk and BP [41-45].

Respiratory system effects

Studies have consistently identified a significant association between using electronic cigarettes and respiratory disorders. At the pulmonary level nicotine acts by activating nicotinic receptors for acetylcholine resulting in bronchoconstriction by action on bronchial smooth muscle. Epigenetic changes resulting in reduced PPAR-gamma (mesenchymal peroxisome proliferator-activated receptor gamma) receptor expression results in an asthmatic phenotype. Lung nicotine levels are more than 200 times the systemic peak in both conventional smokers and vapers [46-48].

Propylene glycol and vegetable glycerine are used as humectants, but repeated inhalation is not associated with significant changes in lung function if the exposure is limited. On the other hand, the new generations of devices, which can reach temperatures above 300 Celsius, can generate toxic carbonyl volatile compounds – formaldehyde and acetaldehyde (at temperatures above 215 Celsius) and acrolein at temperatures above 270 Celsius, two compounds that are associated with exacerbation of bronchial asthma. Diacetyl (2,3 butanedione) a butter flavoring is known to induce irreversible acute bronchiolitis obliterans (long popcorn). Other flavorings such as cinnamaldehyde are known irritants for asthma and benzaldehyde is an irritant for the mucous membranes of the respiratory tract. Yet, benzaldehyde higher levels provided by newer generation ENDS were proven to suppress allergic inflammation in a murine model by decreasing the number of inflammatory cells in lung and nasal tissues [49-53].

Destructive effects on respiratory epithelial cells have been demonstrated in several in vitro studies, as well as on nasal epithelial cells [49-53]. In vivo research has shown reduced lung cancer risk in ENDS users [54-56]. Increased oxidative stress and mitochondrial dysfunction have also been demonstrated in in vivo studies [57-60].

Shortly before the COVID-19 pandemic, a new outbreak occurred in ENDS users- EVALI, with more than 2500 cases reported in the US over 5 months [61-72]. The predominant symptomatology was respiratory – dyspnea, cough, and chest pain, associated also digestive symptoms and fever. Most cases were caused by exposure to tetrahydrocannabinol while the remainder by exposure to vitamin E acetate which was used as a thickening agent. The latter is thought to alter the structure of the surfactant and by heating may generate keten, a toxic compound [73-77]. The introduction of regulations on ENDS status has led to a dramatic decrease in EVALI cases.

DISCUSSION

Apart from the mentioned inflammatory issues and the cardiovascular/respiratory associated entities related to the use of e-cigarettes, other medical consequences have been identified, yet, with various levels of statistical significance and some require further exploration to adequately assess their clinical impact.

For instance, while most studies have focused on pulmonary

and cardiovascular effects, smoking is a well-known risk factor for oral cancer.

Current data shows a similar effect of e-cigarettes when compared to traditional tobacco smoking in terms of oxidative stress, wound healing arrest, promotion of a pro-inflammatory environment by increased pro-inflammatory cytokines, and incidence of periodontal disease, caries, and edentations.

However, the few available data on the carcinogenic effect of e-cigarette use have not led to any relevant practical conclusions, as they did not have a well-defined duration of exposure that enabled an objective risk assessment [78-84]. In addition to the direct effects on the user's health, environmental effects are described, which are attributed to the combination of compounds in mainstream and sidestream smoke.

Finally, as mentioned the most dramatic consequence in relationship with e-cigarette use is EVALI which particularly affects young individuals. While severe COVID-19 forms of infection might mimic an episode of EVALI, during pandemic years a growing number of hospitalizations for this syndrome had been registered.

The medical community increased the level of awareness concerning EVALI and recently various public health resources and scientific papers have been released to address this particular aspect [85-88].

CONCLUSIONS

Although ENDS are promoted as a safe alternative to smoking tobacco, it has been shown that adolescents who use them are more likely to start smoking tobacco again or start smoking it for the first time. Smoking is a significant global public health issue. The methods for quitting smoking have changed over time, but none have achieved perfect success. To combat tobacco addiction, electronic cigarettes are increasingly widely accepted. A global regulatory framework is required to prevent the emergence of an illicit e-cigarette business with detrimental impacts on health.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; methodology, C.N., A.-P.C., A.C., A.-I.T, A.-M.G., and M.C.; software, C.N., A.-P.C., O.-C.S., A.-M.G., and M.C.; validation, C.N., A.-P.C., O.-C.S., A.C., A.-M.G., and M.C.; formal analysis, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; investigation, C.N., A.-P.C., A.-M.G., and M.C.; resources, C.N., A.-P.C., O.-C.S., A.C., and M.C.; data curation, C.N., A.-P.C., and M.C.; writing—original draft preparation, C.N., and A.-P.C.; writing—review and editing, C.N., A.-P.C., and M.C.; visualization, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; supervision, C.N., A.-P.C., and M.C.; project administration, C.N., A.-P.C., O.-C.S., A.C., A.-I.T, A.-M.G., and M.C.; funding acquisition, C.N, and M.C.. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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REVIEW

The Spectrum of Neurological Complications after Bariatric Surgery – Review of the Literature

Florentina Ionita-Radu[1,2], Sandica Bucurica[1,2], Raluca S. Costache[1,2], Mariana Jinga[1,2], Petrut Nuta[2], Mirela Chirvase[1,2]

[1] Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[2] Department of Gastroenterology, Central Emergency University Military Hospital Carol Davila, Bucharest, Romania

Correspondence: Sandica Bucurica, sandica.bucurica@umfcd.ro

Abstract: *Bariatric surgery represents one of the most efficient treatments addressing obesity and its associated complications with an increasing trend. Even if the medical benefits for patients are huge, due to weight loss and despite technical improvements in bariatric procedures, it is still associated with diverse types of complications. Most of the complications appear due to nutrient deficiency, as absorption is compromised after surgical anatomy modification. Neurological complications after bariatric surgery are very diverse with an occurrence within days to years after surgery, most frequently in patients with gastrointestinal or surgical complications. Any segment of the nervous system can be affected with distinct types of manifestations ranging from peripheral axonal neuropathy, nerve palsy secondary to compartment syndrome, encephalopathy, and myelopathy, to psychiatric and demyelinating central nervous system diseases. Identifying patients at risk and systematic screening for nutrient deficiency with proper supplementation is essential for preventing neurological complications and lifelong sequelae. In this paper, we present the spectrum of neurological complications reported after bariatric surgery.*

Keywords: *bariatric surgery; nutrient deficiency; neurological complications*

INTRODUCTION

Bariatric surgery still represents one of the most efficient treatments addressing obesity and because the incidence of severe obesity is rising worldwide, more and more bariatric procedures are done annually in specialized medical centers. Even if the medical benefits for the patients are huge due to weight loss and despite the technical improvements for bariatric procedures, it is still associated with different types of complications.

Most of the complications are due to the modified anatomy which leads to impaired absorption of the nutrient, besides the inflammatory state and mechanical stress induced by surgical procedure. The most common complications are gastrointestinal and surgical complications and include but are not limited to reflux disease, vomiting, dumping syndrome, leaks with high mortality getting up to 15%, stenosis, twists and kinks causing dysphagia, bleeding, enteric perforations, internal hernias, bowel obstruction, ulcerations, and gallstones disease. Other complications seen following bariatric surgery are weight regain, pulmonary thromboembolism, metabolic and hematological disorders, such as anemia, leucopenia, and neuropsychiatric complications. [1]

MATERIALS AND METHODS

The purpose of our paper was to review the existing literature data regarding the neurological complications that occurred after bariatric surgery and the presence of nutrient impairment.

We performed a scoping review of the published literature, and we included original studies, reviews, and a series of cases by searching the Medline, PubMed, and Google Scholar databases. We included articles written in the English language.

We searched for the terms: “bariatric surgery”, “neurological complications and bariatric surgery”, “nutrient deficit and bariatric surgery”, “vitamin deficit and bariatric surgery”, “gastric sleeve”, “gastric banding”, “gastric bypass”, “vertical gastroplasty”, “obesity surgical treatment”.

We excluded papers with respect to bariatric surgery-related complications and pre-operative nutritional deficiencies.

NEUROLOGICAL COMPLICATIONS

Neurological complications after bariatric surgery were reported in 3-16% of cases and usually affected more than one component of the nervous system [2,3].

Neurological disorders are various and affect mainly the peripheral nervous system and less common central nervous system, but both are associated with a poorer quality of life and substantial morbidity. Neurological complications of these patients might occur days to years after surgery and include peripheral neuropathy, compartment syndrome, plexopathy, mononeuropathy, encephalopathy, myelopathy, ataxia, myopathy, myotonia, behavioral and psychiatric disorders [2,3,4].

The physiopathology of neurological impairment comprises nutritional deficiency, rapid weight loss, and compartment syndrome. The vitamin B12 shortage, thiamine deficit, and decreased levels of copper are the most commonly deficient nutrients responsible for neurological conditions development after bariatric surgery. Deficiency of riboflavin, pyridoxine, folate, vitamin D, E, phosphorus, calcium, selenium, zinc, and iodine can also contribute to neurologic dysfunction [3,5].

Impact Of Vitamin and Micronutrient Deficiency After Bariatric Surgery and Neurological Complications

Nutritional impairment after bariatric surgery is quite common and manifests a large spectrum of vitamin and micro-nutrient deficiencies affecting both, the water-soluble vitamins (thiamine, riboflavin, pyridoxine, folate, cyanocobalamin, vitamin C), and the liposoluble ones (A, D, E, K), alongside the fat malabsorption, loss of proteins, and minerals deficiencies (iron, zinc, copper, selenium). All these associate different clinical manifestations within a variable time frame of appearance and usually are overlooked by clinicians. Some bariatric procedures, such as Roux-en-Y gastric bypass and post-biliopancreatic diversion/duodenal switch put the patient at higher risk of developing multiple nutritional deficiencies since the absorptive surface is reduced or bypassed [5,6].

The most common deficiencies reported after bariatric surgery are iron deficiency (which was found in more than 30% of patients) [5], cobalamin and folate deficiency in up to 35%, respectively 39% after five years [6], and thiamine deficiency in 49% of patients [7].

Reports from a French cohort study showed a high rate of overall vitamin B deficiency (74%), with thiamine deficit in more than 47% of the cases [8]. A significant number of patients, up to 30%, already had micronutrient deficiency before bariatric surgery which increased the risk of new complications [9]. Hypovitaminosis D after bariatric surgery was reported in 25-70% of the patients, with calcium deficiency in 10%, but it should be considered that obesity is associated with suboptimal 25-hydroxy-vitamin D and 30% of obese patients could have vitamin D deficiency [10,11]. Vitamin E deficiency was reported up to 65.7% in a recent systematic review, and vitamin A in 5-69% in 2-4 years following surgery. Clinical manifestations of vitamin E deficiency are usually seen after Roux-en-Y gastric bypass, being mainly neurological (ataxia, peripheral neuropathy), but also hemolytic anemia, impaired immune response, ophthalmological disorders, infertility, and nystagmus can occur [12]. Although rarely clinically manifested, deficiency of vitamin C and vitamin K was reported in almost half of the patients [6,12].

Low zinc level after bariatric surgery was associated with high inflammatory status. There are reports of necrolytic migratory erythema with severe bacteremia and optic neuropathy due to zinc depletion [13,14]. The zinc repletion therapy should be done cautiously because it can induce copper deficiency. The copper deficiency occurs mostly after gastric bypass as its absorption occurs in the stomach and duodenum. Many cases of copper deficiency had been reported up till 9 years after bariatric surgery with capricious clinical expression, the majority mimicking vitamin B12 deficiency, consisting of myelopathy, ataxia, peripheral neuropathy, optic neuropathy, blindness, muscle weakness, anemia, and neutropenia [15,16, 17,18].

The diagnosis of copper deficiency usually requires the measurement of serum levels associated with the ceruloplasmin level under 20 mg/dl and C reactive protein because ceruloplasmin is an acute phase protein and levels could be misinterpreted in inflammatory states. MRI study with T2 hyperintensity in the posterior dorsal column of the spinal cord can suggest the diagnosis [19,20].

Most of the neurological complications are often seen in patients with surgical complications, longer hospitalization time, or those with gastrointestinal symptoms, especially protracted vomiting. Moreover, these patients underwent a surgical reintervention in 8-32% of the cases [8,21].

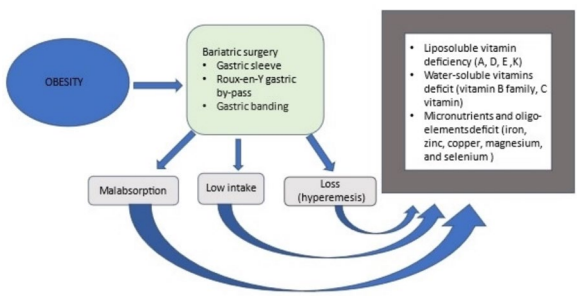

Figure 1: Pathophysiology mechanisms of neurological complications after bariatric surgery and types of nutrient deficiencies

Peripheral nervous system complications

Neuropathy is the most frequent neurological complication described after bariatric surgery and has been reported in 16% of patients after this type of procedure [22]. Its occurrence is induced by vitamin deficiency, especially thiamine, and cobalamin, which are involved in myelin synthesis, but also riboflavin, pyridoxine, and copper deficiency [21].

Another mechanism of occurrence is attributed to entrapment and mechanical compression. Rapid weight loss with subcutaneous protective fat tissue disappearance and inaccurate leg postures can cause compression neuropathy, frequently unilateral. Involvement is usually sensory predominant with late motor progression, chronic and symmetrical, mostly affecting distal limbs, but also small fiber neuropathies and bilateral neuropathies were several times described, contrary to plexopathy which was rarely seen [23,24].

The median nerve compression, the peroneal nerve palsy, and the ulnar nerve palsy were the commonest nerves affected after bariatric surgery. Peroneal nerve palsy is usually unilateral, but cases with bilateral affection were described.

Rapid weight loss with a mean of 45-50 kg reduction in several months is the main risk factor identified for peroneal nerve injury as the nerve's superficial anatomic localization makes it susceptible to impingement [24]. One case of meralgia paresthetica after bariatric surgery was described in an Asiatic report [25]. Rare cases of dry beriberi disease characterized initially by nausea, vomiting, constipation, severe weakness and then progressing to feet tingling and burning were described in the literature with late diagnosis, which was only suggested after performing electromyography [26].

A few cases of acute axonal polyneuropathy resembling Guillain Barre syndrome, rapidly progressive were described in the medical literature after bariatric surgery with good response to vitamin B1 and B12 high dose replacement [27]. There are also recently reported cases of mixed polyneuropathy after bariatric surgery both axonal and demyelinating after five to 24 months post-surgery and some case reports of Guillain Barre syndrome, raising the suspicion that bariatric surgery could induce immunological changes which predispose to autoimmunity [14,28]. Cases of plexopathies affecting the lumbar plexus were reported, manifesting with unilateral severe pain with sensory loss in the hip and thigh region, muscle wasting, patellar reflex loss, and extensive denervation on electromyography study [25].

Several other risk factors for predicting neuropathy development, despite rapid weight loss, were previously reported, such as low albumin and transferrin levels, gastrointestinal complications, surgical complications with long hospitalization, jejunoileal bypass, and lack of nutritional assessment after bariatric surgery [22]. Symptoms of neuropathies include pain, paresthesia, burning feet in small fiber neuropathy, usually progressive weakness, and rarely acute, foot drop, slapping gait, loss of reflection, and objective sensory loss. The diagnosis is confirmed by electromyography studies indicating distal axonal neuropathies. Biopsies of the affected nerves show axonal degeneration and peri-vascular inflammation [24,29].

Treatment includes a high dose of vitamin B12 dose of 1000 ug intramuscular once daily for a week and then weekly, vitamin B1 500 mg daily, intravenous for three days, and afterward 100 mg oral route and acid folic supplementation 5 mg per day, with good response and resolution of symptoms. Vitamin supplementation should be done for long periods and started as soon as possible when diagnosing neuropathy in post-bariatric surgery patients without waiting for confirmation of vitamin deficiency tests since vitamin levels can be normal. Nerve decompression was cited as the most efficient therapy for peroneal nerve entrapment after bariatric surgery [23]. Intravenous immunoglobulin is the treatment of choice for Guillain Barre syndrome, with a good response. Physical therapy can be used as a complementary treatment to vitamin replacement when treating neuropathy [24,29].

Functional impairment is usually mild, but it can be debilitating when the neuropathy becomes chronic or when the patient experiences weakness and foot drop. Also, small fiber neuropathy can evolve into distal neuropathic chronic pain syndrome in all extremities, deteriorating life quality [24,29].

Central nervous system complications (CNC)

The most reported CNS complications after bariatric surgery are encephalopathy, myelopathy, neuropsychiatric disorders, and optic neuropathy [30].

Encephalopathy

The most common type of encephalopathy seen after bariatric surgery is Wernicke encephalopathy secondary to thiamin deficiency, but also different types of encephalopathy are being described, such as hyperammonemia encephalopathy due to high plasmatic levels of glutamine and ammonia, zinc deficiency and urea cycle disorders. Episodic encephalopathy associated with D lactic acidosis is another type of encephalopathy encountered after bariatric surgery, usually precipitated by high carbohydrate intake, and hardly diagnosed by clinicians [31,32].

Clinical manifestation of Wernicke encephalopathy consists of acute confusion, ataxia, nystagmus, sometimes associated with cranial nerve palsies (third and sixth), seizures, dysarthria,

myoclonus, asterixis, papilledema, vestibular symptoms, blurred vision, and polyneuropathy. Almost 80% of patients surviving encephalopathy develop Korsakov syndrome defined by amnesia (retrograde and anterograde), confabulation, and loss of insight, being more common in alcohol consumers. Most cases of Wernicke encephalopathy occur between 14 days to 1-2 years, mostly in women, and are usually precipitated by several factors. Vomit-ing is the most common gastrointestinal symptom precipitating Wernicke encephalopathy, usually preceding thiamine (Gloy VL) deficiency manifestations [8,12,33]. Other factors include rapid weight loss, food avoidance, poor appetite, and lack of vitamin supplementation, as thiamine reserves are depleted in less than 3 weeks without supplementation. In the absence of rapid diagnosis and appropriate treatment, the disease is fatal. Diagnosis of Wernicke encephalopathy is usually clinical, supported by laboratory and IRM studies. Tissue thiamine levels are accurately determined by red blood assays of specific enzymes (erythrocyte transketolase activity coefficient since blood and urine levels are not reliable [34].

IRM changes in Wernicke encephalopathy include symmetrical increased signal on T2/FLAIR phase in dorsomedial thalami, mamillary bodies, tectum plate, periaqueductal area, and around the third ventricle. Treatment of Wernicke encephalopathy consists of intravenous/intramuscular of thiamine 100 mg for 3-5 days minimum, followed by 30 mg twice orally for several months and correction of hypo-magnesemia since magnesium is a cofactor in thiamine-dependent metabolism. Prevention is mandatory and patients at risk should get thiamine supplementation of 100 mg intramuscularly followed by 50 orally once daily. Recovery in Wernicke encephalopathy induced by bariatric surgery after optimum thiamine supplementation is seen in more than half of the cases, the others remaining with cognitive deficiencies, gait disturbances, nystagmus, psychosis, and rarely death [15,30,33].

Hyperammonemia encephalopathy represents a particular type of encephalopathy recognized in the past years in patients who have undergone Roux-en-Y gastric bypass with a high mortality rate, characterized by a high level of ammonia in the absence of liver disease [31]. In a review from Andrew Z. Fenves et all in 2015 a mortality rate of 50% was reported in these patients with a predominance of women as the conditions were most often seen in this category of patients [35].

Laboratory findings include mild to moderate increased levels of ammonia, high levels of plasma glutamine, low albumin and zinc levels, essential amino acids deficiency, and reactive hypoglycemia [36].

The precise mechanism of onset is not yet understood, but several factors were until now described in the hyperammonemia state after bariatric surgery. Most of the lethal cases of hyperammonemia encephalopathy showed genetic predisposition caused by urea cycle abnormalities with a partial ornithine transcarbamylase deficiency or a mild carbamyl phosphate synthetase (CPS) deficiency [32].

One of the predisposing factors for hyperammonemia is the presence of huge nitrogenous residues in the liver. Intake reduction triggers a high catabolic state with protein breakdown. Ammonia level is also increased by altered gut microbiota that produces more urea lithic strains because of small bowel pouches created by bariatric surgery [31,36]. Case reports from patients with this neurological complication after bariatric surgery reported lower citrulline levels in the intestine affecting ureagenic capacity. Moreover, other predisposing conditions as the presence of a splenorenal shunt should be suspected and ruled out in patients developing hyperammonemia [32,35].

Therapeutic options include protein intake reduction, zinc and amino acid repletion, parenteral glucose, lipid supplementation, and oral lactulose. In refractory cases, hemodialysis and setting back of surgical procedures could be an option [37].

D-lactic acidosis encephalopathy is a rare condition reported mostly in short bowel syndrome, but also described years after bariatric surgery, especially after jejunum-ileostomy, and should be taken into consideration in a patient with anatomic changes of the digestive tract who develops confusion syndrome [38]. Confusion episode is associated with behavioral changes, dysarthria, ataxia, lethargy, and weakness, and is usually triggered by high carbohydrate intake. D lactate serum elevation is a result of carbohydrate fermentation in the small bowel or colon and is hardly diagnosed as serum and urine measurement of D lactate is not commonly done, L lactate being the only form that is most frequently checked when assessing lactic acidosis. A level over 3 mmol/l is diagnostic for this pathology and is often associated with a high serum anion gap [38]. Treatment involves metabolic acidosis correction with intravenous bicarbonate, fasting reducing carbohydrate levels, and intravenous amino acids, but also modulating intestinal microbiota with antibiotics which improve neurological symptomatology [39].

Myelopathy is usually seen years after bariatric surgery being one of the most disabling complications. It commonly affects posterior columns with the dorsal ganglia root and can be also associated with neuropathy (myeloneuropathy) or optic neuropathy. Manifestations consist of vibration and

proprioception loss, gait ataxia, hyperreflexia, and Babinsky sign, sometimes accompanied by sphincter dysfunction and limb weakness [40].

When neuropathy occurs sensory changes in glove and stocking patterns are also seen in these patients after bariatric surgery. This complication is caused primarily by cobalamin deficiency but is also attributed to other deficient nutrients such as copper, vitamin E, and pyridoxine. Correction of these micronutrients initially intravenously and then long-term orally or intramuscularly for vitamin B12 improves neurologic symptomatology but without full recovery in some patients. For optimum management copper levels should be regularly checked and supplemented as quickly as needed initially intravenous and then oral administration [41].

Optic neuropathy affecting one or both side nerves is associated with bariatric procedures because of nutritional deficiencies in vitamin B12 and vitamin A, copper, and rarely zinc, causing malfunction of nerve fibers. The onset can vary during a large period and can be acute or progressive causing blindness. As optic neuropathy pathogenesis is linked to demyelination, permanent blindness may not be overcome [22,42].

Central nervous system demyelination presenting as multiple sclerosis which fulfilled the McDonald criteria for diagnosis was reported in a series of cases after various types of bariatric surgery with relapsing-remitting forms [4,43,44], without understanding the mechanism of appearance or the exact relationship between the events, but it might be attributed to rapid and substantial weight loss. Other previous case reports also described multiple sclerosis onset and myasthenia gravis after rapid weight loss induced by bariatric surgery or highly restrictive diets [45]. Two cases of monophasic central nervous system demyelination were also reported by Falah et al in a series of patients with neurological complications after bariatric surgery, and in a Brazilian study [44].

NEUROPSYCHIATRIC DISORDERS

Neuropsychiatric disorders are seen after bariatric surgery, usually consisting of eating disorders (anorexia and bulimia nervosa, binge eating, avoidance eating (post-surgical avoidance eating disorder), reports of acute mania [46], and higher risk of suicide. These disorders frequently appear in preexisting psychiatric conditions, making psychiatric evaluation before surgery mandatory to find patients at risk who need to be followed. Several cases of cognitive complaints were described following bariatric surgery in Roux-en-Y Gastric Bypass induced by focal brain atrophy in the bilateral posterior thalamus [47] but need to be further studied.

Other conditions described after bariatric surgery with higher incidence are stroke, seizures [48,49], spontaneous intracerebral hypotension linked to vitamin A, and D deficiency, and spontaneous leakage of cerebrospinal fluid, but the exact relationship needs to be established in future studies [30].

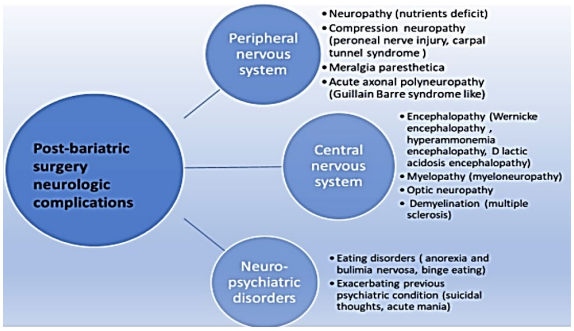

Figure 2: The spectrum of neurological complications described after bariatric surgery. From peripheral to central nervous complications, to neuropsychiatric disorders.

CONCLUSIONS

Complications after bariatric surgery have a broad spectrum of manifestations involving both the peripheral and central nervous system and should be suspected and diagnosed as soon as possible to prevent long-term sequelae. The risk of developing central nervous system demyelination or stroke events after bariatric surgery should be further evaluated in future studies. Patients with rapid weight loss and those who develop gastrointestinal symptoms and surgical complications are considered at high risk for developing neurological complications, which is why nutritional deficiency assessment and supplementation should be done as early as possible.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization: FIR; Methodology: MJ, RSC, PN; Writing - original draft preparation: MC; writing-review and editing SB, MC; Supervising: FIR. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

Roadmap of Photoplethysmography Technology in Advanced Cardiovascular Assessment

Lucian Evdochim[1], Adrian Florescu[2], Lidia Dobrescu[1]

[1] Department of Electronic Devices, Circuits and Architectures, Faculty of Electronics, Telecommunications and Information Technology, University Politehnica of Bucharest, Romania
[2] Elias Emergency University Hospital, Bucharest, Romania

Correspondence: Lucian Evdochim, lucian.evdochim@stud.etti.upb.ro

Abstract: *This review explores the trajectory of photoplethysmography (PPG) technology from its inception in 1934 to its integration into smart devices in 2013. While PPG has proven effective in estimating left ventricular ejection time (LVET) and distinguishing between hypertensive and normotensive patients, challenges persist. The need for a robust mathematical model to explain physiological behaviors, address calibration protocols, and handle waveform variability is emphasized. Despite limitations, PPG is on the cusp of achieving clinical-grade confidence, particularly in estimating blood pressure and tracking vasomotor states. Integration into wearable devices is a prominent trend, with major companies exploring applications for improved health monitoring. Open-source databases and the availability of physiological data aim to enhance understanding, paving the way for universally accepted protocols. As PPG advances, it holds promise for personalized healthcare, revolutionizing treatment evaluations and preventive measures. However, potential disagreements over protocols may impede progress. Nevertheless, the technology's potential to remotely monitor cardiovascular markers could reduce physicians' workload for routine tasks. PPG stands as a beacon for the future of noninvasive cardiovascular assessment.*

Keywords: *photoplethysmography roadmap; cardiovascular assessment; cardiovascular disorders; left ventricular ejection time; blood pressure estimation*

INTRODUCTION

Cardiovascular diseases are, according to WHO, the most common cause of death globally. There are made continuous efforts to reduce the incidence, morbidity, and mortality of these conditions. All over the world, the physicians involved in preventing, detecting, and treating cardiovascular diseases are insufficient and subjected to an overwhelming workload: theoretically, after a certain age, virtually all individuals have a certain risk of developing cardiovascular conditions and these patients, after diagnosis, need periodical evaluation for monitoring, assessment of treatment efficacy and, also, management of complications. Therefore, in the context of nowadays technology capabilities, it has raised the question if there could be developed some methods to monitor these patients over a distance, using suitable medical technologies to non-invasively measure some biomarkers.

At present, it is available to remotely measure, using various wearable devices, pulse, SpO_2 and to record an ECG. However, in detecting or monitoring the risk of adverse events, it would be useful to be able to remotely monitor arterial blood pressure, hypertension being the most prevalent cardiovascular risk factor, or, more advanced, to monitor various parameters of heart performance.

Photoplethysmography (PPG) technology has already demonstrated promising efficiency in distinguishing between normotensive and hypertensive patients or in estimating left ventricular ejection time. In our opinion, with more research in this field and with better mathematical models that can explain all physiological behaviors, PPG could help healthcare professionals offer personalized care regarding the prevention, treatment, and follow-up of cardiovascular diseases.

PHOTOPLETHYSMOGRAPHY APPLICATIONS

Short History

The founding concept of the PPG technique is older than a

century when German physicist M.R. Bonnsman invented a photocell device in 1934 intended for peripheral blood volume evaluation [1]. This novel method was taken over by Alrick Hertzman [2,3] for further investigations in the physiological domain. Based on numerous experimental investigations, but limited by the technology capabilities at that time, he proposed blood volume variation as the origin of the PPG signal. No new progress in this technique has been made in the next years since the associated sensor was more of a tool in circulation research.

A valuable clinical application emerged in the form of blood oxygen saturation ratio (SpO_2) [4], pioneered by Takuo Aoyagi in the 1970s [5]. The device, now known as a pulse oximeter, was introduced into U.S. clinical monitoring a few years later, confirming its utility. Following this significant advancement, there was no immediate surge in research within the PPG technology domain; instead, efforts were concentrated on enhancing the accuracy of SpO_2 measurements. The pulse oximeter device became commercially accessible in the 2000s, owing to technological advancements and its availability in the open market [6].

Technology nowadays

The subsequent period that drew attention to PPG was marked by the integration of this technology into the first smartwatches in 2013 [7]. This significant development raised awareness among both academic and industry researchers regarding the potential for cardiovascular monitoring through wearable devices. While the well-established SpO_2 measurement and heart rate determination are valuable for assessing heart performance during resting periods or intense activity [8,9], they do not constitute a comprehensive tool for physicians to remotely monitor a patient in terms of cardiovascular disease (CVD) prevention or tracking.

Therefore, intense research has commenced in the PPG domain over the last decade, exploring various capabilities as depicted in Figure 1. One sought-after application is noninvasive arterial blood pressure (ABP) monitoring, considered a crucial hemodynamic marker in the evaluation of cardiovascular diseases (CVD) [10,11]. By incorporating this targeted assessment into smart devices such as watches, bracelets, and rings, personalized healthcare becomes accessible for end-users. The confidence in this feature has prompted major companies like Samsung [12] to integrate it into their devices. Start-ups such as Valencell [13] and Aktiia [14] assert their ability to provide this measurement using Machine Learning (ML) techniques. Additionally, numerous individual research entities report significant progress in this direction, leveraging artificial intelligence (AI) networks [15].

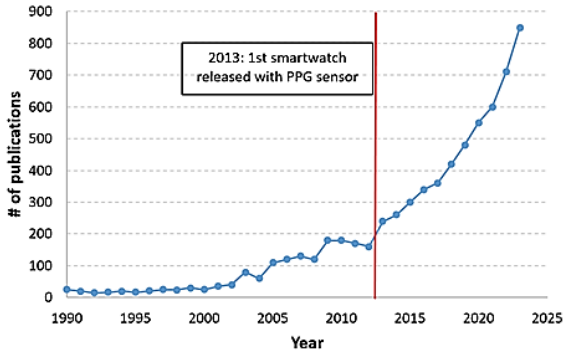

Figure 1: A timeline of PPG-related publications every year from 1990 to 2023. Data obtained from Google Academic, last accessed on 15th December 2023

The key factors contributing to the popularity of PPG encompass the mitigation of motion artifacts, the precision of SpO_2 readings and heart rate measurements, initiatives for extracting arterial compliance, and the estimation of blood pressure. Furthermore, a novel approach, remote photoplethysmography (rPPG) [16], has emerged in recent years, focusing on extracting hemodynamic information through facial recognition. The advancement of information technology, notably the internet and easy access to information, has played a significant role in augmenting the popularity of PPG.

However, as depicted in Figure 1, the upward trend in the number of publications does not align with the maturity of the technology. Regrettably, numerous topics are repetitive, merely contributing to the validation of specific assessments rather than yielding outstanding discoveries or breakthrough research. As will be presented in the next section, a strategy focused solely on PPG signal interpretation is not sustainable. Reflective sensors are commonly employed for wearable applications, as illustrated in Figure 2, in contrast to the transmissive ones more prevalent in clinical settings.

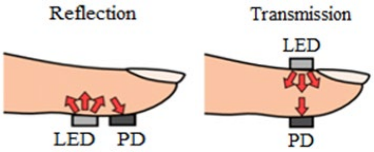

Figure 2: Two types of PPG modes: reflective and transmissive. In wearable devices, the first mode is prevalent, while in clinical settings the second one is present.

Eligible models must be developed to comprehensively explain all observed physiological behavior, and the correlation or causation with arterial blood pressure needs to be elucidated. Without such a focused direction, the repetition of topics will persist without yielding new and significant discoveries.

PPG as a standalone biomarker

As a standalone biomarker, information extracted from PPG waveform contour analysis becomes crucial. Following this approach, significant features such as the tidal wave (TDW) [17] and the dicrotic wave (DCW) [18] can be associated with the vasomotor state. The latter waveform component proves to have a strong correlation in estimating vasomotor tone, as observed in Figure 3, where the dicrotic notch sharpens proportionately with the level of vasodilation [19]. When combined with other waveform markers, such as the ratio between systolic upstroke and downstroke, and aided by Machine Learning (ML) models, the hypertensive state can be reliably detected [20].

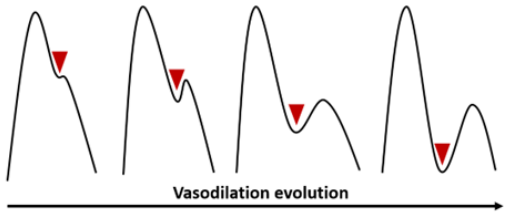

Figure 3: Evolution of PPG waveform during vasodilation progress, by highlighting incisura or DCW feature. Reconstructed after the findings on [19]

Another application involves the myocardial performance index (MPI or TEI) [21], as the PPG waveform encompasses information about isovolumetric contraction time (IVCT) [22], isovolumetric relaxation time (IVRT), and left ventricular ejection time (LVET) [23]. Despite the potential for overestimation and underestimation of measurements due to the hemodynamic state, these values closely align with the golden standard method of Doppler echography.

PPG in sensor fusion

One popular technology in this category is represented by the pulse pressure transit time (PTT) method [24], developed for arterial compliance and blood pressure assessment. The current literature protocol [25] guides the measurement of this marker from the electrocardiogram (ECG) R peak to the PPG systolic point, as depicted in Figure 4.

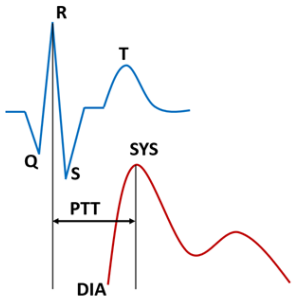

Figure 4: PTT protocol based on guidelines [25]

Based on the physical equation developed by Moens–Korteweg [26], which describes the traveling behavior of mechanical pulse pressure, blood pressure estimation can be extracted. Thus, a stiffer system will promote rapid traveling of the pulse wave, resulting in a shorter PPT time. Conversely, an elastic system, due to its ability to absorb mechanical energy, will slow down the traveling mode, leading to a longer measured time.

Another application that combines PPG capabilities involves fusion with a medical cuff. During cuff inflation, PPG acts as a monitor of blood supply at the targeted site. After a certain point, the externally induced pressure will trigger blood vessel occlusion, which is reflected by a missing PPG waveform. This measurement approach was embedded in the Omron smartwatch [27] to enhance the accuracy of blood pressure assessment.

A recent application involves remote photoplethysmography [16], which targets face recognition in combination with pulsatile detection of facial skin. By selecting two separate regions of interest (ROI), such as the chin and forehead, equivalent approaches to the PPT technique are employed to compute and estimate blood pressure, as depicted in Figure 5.

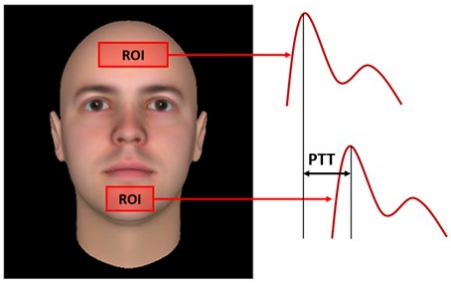

Figure 5: Principle of rPPG. Two locations of skin pulsation generate PPG waveform but with a time delay in between. This delay is exploited by a similar to the PPT method to extract blood pressure estimation

PHOTOPLETHYSMOGRAPHY LIMITATIONS

Physiological model

Despite the important and useful applications of the PPG technique, either standalone or in conjunction with other methods, the technology has demonstrated some limitations.

The first significant limitation pertains to the proper understanding of PPG origin, particularly in relation to pulse formation. While Alrick Hertzman proposed blood volume variation as a working model in the 1930s, recent investigations have revealed some drawbacks. One common limitation arises during reduced SpO_2 readings [28], which actually represent the ratio between oxygenated and deoxygenated blood volume. Additionally, the hand position

concerning the heart level [29] modulates the PPG waveform amplitude according to specific rules, contrary to the initially proposed volume variation model. Therefore, significant progress in the hemodynamic domain cannot be achieved without a suitable PPG model that can explain and predict different physiological states. At this juncture, two mathematical models are required: one to elucidate the optical nature of the recorded signal and another to describe the mechanical nature that provides a specific waveform morphology. Training Machine Learning (ML) or Artificial Intelligence (AI) systems with a large set of hemodynamic data will not unveil the true relationship between these two physical quantities. Instead, it serves as an interim step to understand which waveform components are predominant in blood pressure assessment.

A recently developed model proposes the red blood cell deformation effect as the origin of PPG formation [30]. The mere presence of erythrocytes in the measurement area modulates the direct component or baseline of the recorded waveform. Interestingly, as cells move under a defined velocity field, they deform in the capillary regime. Thus, targeting red blood cells with light results in the absorption of radiant flux proportional to the deformation level. Given that many publications attempt to correlate arterial blood pressure (ABP) waveform with PPG, it is observable that the physiological stimuli contributing to PPG formation are the blood flow velocity at the capillary level, ranging from 50 µm/s to 2000 µm/s [31]. As a result, as depicted in Figure 6, the PPG waveform is exclusively modulated by the velocity values through the capillary network, reflected in the final waveform by red blood cell deformation.

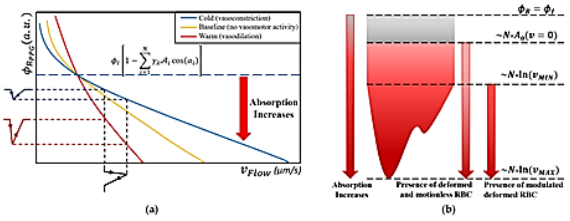

Figure 6: RBCM MODEL. Reused from [30]

It is important to note that both the mechanical blood pressure pulse and red blood cell deformation—precursors for PPG—are driven by blood flow velocity. Therefore, this physical quantity serves as the same input stimulus, the causal factor, for both associated waveforms. In this context, a correlation rather than direct causation exists between arterial blood pressure (ABP) and PPG information. From this standpoint, estimations of hemodynamic markers can be extracted with the aid of wearable technologies. However, due to the logarithmic nature of PPG origin, as depicted in Figure 6, the hemodynamic information will undergo modifications that could lead to wrongly interpreted results.

Finally, without a proper optical model, the improvement of pulse oximeter sensors becomes challenging. The most critical design considerations are related to mechanical features, such as the frequency of the LED used, initial light flux power, and the arrangement of the photodiode. In terms of signal processing, important design inputs include signal amplification, sampling frequency, and filtering stages.

Measurement protocols

All the aforementioned PPG measurement capabilities require a universally agreed-upon protocol to ensure that different devices provide consistent results for the same user or patient. This requirement is crucial for accurate measurements to be eligible for clinical applications. Moreover, regardless of the device's manufacturer, doctors need to rely on the measured data to provide correct treatment or formulate an observational plan.

While the PPT method has demonstrated high-confidence results in estimating blood pressure with the defined protocol, there is still an applicability problem on the user end. Measuring PPT involves two sensor readings simultaneously: EKG and PPG, as discussed earlier. Even though the latter doesn't require user intervention, the former necessitates the user to close the electrical circuit of their body to perform an EKG. Consequently, the measurement cannot be performed frequently without user intervention, for example, during sleep or physical exercise. In essence, it shares the same measurement limitations as a traditional oscillometric blood pressure device [32].

For assessments that involve standalone PPG sensors, a major limitation is the calibration protocol process. Given the optical nature of the recorded quantity, the readings need to be calibrated with a mechanical reference, especially for applications intended for blood pressure estimation. In the assessment of vasomotor state, where absolute values aren't critical, a protocol should outline which PPG waveform components, besides DCW, need to be evaluated for high-confidence results. This is necessary due to the various waveform morphologies that appear in the real world, apart from the idealized perfect shape.

The same approach is necessary for measurements involving time values, such as left ventricular ejection time (LVET) determination. While the association with the TDW feature yields good results, a common agreement on the onset and ending points is essential. Given that the TDW component comprises a finite disturbance event in combination with different morphologies, a protocol is also required for

consistency in interpretation.

Finally, from a numerical standpoint, all the measured hemodynamic markers should be evaluated by a specialist within the proper context. The obtained data must be assessed to determine if the measurements were intended for prevention, treatment evaluation, or cardiovascular disease monitoring.

PHOTOPLETHYSMOGRAPHY FUTURE

An important stride in pulse oximeter technology has already been taken by integrating it into wearable devices. The increased computational power per integrated circuit in these devices holds the potential to enhance the signal processing part of the technology.

The availability of open-source databases containing hemodynamic and physiological data intended for researchers [33,34] will contribute to a proper understanding of PPG technology. In the final analysis, having such resources will help reduce, mitigate, or avoid the discussed drawbacks with a supportive tool.

The existing technological capabilities are poised to elevate PPG technology to clinical grade, enabling it to provide high-confidence measurements. Consequently, cardiovascular markers such as LVET and MPI can be remotely monitored, reducing the need for unnecessary physician office visits. This advancement holds potential benefits for both patients and doctors.

The capability to monitor patients' markers with a higher frequency rate would enhance the evaluation of the efficiency of the treatment plan. Additionally, cardiovascular diseases (CVDs) could be prevented or tracked more effectively, enabling better control and management.

An important assumption pertains to future protocols. The competition to embed hemodynamic markers into wearable devices will likely lead the first entity with proven and credible tracking capabilities to establish standalone protocols. Consequently, the community researching in the respective domain will likely follow the defined rules. However, a potential drawback is that this could trigger disagreements over a common, acceptable protocol, which may delay the accessibility of personalized healthcare [35].

CONCLUSIONS

The age-old noninvasive method, photoplethysmography, holds immense potential for enhancing cardiovascular assessment. However, realizing this potential requires the development of a proper mathematical model that can link and explain all physiological behaviors.

While the interest in PPG surged with the advent of the first smart devices in 2013, no disruptive progress has been achieved since then. Instead, some recurrent topics have been validated rather than proposing novel waveform markers for association with clinical applications. Blood pressure estimation and vasomotor state tracking are currently receiving the most attention, with popular companies piloting projects to integrate them into wearable devices.

Despite current limitations, PPG has shown validity, especially in estimating LVET and in binary detection between hypertensive and normotensive patients. Given the current context of technological advancement, there is optimism that this technology will achieve clinical-grade confidence. Consequently, it is anticipated to become an integral part of human lives by providing easy accessibility to personalized healthcare. This, in turn, will unlock improved health monitoring and contribute to a reduced workload for physicians in handling less critical tasks.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, L.E. and L.D; methodology, L.E.; software, L.E.; validation, L.E., A.F and L.D.; formal analysis, L.E; investigation, L.E.; re-sources, L.D.; data curation, A.F.; writing—original draft preparation, L.E.; writing—review and editing, A.F; visualization, L.E.; supervision, A.F.; project administration, L.D.; All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Ethics approval and consent to participate

Not applicable.

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