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The Updating and Individualizing of Sleep Hygiene Rules for Non-clinical Adult Populations

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Abstract: Sleep hygiene is essential for the prevention of somatic and mental disorders, including the prevention of sleep disorders. However, it does not typically address individual differences. The aim of this review is threefold: first, to outline the empirical evidence for particular components of sleep hygiene rules; second, to indicate the importance of individualized sleep hygiene application with regard to the varying degree of validity of sleep hygiene rules in the population; third, to highlight a new field of sleep hygiene, namely light hygiene. PubMed and Google Scholar were used to identify studies that were published between 2007 and 2022. A search was conducted for studies related to sleeping rules topics: sleep regularity, regular exercise, alcohol, caffeine, napping, relaxation and meditation, food intake and light exposure. In applying these sleep hygiene principles, it is essential to pay attention to individual variables such as age, genetic predisposition, health status, and substance (caffeine, alcohol) possible dependence.

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Introduction

Sleep hygiene (SH) is defined as a set of behavioural and environmental recommendations designed to promote healthy restorative sleep. SH usually focuses on the following areas: *daytime/evening routine, sleep environment and consumption of substances that affect sleep*. The first references to this topic date back to the second half of the 19th century (Gigli and Valente, 2013). One of the earliest modern comprehensive concepts of SH rules was defined by Peter J. Hauri in the 1980s (Chung et al., 2018). In 1991, this list of recommendations was updated, and since then, several further modifications have been made. SH rules are not only used to prevent sleep difficulties in the general population but are also used for patients with sleep disorders as part of a treatment approach. Currently, there are several versions of sleep hygiene rules that vary in their directive and detailed nature (Chung et al., 2018).

Sleep hygiene represents an important component of disease prevention and of a healthy lifestyle for the following several reasons: (1) it is easily accessible, (2) it does not require direct long-term involvement of a professional, (3) it is relatively inexpensive, and (4) it can serve as a first-choice intervention for sleep problems or as a prevention of sleep disorders. Following the rules of SH can positively affect not only the quality of sleep itself but also daytime functioning (Brick et al., 2010; Yazdi et al., 2016). On the other hand, awareness of SH does not necessarily mean better sleep (Voinescu and Szentagotai-Tatar, 2015). Moreover, potential users may be discouraged and confused by multiple versions of SH rules and overgeneralization within them (Irish et al., 2015). Therefore, updating the SH rules and maintaining consistency of the SH approach is important for both nonclinical and clinical populations. No less important is a focus on individuality, as SH is usually defined in general terms, without considering the influence of age, chronotype, morbidity or genetic disposition of the individual.

The main aims of this review are to (1) critically review the empirical evidence for the different components of SH and clarify specific guidelines for optimal sleep support; (2) incorporate individual aspects of SH rule application; and (3) highlight the importance of light hygiene as a new field of SH. A summary of the results and recommendations based on recent empirical studies are presented in Table 1.

PubMed and Google Scholar were used to identify studies that were published between 2007 and 2022. A search was conducted for studies related to sleeping rules topic: sleep regularity, regular exercise, alcohol, caffeine, napping, relaxation and meditation, food intake and light exposure. The choice of rules was inspired by the original letter of sleep hygiene by Peter J. Hauri (1991).

Sleep regularity

One of the most important rules of sleep hygiene is a regular sleep schedule, which means going to bed and arising at the same times each day, including weekends. This is based on the effort to maximize synchronization between homeostatic sleep pressure, circadian rhythm, and the sleep period (Zuraikat et al., 2020).

Table 1 – Summary of empirical results and sleep hygiene recommendations for a healthy population

Sleep hygiene recommendations	Summary of recommendations and research results
Regular sleep timing	• Irregular sleep patterns disrupt sleep. A regular sleep schedule throughout the week, including weekends, is important for everyone. • Adolescents and young adults who do not sleep in accordance with their biological needs are the most vulnerable group in terms of physical and mental health effects.
Regular exercise	• Regular as well as irregular exercise improves sleep. The following factors should be considered: age, chronotype, regularity, timing, type of exercise and health status. • Exercise affects the circadian rhythm; late exercise can cause a sleep delay. • Morning exercise is good for synchronizing circadian rhythms; evening exercise does not have as much of a negative impact on "night owls" as it does on "early birds". • Evening low-intensity exercise or yoga is recommended, especially for people suffering from anxiety and depression. However, intensive exercise is not recommended within 2–3 hours before bedtime.
Alcohol avoidance	• Sleep disruption due to alcohol is dependent on the age and sex of the individual and the amount of alcohol consumed. • Acute alcohol use before bedtime reduces sleep latency but increases the number of awakenings in the second part of the night. • Chronic alcohol use is associated with difficulty initiating sleep, poor sleep quality and daytime sleepiness. • One shot of liquor is sufficient to negatively affect sleep in women.
Caffeine avoidance	• All sources of caffeine intake should be considered (tea, coffee, chocolate, medications, etc.). • Caffeine doses of 100–150 mg consumed within 3 hours before bedtime significantly disrupts sleep (objectively and subjectively). • The effects of variable individual metabolisms (polymorphism of cytochrome CYP1A2), which affect the rate of caffeine metabolism and susceptibility to insomnia, should be considered. • Caffeine receptor sensitivity increases with age and caffeine metabolism decreases with age. • Pregnant women should avoid caffeine due to its slow metabolization during pregnancy.
Napping	• The benefits of napping are dependent on the nap timing and duration and the age and health status of the individual. • The ideal length of a nap is approximately 15 minutes; there is no subsequent sleep inertia and this length of nap has a positive impact on nighttime sleep. • No significant negative sleep effects from early napping up to 50 minutes have been shown for adults who are elderly.

Sleep hygiene recommendations	Summary of recommendations and research results
Relaxation and meditation	• These are especially recommended for people with stress, insomnia and anxiety. • The most effective modes are progressive muscle relaxation, mindfulness, yoga nidra, etc.
Food intake	• Food is one of the cues for the circadian rhythm (whether it occurs in the day or night). The timing, amount of food ingested, and the composition of the diet are important: vitamins B1 a B2 synthesis promote sleep, amino acid such as L-theanine is also known to induce sleep. • It is recommended to abstain from eating for 2–3 hours before bedtime. • Time-restricted eating (10 hours of eating and 14 hours of fasting) is beneficial for human circadian rhythms (it serves as a de/synchronizer).
Light exposure	• Light is the strongest synchronizing agent (zeitgeber) for the circadian system. • The timing, intensity and duration of light exposure, the light's wavelength, and contrast of light intensity during the day versus light intensity during the evening influence sleep. • Exposure to blue light between 30–60 minutes before bedtime in a healthy individual leads to a shift in sleep time of up to 30 minutes and a reduction in melatonin secretion. • Natural light exposure during the first hour after waking (to synchronize circadian rhythms) is advisable, along with exposure to light of reduced intensity and wavelength in the evening. • The sensitivity to evening light varies by more than 50-fold among individuals.

Sleep irregularity has a number of health implications. The highest sleep pattern variability is evident in young adults and adolescents, who spend more time in bed on days off compared to school/work days. The magnitude of the difference in sleep timing between work and nonwork days (so-called social jet lag) is related to weight gain and higher insulin resistance (Roenneberg et al., 2012; Bailey et al., 2014; Taylor et al., 2016). Weekend catch-up sleep is also known to be less effective in optimizing neurobehavioral function than the recommended 8–10 hours of sleep at that age (Lo et al., 2017). Irregular sleep rhythms are associated with lower work performance (Phillips et al., 2017), lower ratings of subjective sleep quality, mood declines, and a higher risk of depression (Fang et al., 2021). It is also important to sleep regularly in our biological rhythm. Studies show sleeping in chronotype schedule improves sleep quality, impacts REM (rapid eye movement) sleep and total sleep time (Reiter et al., 2023).

Current clinical sleep therapies also require regularity in wake time but allow or even encourage variability in the time of going to bed at a preferred time when

patients feel sleepy (Johnson et al., 2016; Maurer et al., 2021). Sleep irregularity among shift workers is a major problem that goes beyond the scope of the text.

Recommendation: Go to bed and arise at the same hours throughout the week as much as possible.

Regular exercise

To assess the effect of exercise on sleep quality, several variables should be taken into account. In particular, age, chronotype, exercise consistency, exercise timing, exercise mode and health status were assessed.

Regular exercise in healthy adults has a positive impact on subjective sleep quality, measured by the Pittsburgh Sleep Quality Index (PSQI) as well as objective sleep parameters and sleep duration and can even lead to a reduction in prescribed medications (Kredlow et al., 2015). Regular exercise generally provides higher benefits than occasional exercise, but longitudinal studies on this topic are still lacking (Park et al., 2021).

At the beginning of the 21st century, there was a clear recommendation to avoid exercise in the evening due to increased arousal (Youngstedt et al., 2021), but a number of recent studies underline the importance of evening low-intensity exercise, light stretches or yoga, especially in older adults (Seol et al., 2021) and people suffering from anxiety and depression (Flausino et al., 2012). Late-night low-intensity exercise is contraindicated for individuals who do not engage in physical activity during the day (Youngstedt et al., 2021). It is also important to remember that exercise can influence the circadian system. Engaging in moderate to heavy physical activity in the evening can cause a phase delay, but this does not apply in all cases (Youngstedt et al., 2019). For late chronotypes, both morning and evening exercises induce phase advance. In contrast, in morning chronotypes, phase advance is experienced due to morning exercise, but phase delay is experienced due to evening exercise (Thomas et al., 2020). Morning exercise may reduce circadian desynchronization in the general population and especially in young adults, who are more likely to have the evening chronotype (Lang et al., 2022).

Recommendation: Exercise has a positive effect on sleep, but it is important to consider individual differences in chronotype and adjust the time, intensity and type of exercise accordingly.

Alcohol

Alcohol is known to be a potent somnogen, apparently inducing sleep by increasing adenosine levels and disrupting the sleep architecture. The negative effect of alcohol on sleep is repeatedly mentioned in the literature (Bando and Hotate, 2012; Ebrahim et al., 2013). However, it is one of the most commonly used "over-the-counter" sleep aids (Thakkar et al., 2015; Kurhaluk, 2021). In terms of sleep hygiene, it is necessary to consider the timing, amount of alcohol, type of alcohol intake and sex of the consumer (van Reen et al., 2011). Alcohol absorption runs for a number of

hours and can be inhibited by eating at the same time (Jiang et al., 2020). Research results confirm the negative effect of alcohol, even in small doses (e.g., one shot of liquor 1 hour before sleep) (Bando and Hotate, 2012).

Acute alcohol intake alters the secretion of melatonin and cortisol and alters body temperature in cases of dependency and even in the withdrawal period (Meyrel et al., 2020). The consumption of one shot of liquor 1 hour before bedtime suppresses melatonin secretion by up to 20% (Rupp et al., 2007). Acute alcohol intake in occasional alcohol users disrupts sleep architecture and significantly fragments the second half of the night (Thakkar et al., 2015). Chronic alcohol use is associated with difficulty initiating sleep, poor sleep quality and daytime sleepiness. Particularly problematic is so-called "binge drinking" (occasions of consuming 4 drinks in one day), which is associated with a higher risk of insomnia, especially in people over the age of 50 (Canham et al., 2015). There is a relationship between alcohol consumption and increased inflammatory processes (Wilkinson et al., 2018).

Other individual variables, such as sex, also need to be taken into account (Wall et al., 2016). Alcohol use affects sleep systems differently in men and women. Women show greater sleep disruption than men (Inkelis et al., 2020).

Recommendation: Avoid alcohol consumption in the evening (at least 3 hours before going to bed, 6 hours at best). Although the person may not be aware of it, alcohol has a negative impact on sleep even in small doses, such as one shot of liquor 1 hour before sleep.

Caffeine

Caffeine is the most widely used psychoactive substance. Approximately 80% of the world's population uses coffee daily, and its daily use gradually increases, while the age of consumption begins to decrease (Heckman et al., 2010). Caffeine takes effect in the plasma within 30 minutes on average, and the half-life of consuming one cup of coffee is 3–7 hours. The effect is highly dependent on receptor sensitivity and adaptation, age and other lifestyle factors (Irish et al., 2015).

Generally, 100–150 mg of caffeine (1 espresso contains approximately 90 mg) 3 hours before sleep causes significant sleep disruption in several different ways: increased sleep latency, decreased total amount of sleep, increased amount of superficial sleep stages and a reduction in the duration of the deep and REM sleep stages, as well as multiple sleep stage shifts (Burdan, 2015; Watson et al., 2016). Caffeine consumption also affects melatonin secretion and may delay the onset up to 40 minutes (Burke et al., 2015). There is evidence that regular consumers develop a tolerance to caffeine (Weibel et al., 2020). Morning consumption (approximately 2 cups of coffee) affects the architecture of sleep in low consumers but not in regular consumers (Porkka-Heiskanen, 2011). There are also clear differences in the sensitivity of the caffeine effect on sleep related to the polymorphism of cytochrome CYP1A2. This is characterized by different metabolism speeds (Nehlig, 2016; Tennent et al., 2020) CYP1A2 activity is affected not only by genotype, sex and

Table 2 – Overview of caffeine content in certain beverages and foods

Type of beverage/food	Average concentration	Range of caffeine (mg)*
Filtered coffee	85 mg/125 ml	60–135
Instant coffee	65 mg/125 ml	35–105
Decaffeinated coffee	3 mg/125 ml	1–5
Espresso	60 mg/30 ml	35–100
Tea	32 mg/150 ml	20–45
Iced tea	20 mg/ 330 ml	10–50
Hot chocolate	4 mg/150 ml	2–7
Caffeinated beverages without alcohol	39 mg/330 ml	30–48
Cola	41 mg/330 ml	26–57
Energy drinks	80 mg/330 ml	70–120
Chocolate bar	20 mg/30 g	5–36
Dark chocolate	60 mg/30 g	20–120
Milk chocolate	6 mg/30 g	1–15

*it depends on the brand, but, for coffee and tea, it also depends on the type, length of steeping, filtration, temperature and preparation method; data from: http://www.coffeeandhealth.org

age but also by smoking, which accelerates caffeine metabolism. In contrast, caffeine metabolism is slower in women and pregnant women. By the third trimester, it can take up to 18 hours to metabolize caffeine (Tennent et al., 2020). People who are older are more sensitive to caffeine due to age-related changes in adenosine receptor transmission (Frozi et al., 2018).

Recommendation: Avoid caffeine intake 6–8 hours before sleep. It is important to remember that caffeine is also contained in other products besides tea and coffee. The combination of individual items consumed during the day can easily lead to overconsumption of caffeine and thus overstimulation (Table 2).

Napping

The benefits of napping depend on the length and timing of the nap. Another important nap-related variable is age. In terms of length, research studies suggest that the ideal length of a daytime nap is approximately 15–20 minutes due to minimal sleep inertia, as individuals do not go into deep sleep stages (Hilditch et al., 2017). In terms of time suitable for daytime sleep and age, even 50 minutes of midday sleep does not negatively affect nighttime sleep in individuals who are elderly (Faraut et al., 2017). In the population of older adults, evening napping is, surprisingly, not necessarily associated with negative effects on sleep (Dautovich et al., 2008), but it may cause earlier rising in the morning (Faraut et al., 2017). Early afternoon napping was shown to have shorter sleep latency and increased nocturnal sleep efficiency with higher slow-wave activity compared to late afternoon napping (Faraut et al., 2017).

It is important to distinguish between healthy populations and patients suffering from insomnia. The negative effect on nighttime sleep in insomnia sufferers is due to

reduced sleep pressure, regardless of the timing or length of daytime sleep (Jang et al., 2018).

Recommendation: If necessary, take a 15–20 minutes nap in the early afternoon. For individuals who are elderly, it is possible to extend that time, but the subsequent times of falling asleep and arising should be monitored. Individuals with sleep problems should avoid naps.

Relaxation

Petit et al. (2003) in their sleep education guidelines, recommend setting aside time to "wind down" and use relaxation techniques before bedtime, because those techniques can balance sympathetic overactivity and hyperarousal and reduce stress and tension (Jerath et al., 2019). One of the most commonly used relaxation techniques is Jacobson's progressive muscle relaxation (PSR), and more recently, mindfulness techniques have been widely used. Both of these techniques have shown a positive effect on reducing stress and tension or anxiety while increasing subjective sleep quality regardless of age in students as well as in seniors (Örsal et al., 2014; Black et al., 2015). No negative effect was observed regardless of the participants' ages (Mirzanah et al., 2020). These methods are equally effective in patients with chronic insomnia (Hubbling et al., 2014) or in those with other clinical conditions (Harorani et al., 2020). Self-relaxation training relieves pain, resulting in higher subjective well-being, which is essential for easily falling asleep (Sun et al., 2013). Recently, the effect of yoga nidra has also been investigated. Yoga nidra appears to be a very promising method that generally improves well-being (Gulia and Sreedharan, 2022) and, according to objective sleep measures, increases sleep efficiency and the amount of deep sleep stages while simultaneously reducing cortisol levels (Datta et al., 2021).

Recommendation: Relaxation techniques, such as progressive muscle relaxation, mindfulness or yoga nidra, result in positive effects regardless of sex or age. The most effective relaxation technique seems to be yoga nidra, which even increases the amount of deep sleep. These techniques can be performed before bedtime or during the day.

Food intake

Food intake is another important factor in sleep hygiene. The three most relevant aspects of food intake are timing, duration of food intake during the daytime and composition of the diet.

The timing of food consumption plays a role in the synchronization of peripheral circadian rhythms. Inappropriate timing of food intake can destabilize metabolic processes (Stenvers et al., 2012; Bo et al., 2017) and generally desynchronize circadian clocks (Mukherji et al., 2015). Food consumption later than 2–3 hours before bedtime is known to reduce sleep quality, slow digestion, and increase heartburn and reflux. Nocturnal awakenings and lower sleep duration are also potential consequences (Chung et al., 2020).

The total time spent eating within a 24-hour period is highly relevant. Studies suggest that extended eating over 15 hours may be a factor contributing to metabolic disorders (McHill et al., 2017; Réda et al., 2020). Conversely, time-restricted eating (10 hours of eating and 14 hours of fasting) reduces weight, blood pressure and lipid levels (Wilkinson et al., 2020).

What we consume is also important. Nutrients that affect tryptophan availability, such as protein (Nongonierma and FitzGerald, 2015), or serotonin and melatonin synthesis, vitamins B1 and B2 (Vernia et al., 2021), promote sleep. Amino acids such as L-theanine (which is found in green tea or can be taken as a supplement) are also known to induce sleep (Kim et al., 2019). The amount of fats is associated with both better and worse sleep quality. Type of fat appears to be a significant factor. Unsaturated fats, such as those found in fatty fish, avocado, etc., are associated with better sleep quality (Frank et al., 2017; Wilson et al., 2022). Dietary recommendations for the general population favour vegetable oils, which are low in saturated fat (St-Onge et al., 2016). High-fat meals are not recommended for people with sleep-disordered breathing. Studies involving patients with obstructive sleep apnea have shown that a fatty dinner can increase disease severity (Trakada et al., 2014; Wilson et al., 2022).

Recommendation: Avoid any food consumption 2–3 hours before bedtime. Try to consume foods and nutrients that promote the production of serotonin and melatonin, affect tryptophan availability, and induce sleep (e.g., L-theanine).

Light hygiene

The most up-to-date sleep hygiene recommendations include light hygiene, which is why it is given the most attention in this paper. Light is the strongest synchronizer of our circadian rhythms. Light intensity in nature can reach up to 100,000 lx in direct sunlight and ~25,000 lx out of direct sunlight during the day, decreasing to an intensity of 0.1–0.3 lx during the night, even during a full moon (Rumanova et al., 2020). However, natural conditions are very different from artificial lighting environments. In buildings, light intensity is much lower during the day, with intensity ~500 lx (Blume et al., 2019). Conversely, in the evening and during the night, we are exposed to much more indoor light than outdoors; ~5–15 lx is reached by normal street lamps in cities, ~100–300 lx is the light in normal living rooms, and a PC screen emits ~40 lx, depending on its size (Bedrosian and Nelson, 2017). The contrast of light and dark occurring under natural conditions, i.e., high intensity during the day and darkness in the evening, is important for the proper functioning of the circadian system.

The timing, intensity, duration, and wavelength of light modulate the photic settings of the circadian system (Chang et al., 2011) and affect sleep quality, cognitive performance, alertness (Wahl et al., 2019) and the secretion of hormones that exhibit circadian rhythmicity, such as melatonin or cortisol (Schmid et al., 2021). Exposure to blue light even at low intensities right before bedtime can have serious

consequences for sleep quality, including delay and suppression of REM sleep or sleep timing (Cho et al., 2013; Wahl et al., 2019).

Dimmed artificial light in the evening helps to balance circadian rhythms and positively affects sleep and even short-term memory (Tam et al., 2021). Exposure to orange light in the evening causes less sleep phase shift and more evening sleepiness than exposure to blue light (Münch et al., 2017). Nocturnal sleeping with the light on (~40 lx from a lamp or a TV) has a significant negative effect on sleep architecture and quality compared to sleep in the dark (Cho et al., 2013). The effects of blue light may become less pronounced with increasing age (Daneault et al., 2016), and children and young adults have a more intense response to melatonin suppression by blue light than adults who are elderly (Bedrosian and Nelson, 2017).

In general, there is considerable variability in the sensitivity of the circadian system to light. On average, people are highly sensitive to evening light. Fifty percent suppression of melatonin occurs when illuminance is < 30 lx, but this sensitivity to evening light varies by more than 50-fold among individuals (Phillips et al., 2019). Interindividual differences in light sensitivity may explain differences in vulnerability to disruption of the circadian system and the subsequent impact on human health.

Recommendation: It is advisable to expose oneself to natural bright blue light in the morning and, conversely, to avoid exposure not only to blue light but also to high-intensity light (more than 10 lx) in the evening. Individuals should sleep in a dark room (using blackout curtains) and wear comfortable sleeping masks, if necessary.

Discussion

The presented text summarizes recent research that provides the basis for sleep hygiene rules as they are currently understood. The basic principles and general recommendations for healthy sleep have not changed substantially over the past 40 years. Unfortunately, difficulties in their application are gradually becoming apparent due to the lack of individualized approaches.

Future studies addressing recommendations for healthy sleep should be fully relevant to current life circumstances. They should reflect common behavioural patterns (not sleep alone) while incorporating tolerance to substance (caffeine, alcohol) or habit-related issues associated with those patterns (Weibel et al., 2020). For example, daytime nap is not recommended to people with insomnia, but daytime nap about 15 minutes can be beneficial (positive effect on short-term improvements in physical performance, memory, emotional processing) and without side effects for healthy people (Mantua and Spencer, 2017). It would also be desirable to address the interaction between different habits. For instance, a nap after lunch may reduce the need for caffeine, and the consideration of the sleep-disrupting effects of substance withdrawal could be important in people who are struggling with addiction (Irish et al., 2015). Another example is the interplay between caffeine metabolism and tobacco use. Tobacco use accelerates caffeine metabolism by increasing the activity of the enzyme CYP1A2 in the liver (Tennent et al., 2020). Some rules are

applicable to the whole population with only minimal exceptions, for example the importance of exposure to light in the morning or avoiding light of high intensity in the evening. Light hygiene plays a preventive role not only in sleep disorders but also in mental health. The adoption of habits related to exposing ourselves to natural light and, conversely, avoiding light at times when it is naturally dark, may seem trivial, but it is absolutely essential for the optimal functioning of our bodies, and it has an above-average effect on our sleep (Swanson and Burgess, 2017). On the other hand, inter-individual differences in light sensitivity must also be taken into account in this case (Phillips et al., 2019).

Sleep hygiene is one of the pillars of health and is part of a healthy lifestyle. It is important to explain these rules to individuals and take into account their lifestyle and possible inter-individual differences. In addition, it is advisable to be flexible in demanding strict observance of sleep hygiene rules.

Conclusion

Individual factors have been shown to play an important role in sleep hygiene, including age, chronotype, genetic predisposition or health status. If sleep hygiene rules are offered without an individualized approach, they may not yield the desired results or may not be applicable in a particular case and may therefore be rejected by the recipient because the rules appear generally impractical.

Sleep hygiene remains essential for the prevention of somatic and mental disorders, including the prevention of sleep disorders. Non-compliance with sleep hygiene rules can cause serious sleep problems or lead to progression to clinical conditions.

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Potential Mechanism of Platelet-rich Plasma Treatment on Testicular Problems Related to Diabetes Mellitus

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Abstract: Diabetes mellitus is a condition of continuously increased blood glucose levels that causes hyperglycemia. This condition can result in disorders of various organs including testicular problems. The use of platelet-rich plasma (PRP) which is contained in several growth factors shows its potential in overcoming testicular problems. This literature review study was conducted to identify the potential of PRP in overcoming various testicular problems due to diabetic conditions.

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by a continuous increase in glucose levels known as hyperglycemia. Impaired insulin secretion and action lead to hyperglycemia, manifested in carbohydrate, fat, and protein metabolism disturbances (American Diabetes Association, 2014). The increasing prevalence of DM is a global health challenge and can affect individuals, families, and communities' well-being. International Diabetes Federation states that in 2021 as many as 537 million adults (20–79 years) have diabetes, and as many as 3 out of 4 adults with diabetes live in low- and middle-income countries (IDF, 2021). The cases of DM are predicted to increase, and it is estimated they will reach 578 million cases in 2030 and 700 million cases in 2045 (Saeedi et al., 2019). The prevalence of DM in Southeast Asia in 2021 is estimated to reach 90 million and predicted to rise to 152 million in 2045 (IDF, 2021). DM is the 3rd disease with high mortality in Indonesia, with a prevalence of around 10 million people in 2018. Its prevalence is expected to increase two to three times in the next 10 years (KEMENKES, 2018).

Reproductive system problems caused by DM have been reported in several studies. Sperm analysis in men with DM showed lower results on concentration, sperm motility, seminal fluid volume, and testosterone levels compared to healthy men (Maresch et al., 2017; Long et al., 2018). The imbalance between reactive oxygen species (ROS) production and the ability to deoxidation results in increased oxidative stress in the reproductive system of men with DM. This condition initiates the impairment of the protein and DNA structure of the cells which results in damage to cellular function. Previous studies revealed that increased oxidative stress in DM can cause testicular DNA damage, disturb the reproductive cell survival and spermatogenesis function, and further cause male infertility (Shrilatha, 2007; Shrilatha and Muralidhara, 2007).

Platelet-rich plasma (PRP) has been widely used to treat several health problems, including aesthetic dermatology as a skin rejuvenation agent, acne scar treatment, hair loss treatment, treatment for skin pigmentation problems, and several other skin problems (White et al., 2021). Several studies have been conducted to determine the therapeutic effect of PRP on male reproductive problems treatment. A previous study by Dehghani et al. (2019) showed that intratesticular injection of 80 µl of PRP in rat model of male infertility induced by Busulfan, increased the number of spermatogenic stem cells, sperm count, motility, tail length, and testosterone level. This shows that PRP has the potential to repair the testicular structure and function damage in infertility animal models (Dehghani et al., 2019). The aim of this literature review is primarily to discuss the potential mechanism of PRP to treat and improve testicular problems as a result of DM.

Molecular pathophysiology of diabetes mellitus

This condition may be due to insulin secretion disturbance resistance in responding to insulin peripheral action, or both (Zheng et al., 2018). Generally, DM is

categorized into 3 subtypes namely: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and gestational diabetes (GDM) (American Diabetes Association, 2009). Sedentary lifestyle, aging, and contamination of chemicals toxicity have led to an increase in the prevalence of T2DM in society with a proportion of 90% of all DM cases (Hu and Jia, 2019). DM will cause various organs disturbances such as the kidneys, heart, eyes, blood vessels, nerves, and reproductive organs. Several studies stated the adverse impact of DM on testicular function so that it can affect fertility status (Condorelli et al., 2018; Galicia-Garcia et al., 2020). Excess nutrition and obesity cause hyperglycemia and dyslipidemia which have an impact on chronic and systemic inflammation which in turn develops into complications of diabetes in various organs. Macrophages and dendritic cells will be activated as a result of chronic hyperglycemia and dyslipidemia resulting in increased release of proinflammatory cytokines such as IL-1β and TNF-α. Chronic inflammation that occurs will activate various signalling pathways including NF-κB and p38 MAPK which results in the development of several DM complications including diabetic kidney disease, diabetic retinopathy, and diabetic peripheral neuropathy (Kong et al., 2021).

Diabetes progressivity and its complication development are also known to have close relation with the role of advanced glycation end products (AGEs). AGEs production is induced by uncontrolled hyperglycemia which in turn leads to the development of diabetic complications (Peppa et al., 2003; Cole and Florez, 2020). Irreversible damage caused by AGEs on structural and functional integrity through intermolecular and intramolecular cross-linking is believed to be the principal mechanism. Adjacent AGE molecules can bind to each other and to certain proteins resulting in changes in protein structure and function. The disruption caused by this binding affects in inactivation of proteins and enzymes, increased resistance to proteolytic digestion, ROS formation facilitation, increased inflammatory reactions and disrupted intracellular signalling resulting in metabolic and biochemical changes (Khalid et al., 2022). Several cell-mediated pathophysiological responses may arise as a result of interactions between AGEs and various cell surface receptors that are thought to contribute to the onset of DM. Activation of various signalling pathways is triggered by binding between AGEs and their cognate receptors that initiate changes in cellular function and metabolism through increased inflammatory reactions and oxidative stress (Asadipooya and Uy, 2019).

Diabetes mellitus and testicular problems

AGEs formation due to long-term uncontrolled hyperglycemia can indirectly cause sperm disorders in diabetes. Individuals with DM often experience increased levels of AGE in their blood. This triggers further damage processes through the general mechanisms of inflammation, oxidative stress, and apoptosis (Kong et al., 2021). Cellular damage caused by AGEs in the testes has been studied, and it is known to reduce testosterone production leading to spermatogenesis disturbances (Zhao et al., 2016b; Chen et al., 2019; Rizal et al., 2019). AGEs are able to produce ROS

through modification of macromolecular functions independently or through binding to AGEs receptors (RAGE) (Yamagishi, 2008).

Human spermatozoa have less adequate repair mechanisms; besides that, the high content of polyunsaturated fatty acids makes them vulnerable to oxidative stress (Lewis and Aitken, 2005). ROS formation causes membrane lipid peroxidation which results in an increase in malondialdehyde (MDA) levels as a biomarker of lipid peroxidation in spermatozoa (Aitken et al., 1989; Tavilani et al., 2005). Spermatozoa fertilizing ability is strongly influenced by oxidative stress conditions due to increased ROS via the production of lipid peroxides from unsaturated fatty acids which accumulate in large quantities in the sperm cell membrane phospholipids (Nakamura et al., 2002; Mahfouz et al., 2009). A study by Karimi et al. (2011) showed an increase in MDA levels of sperm and seminal plasma in diabetic men with normozoospermic that led to elevated of cell damage. This may lower the fertility capacity in diabetic men with normal semen analysis (Karimi et al., 2011). Seminal plasma has antioxidant capacity both enzymatic and non-enzymatic which can protect spermatozoa from oxidative stress (Micheli et al., 2016). Seminal total antioxidant capacity (TAC) was shown to be lower in diabetic men compare to the non-diabetic (Karimi et al., 2011). The probability of male infertility was increased regarding the reduced seminal TAC due to high levels of ROS (Mahfouz et al., 2009). The decrease in TAC level is in line with the increase in MDA level in diabetic men. Besides that, the study also revealed that AGEs negatively correlated with seminal TAC indicated the increase in stress oxidative in the semen of diabetic men (Karimi et al., 2011).

Platelet-rich plasma (PRP) in reproductive system

Definition of PRP

Platelets are part of the blood cells with the smallest density compared to other blood components. Platelet diameter is 2 μM with normal counts ranging from 150,000–400,000 platelets per μl (Daly, 2011; Williams and Sergent, 2022). The key function of platelets is in the process of aggregation and bleeding prevention through the formation of plugs in the area of damaged blood vessels. It stimulates the secretion of blood clotting factors to induce the coagulation process. The high content of growth factors in platelets also plays a role in inflammatory processes, angiogenesis, stem cell migration, and cell proliferation (Periayah et al., 2017).

Platelet-rich plasma or platelet-rich growth factor is an autologous blood product that is rich in platelets in a small volume of plasma, above baseline obtained by centrifugation (Alves and Grimalt, 2018). PRP has been used extensively in the medical field as a therapeutic option in managing various health problems. Several studies reported PRP could improve the wound-healing process by accelerating the increase in fibroblasts, macrophages, and collagen in maxillofacial surgical interventions (Menchisheva et al., 2019). In addition to the field of aesthetic dermatology, PRP is reported to have a positive effect on treating osteoarthritis by

preventing the severity of articular cartilage damage (Bansal et al., 2021). The use of PRP for the treatment of muscle injuries has been reported in several clinical studies. The PRP treatment showed a positive response to the healing process, reduced pain, and shorten the return to play in some athletes (Setayesh et al., 2018).

PRP classification

Based on the cell components and fibrin architecture, PRP is classified into four major families (Table 1) (Dohan Ehrenfest et al., 2009).

Table 1 – Platelet-rich plasma classification

Pure platelet-rich plasma (P-PRP)	1. Also known as leukocyte-poor PRP 2. Preparation without leukocyte content 3. Low-density fibrin network after activation
Leukocyte- and PRP (L-PRP)	1. Preparations with leukocytes 2. Low-density fibrin network after activation 3. The most widely used in various commercial and experimental protocols
Pure platelet-rich fibrin (PRF)	1. Also known as leukocyte-poor platelet-rich fibrin 2. Preparations without leukocytes 3. High-density fibrin network
Leukocyte- and platelet-rich fibrin (L-PRF)	1. Also known as second-generation PRP 2. Preparations with leukocytes 3. High-density fibrin network

PRP preparation

The PRP production was carried out by differential centrifugation. Sedimentation of certain cellular constituents was obtained by adjustment of acceleration force based on different specific gravity (Dhurat and Sukesh, 2014). For PRP preparation the buffy-coat method was employed, as described below:

1) PRP-method

Centrifugation was conducted two times. Separated red blood cells (RBCs) were obtained from the first centrifugation, while the second ones resulted in a concentrated platelet suspended in the small volume of the plasma.

As an initial step, the whole blood (WB) was collected in the anticoagulants-contained tube. Constant acceleration centrifugation was conducted for RBC separation. This step resulted in three layers: an upper layer contains platelets and white blood cells (WBCs), a middle thin layer namely buffy-coat that is rich-contained of WBCs, and the bottom layer consist of RBCs (Dhurat and Sukesh, 2014).

The upper layer was discarded from the tube, remaining the buffy-coat and RBCs continues to the second centrifugation. The second centrifugation was carried out

at a sufficient speed to separate the buffy coat into PRP and residual RBCs (Dhurat and Sukesh, 2014; Mijiritsky et al., 2021). The upper 2/3rd portion of the volume that mostly contained PPP (platelet-poor plasma) was discarded, and the remaining 1/3rd volume of plasma (±5 ml) was homogenized to prepare the PRP (Dhurat and Sukesh, 2014).

2) Buffy-coat method
Before the high-speed centrifugation, the WB should be stored at 20 °C to 40 °C. There are three layers produced by the high-speed centrifugation: the bottom layer consisting of RBCs, the middle layer consisting of platelets and WBCs and the top PPP layer. The supernatant plasma at the top of the tube was removed and the buffy-coat layer was then transferred to another sterile tube. The separation of WBCs was conducted by low-speed centrifugation or use Leukocyte filtration filter. In the buffy-coat method, as much as 10 ml of the whole blood can produce a very thin layer of buffy coat, making it difficult to separate WBC and platelets from the underlying RBC layer (Dhurat and Sukesh, 2014).

Growth factors component of PRP
Platelets contain three types of granules, namely alpha, delta, and lambda, which secrete various proteins and substances needed in the process of tissue repair. Granules make up about 10% of the platelet volume, with about 50–80 α-granules in each platelet. These granules consist of membrane-bound proteins (including integrin [αIIb, α6, β3], platelet endothelial cell adhesion molecule [PECAM], leucine-rich repeat family receptors [GPIb-IX-V complex], immunoglobulin family receptors [glycoprotein VI] and other receptors [CD36, Glut-3]) and soluble proteins that are released into extracellular space (Maynard et al., 2007). The majority of components of delta granules are beneficial for the clotting process such as calcium, magnesium, adenosine, and bioactive amines (serotonin and histamine) (Jedlitschky et al., 2004). Lambda granules as lysosomal-type organelles contained enzymes that have a critical role in the protein, lipid, and carbohydrate degradation process. They have also a role in the debris and infectious agents removal in the tissue damage (Boswell et al., 2012). Activated platelet-rich plasma will release several growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor β (TGF-β), fibroblast growth factor (FGF), insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF) (Marck et al., 2019).

1) Platelet derived growth factor (PDGF)
PDGF is involved in stimulation of cell proliferation and migration in the process of embryogenesis (Andrae et al., 2008; Demoulin and Essaghir, 2014). The PDGF family consists of four polypeptide chains encoded by four different genes, namely PDGFA, PDGFB, PDGFC, and PDGFD genes that are located on chromosomes 7, 22, 4, and 11, respectively (Fredriksson et al., 2004). This protein acts via two receptors,

namely PDGFRα and PDGFRβ which have different roles in various human biological processes. PDGFRα-dependent signalling regulates the gastrulation and development of lungs, intestines, skin, testes, kidneys, and other organs. Meanwhile, the PDGFRβ is involved in the process of early hematopoiesis and blood vessel formation (Andrae et al., 2008).

2) Transforming growth factor β (TGF-β)
TGF-β consist of three isoforms, namely TGF-β, TGF-β1, TGF-β2 and TGF-β3 (Davis et al., 2014). This growth factor has a crucial role in stimulating collagen production and in protecting it from damage. In addition, TGF-β1 is also involved in the process of angiogenesis, connective tissue regeneration, and immune cell chemotaxis (Gurtner et al., 2008).

3) Vascular endothelial growth factor (VEGF)
VEGF is involved in the process of angiogenesis, nutrient transport, and increases blood flow to the place of injury. The increase in VEGF secretion is interrelated with other growth factors such as PDGF, TGF-β and EGF (Barrientos et al., 2008; Dhillon et al., 2012).

4) Epidermal growth factor (EGF)
EGF is reported to have a close correlation to the action of mesenchymal and epithelial cells in secreting cytokines. The presence of EGF can stimulate mesenchymal cells to undergo mitotic division. The processes of angiogenesis and chemotaxis in endothelial cells are also known to be stimulated by EGF secretion. Other studies also state that EGF can accelerate the epithelialization and healing process (Barrientos et al., 2008; Berlanga-Acosta et al., 2009; Knezevic et al., 2016).

5) Insulin-like growth factor (IGF)
During platelet activation, IGF was released and has a role in the stimulation of differentiation and mitogenesis of mesenchymal cells (Marques et al., 2015). IGF is known to have critical roles in cell growth, differentiation, and together with PDGF it stimulates the collagen synthesis (Pavlovic et al., 2016).

6) Fibroblast growth factor (FGF)
FGF as the most potent mitogen, is involved in various actions in various cell types, especially mesenchymal cells, chondrocytes, and osteoblasts. FGF has a crucial role in the growth and differentiation of chondrocytes and osteoblasts and supports the process of angiogenesis along with VEGF (Barrientos et al., 2008; Oliveira Filho et al., 2010).

PRP treatment in reproductive system
PRP administration in the management of human reproductive problems has been reported in several studies. A previous study reported that PRP has been used

to manage poor ovarian response (POR) (Dawood and Salem, 2018). PRP is also used in assisted reproductive technology, especially for the treatment of premature ovarian failure (POF), which is a condition where the ovary fails to respond to stimulation for follicle production. Based on the study conducted by Pantos et al. (2019), an increase in folliculogenesis and improvement in ovarian function is reported from 8 perimenopausal women/POF after PRP administration.

A study by Bader et al. (2020) showed that the use of autologous PRP in male infertility management showed an improvement in vitality and motility, and a significant reduction in vacuolization, DNA fragmentation, and ROS levels. As much as 2% PRP treatment was reported to improve sperm parameters and prevented cell death in H_2O_2-exposed spermatozoa compared to a fresh-collected semen sample (Bader et al., 2020). Another study conducted by Samy et al. (2020) showed that PRP has an essential role in preventing testicular degeneration in testicular-detorsion-induced rats as indicated by increasing antioxidant markers (TAC, GSH, GST), increasing Bcl2 expression, and decreasing IL-1β, TNF-α, and caspase-3.

Potential mechanism of PRP in treated the testicular problems related to DM

Anti-inflammation

PRP is known to contain high concentrations of growth factors which play an important role in anti-inflammatory activity through inhibition of the nuclear factor-kB (NFκB) cascade. In line with these, there is a decrease in the expression of inflammatory mediators accompanied by a decrease in COX-2 expression (Thursina et al., 2022). The growth factors contained in PRP also facilitate cellular anabolism, increasing the release of inflammatory mediators and modulators resulting in anti-inflammatory and analgesic effects (Xie et al., 2014). This is also supported by research results which show that PRP can counteract the inflammatory cascade by increasing various anti-inflammatory factors such as hepatocyte growth factor (HGF) (Wu et al., 2011; Thu, 2022). The HGF content in PRP is known to inhibit the translocation of the NF-κB-p65 subunit into the nucleus resulting in downregulation of the transcription of several proinflammatory factors such as MMPs, ADAMTs, and IL-1β (Camargo Garbin and Olver, 2019). Apart from HGF, several growth factors contained in PRP are involved in inhibiting the inflammatory process, including PDGF, TGF-β, and IGF (Andia and Maffulli, 2013). The schematic of growth factors contained in PRP in regulating the inflammatory process is depicted in Figure 1.

Anti-apoptosis

PRP is known to have an anti-apoptotic effect, this was shown through a study conducted by Samy et al. (2020) using a rat model of testicular torsion. Testicular torsion is known to reduce Bcl-2 expression as an anti-apoptotic factor. Administration of PRP is known to significantly increase Bcl-2 expression and decrease Caspase-3 expression which indicates PRP's ability to ameliorate the tissue

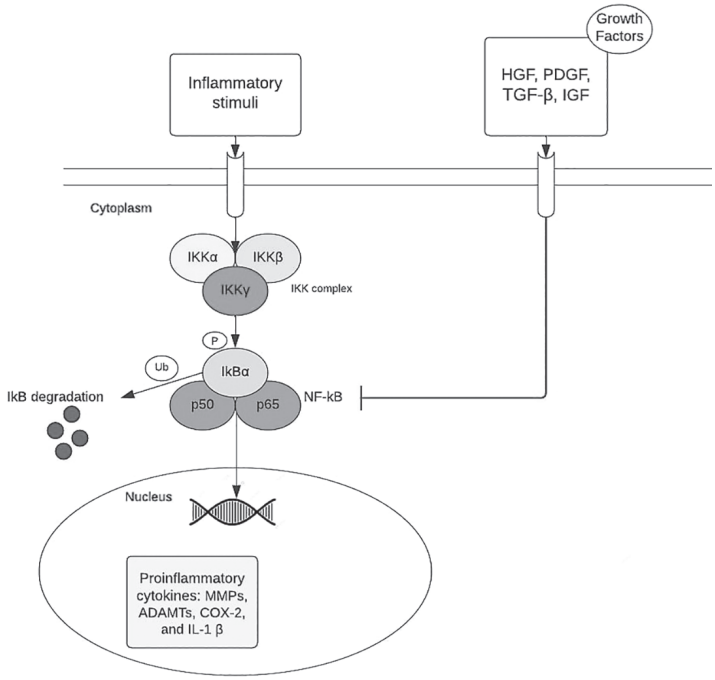

Figure 1 – Platelet-rich plasma (PRP) involvement in inhibitory of inflammation process. Extracellular inflammatory stimulus activates the IκB kinase (IKK) enzyme through binding to integral membrane receptors. This activation causes the IκBα protein to be phosphorylated, resulting in ubiquitination and dissociation of NF-κB which will then be degraded by the proteosome. An activated NF-κB will enter the nucleus for binding to specific DNA sequences and affect several cell functions. Administration of PRP containing growth factors can inhibit the translocation of activated NF-κB into the nucleus resulting in downregulation of various pro-inflammatory factors (Brasier, 2006; Gilmore, 2006; Perkins, 2007; Camargo Garbin and Olver, 2019).

repair ability of testis regarding the ischemia/reperfusion effects due to testicular torsion (Samy et al., 2020) (Figure 2). PRP contains VEGF which has a critical role in cell proliferation, cell cycle maintenance, and germ cells apoptosis prevention (Tunçkiran et al., 2005; Caires et al., 2012; Tao et al., 2017). The overproduction of ROS will be suppressed by PRP administration through the activation PI3K/Akt signalling pathway, resulting in the downregulation of NFκB (Salem et al., 2018).

Antioxidant (ROS)

Vascular endothelial growth factor (VEGF) as one of the growth factors contained in PRP exhibits antioxidant capabilities via activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway *in vitro* (Kweider et al., 2011; Tohidnezhad et al., 2014) as depicted in Figure 3. During the processes of spermatogenesis and fertilization, the Nrf2/antioxidant response element (ARE) signalling pathway and its regulatory antioxidants, play an important role in countering cellular oxidative stress (Kensler et al., 2007; Nakamura et al., 2010). The antioxidant enzymes Zn/

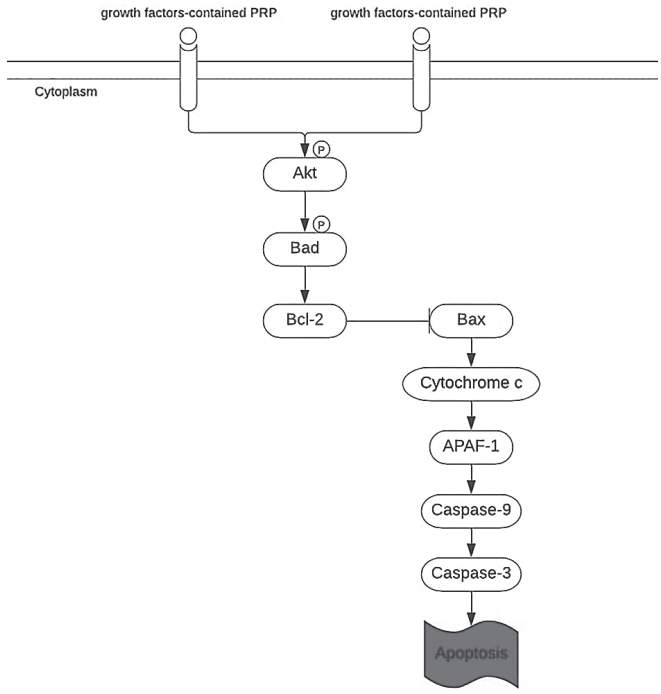

Figure 2 – Schematic inhibition pathway of apoptosis by platelet-rich plasma (PRP). PRP administration has an anti-apoptotic potential by means of activation of the Akt/Bad/Bcl-2 signalling pathway (Tao et al., 2017).

Cu SOD (superoxide dismutase) contained in PRP play a crucial role in protecting sperm motility by increasing the integrity of the sperm membrane. As an important role holder in the ROS scavenger system, this enzyme works by reducing DNA fragmentation due to exposure to H_2O_2 through inhibition of lipid hydroperoxide (LPO) of spermatozoa (Perumal, 2014; Lee et al., 2016; Zhao et al., 2016a).

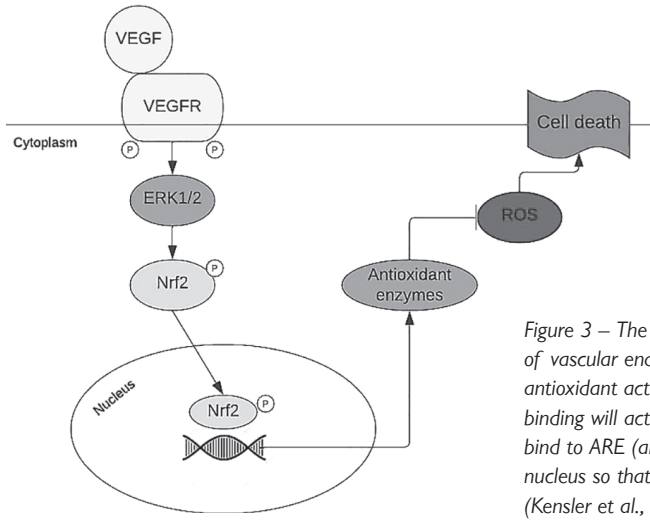

Figure 3 – The potential of signal transduction of vascular endothelial growth factor (VEGF) in the antioxidant activity regulation. VEGF-VEGF receptors binding will activate the Nrf2 pathway which can bind to ARE (antioxidant response element) in the nucleus so that it can regulate antioxidant enzymes (Kensler et al., 2007; Nakamura et al., 2010).

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Complete Denture – Border Molding Technique Using a Laboratory Condensation Silicone Putty: Review

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Abstract: During the fabrication of a complete denture, functional impression is taken. Literature studies show that polydimethylsiloxane (condensation silicone) has not been reported by United States dental schools to perform border molding. Thus, the purpose of this article is to review the functional impression technique when border molding is performed with a laboratory condensation silicone putty.

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Introduction

One of the clinical steps to fabricate a complete denture is the functional impression (Zarb et al., 2004). This step can be performed using the selective pressure technique developed by Boucher (Boucher, 1944; Petropoulos and Rashedi, 2003; Özkan, 2017). The selective pressure technique combines pressure and non-pressure techniques (Boucher, 1944; Petropoulos and Rashedi, 2003; Duncan et al., 2004; Özkan, 2017). After using the wax to create relief on flaccid and retentive areas of the plaster cast, the individual (custom) tray is made. The relief sites will create areas of space between the tray and the mucosa. In these areas, the impression material will not exert pressure on the mucosa, while in other areas, the impression material will exert minimal pressure on it. According to Duncan et al. (2004), "areas that are anatomically favorable to withstanding pressure, such as the buccal surface of the maxillary alveolar process, lateral palate, or buccal shelf in the mandible, are loaded. These areas are supported by dense cortical bone. The rugae, midline raphe, mandibular alveolar ridge, and areas of movable tissue are relieved because they do not provide the same favorable anatomic quality for withstanding functional load (Duncan et al., 2004)".

For complete dentures, the functional impression (selective pressure technique) can be made in two or three steps using an individual tray: Step 1 (Border molding) – It is performed to obtain peripheral sealing of the future complete denture. In this step, one of the following materials can be used: low fusing impression compound, condensation silicone, polyether, polysulfide, wax, or addition silicone. Step 2 (Final impression, *also called* "*corrective impression*") – It is made to copy the oral mucosa of the ridge, and it can be made with the zinc oxide eugenol paste or a fluid elastomer (polyether, polysulfide, or addition or condensation silicone) (Smith et al., 1979; Chaffee et al., 1999; Gennari Filho et al., 2002; Zarb et al., 2004; Solomon, 2011a; Goiato et al., 2011a, 2012, 2013; Carlsson et al., 2013; Mehra et al., 2014; Yilmaz and Özçelik, 2014; Arora et al., 2015; Özkan, 2017; Jayaraman et al., 2018). In addition, it is possible to make the posterior palatal seal or "post damming" (Step 3) in two ways depending on the material that will be used for the final impression (Ansari, 1997; Solomon, 2011a, Carlsson et al., 2013); i.e.:

1) "post damming" after final impression – *when zinc oxide eugenol paste will be used*, posterior palatal seal can be made, after making the final impression, using wax (Ansari, 1997; Goiato et al., 2013);
2) "post damming" before final impression – *when a fluid elastomer will be used*, the posterior palatal seal is made at the same time as the border molding, using an impression compound (Solomon, 2011a) or a silicone putty (Solomon, 2011b). It is important to emphasize that every time an elastomer is used for molding, it is necessary to previously use an adhesive to "glue" it to the individual tray.

There is yet another way to accomplish this step. Before making the individual tray, the "soft palate vibration region of the plaster cast" must be subtly worn down (Solomon, 2011a). Later, during the making of the individual tray, the acrylic resin fills

the worn region of the plaster cast (Solomon, 2011a). Thus, during border molding, the "post damming" is performed by the individual tray.

Functional impression, based on the selective pressure technique, has functions that include: 1) to delimit the area of the ridges where the complete dentures will be supported; 2) to allow mucosal blood flow while the patient wears their complete dentures, promoting comfort and health to the patient; 3) to avoid excessive compression of the ridge, as well as, theoretically, bone resorption; and 4) to allow adequate adaptation, stability, and retention of the complete dentures over the ridges (Chaffee et al., 1999; Petropoulos and Rashedi, 2003; Duncan et al., 2004; Özkan, 2017; Jayaraman et al., 2018). Thus, this step is very important for the fabrication of a complete denture, and it should not be abandoned.

Studies were carried out in dental schools in the United States (US) to verify which materials were used by them, for border molding, to fabricate the complete dentures (Petropoulos and Rashedi, 2003; Petrie et al., 2005; Mehra et al., 2014):

- In 2001, a survey in 41 dental schools in the US showed that the border molding materials used by them included impression compound (28 schools), polyether (2 schools), polyvinylsiloxane (1 school), polysulfide (1 school), Adaptol (Jelenko) (1 school), impression compound and polyvinylsiloxane (5 schools), impression compound and polyether (2 schools), and impression compound and polysulfide (1 school) (Petropoulos and Rashedi, 2003).
- In 2003, questionnaires were mailed to all 1,762 active ACP (The American College of Prosthodontists – US) members and chairpersons of prosthetic/restorative departments in the 54 US dental schools. Nine hundred and forty-five questionnaires were returned by members of the ACP (54% return rate) and 42 questionnaires were returned by the US dental schools (78% return rate). The most popular material used for border molding was impression compound (67% of reporting ACP members, and 95% of the responding dental schools). Other materials used by individuals surveyed, for border molding, were polyvinylsiloxane, polyether, and Adaptol (Jelenko) (Petrie et al., 2005).
- In 2014, an online survey was sent to all program directors of US postdoctoral prosthodontic programs (the overall response rate for the survey was 87%). It was found that 71% of dental schools used the impression compound, 10% of the schools used wax, and the rest of them used polyether, polyvinylsiloxane, or Adaptol (Jelenko) (Mehra et al., 2014).

Therefore, in all these surveys, the material most used for border molding was impression compound (Petropoulos and Rashedi, 2003; Petrie et al., 2005; Mehra et al., 2014). Another situation observed was that polydimethylsiloxane (condensation silicone) is not used by dental schools in the US.

For clinical fabrication of a complete denture, border molding using a laboratory condensation silicone putty has been previously reported by two studies (Gennari

Filho et al., 2002; Goiato et al., 2013). Despite this, the step by step of this technique was not reported in English (Gennari Filho et al., 2002; Goiato et al., 2013). In addition, based on the fact that US dental schools have not reported the use of polydimethylsiloxane for border molding (Petropoulos and Rashedi, 2003; Petrie et al., 2005; Mehra et al., 2014), the purpose of this article is to review the functional impression technique when border molding is performed with a laboratory condensation silicone putty.

Material and Methods

A search was performed on the PubMed website in 2022 using combinations of the following keywords: "complete denture" with "functional impression" or "border molding". The search was expanded as needed. Books in English and Portuguese, on the subject of the article, were included when reporting important information. In addition, a Google search was also performed using keywords in Portuguese ("artigo" e "moldagem functional"). Only references in English and Portuguese (Brazil) were considered.

Review

Clinical steps to manufacture and deliver complete dentures
(Collett, 1970; Tamaki, 1983; Zarb et al., 2004; Telles, 2009; Goiato et al., 2011a, 2014; Özkan, 2017)

(I) Anatomical or preliminary impression.
(II) Functional impression.
(III) Wax rims phase (adjustment of the upper wax rim [#]; selection of artificial teeth [size, width, shape, shade and material [# #]; assembly of the upper plaster cast on the semi-adjustable articulator using the facebow; intermaxillary registration [# # #]; and finalization of the assembly of the plaster casts on the semi-adjustable articulator).
(IV) Aesthetic and functional try-in of the wax-attached acrylic teeth; and artificial gingiva shade selection [# # # #].
(V) Delivery of complete dentures to the patient, and adjustments of acrylic bases and occlusion of artificial teeth [# # # # #].
(VI) Control dental appointments (adjustments of acrylic bases and occlusion of artificial teeth, when necessary).

Note 1 [#]: Buccal corridor, level of wax exposure with the upper lip at rest, upper lip support, and adjustment of the occlusal plane with a Fox ruler.

Note 2 [# #]: Selection of the length and width of the artificial teeth can be done using the Clapp technique. The acrylic teeth shape selection can be according to the patient's face shape (e.g., oval, square, or triangular). The material for the artificial teeth can be acrylic resin or ceramic. The selection of the shade of artificial teeth is also carried out at this stage.

Note 3 [# # #]: The intermaxillary registration refers to the restoration of the vertical dimension of occlusion (using the Pleasure or Willis technique) and centric relation.

Note 4 [# # # #]: This step is performed using an artificial gingiva shade scale. The artificial gingiva chosen for the patient should have a shade similar to that of the inner surface of the upper lip.

Mechanical, functional, aesthetic and phonetic aspects must be evaluated in this functional and aesthetic test phase:

- Mechanical: Check the adaptation of the acrylic bases to the alveolar ridges (the bases must not be extremely relieved); contour, volume, and shape of acrylic bases; thickness of acrylic edges; level of union between artificial teeth and wax (artificial teeth must be well attached to the wax); wax sculpture of the gingival part; and thickness of the acrylic base in the palate region.
- Functional: Check the centric occlusion; disocclusion during protrusion and laterality (bilateral balanced joint); free functional space and vertical dimension of occlusion; lip mobility; occlusal plane; and incisal edges of the anterior artificial teeth of the mandibular prosthesis, which should be at the level of the patient's lower lip, when his mouth is slightly open.

According to Zarb et al. (2004)
"In most patients, the incisal edges of the natural lower canines and the cusp tips of the lower first premolars are even with the lower lip at the corners of the mouth when the mouth is slightly open. If artificial lower anterior teeth are located above or below this level, their vertical positioning will probably be incorrect .... When the lower teeth are above the lip at the corners of the mouth, any one or a combination of the following may exist: (1) the plane of occlusion may be too high; (2) the vertical overlap of the anterior teeth may be too much; and (3) the vertical space between the jaws may be excessive. When the lower teeth are below the lip at the corners of the mouth, the opposite situations may exist".

- Aesthetic: Check the exposure of the upper incisors with the upper lip at rest; and shade, shape, disposition, and position of the artificial teeth; lip support; size and alignment of the teeth; exposure of the artificial gingiva during the smile; smile curve; buccal corridor; and midline.
- Phonetic: The dentist must be aware of whistles and other strange sounds during the patient's speech, as they are indicative of invasion of the phonetic space.

Note 5 [# # # # #]: Adjustment of the acrylic base can be done using the white paste of the zinc oxide eugenol impression material. This adjustment aims "to remove areas" of the base of the prosthesis that generate greater pressure on the alveolar ridge. In this phase, only the adjustment of the artificial central occlusion is recommended, due to the patient's neuromuscular limitations. Later, in the control

appointments, occlusal adjustments can be made during the patient's protrusion and laterality.

If the acrylic area of the palate of the prosthesis is too thick, making it difficult for the patient to pronounce sounds, it can be adjusted using the palatography technique.

Anatomical or preliminary impression

(Tamaki, 1983; Tanaka et al., 1994; Telles, 2009; Guiraldo et al., 2012; Anusavice et al., 2013; Chain, 2013; Guiotti et al., 2016; Özkan, 2017; Moreno et al., 2020)

The anatomical or preliminary impression is the first step in making a complete denture. This procedure is performed using a metal tray for a totally edentulous maxilla or mandible. The manufacturers of these types of trays produce them in different sizes so that the dentist can choose the best option for his patient.

Impression compound, alginate, or condensation or addition silicone putty can be used to make the anatomical impression. Impressions using alginate may require pre-customizing the stock tray with wax strips. This happens when the edges of the stock tray are too far from the base of the vestibule and the floor of the mouth. Thus, these strips are mechanically attached to the edges of the stock tray to increase their length and provide support for the alginate. For the other impression materials mentioned above, customizing the stock tray with wax is contraindicated – because the temperature of ~50 °C required to use the impression compound would melt the wax, and the consistency of both silicones mentioned above would crush or remove the wax from the edges of the tray during the making of the anatomical impression.

To make the anatomical impression of the maxilla or mandible arch, the dentist must perform functional movements of the patient's lips and cheeks; furthermore, for the mandibular arch, the dentist must ask the patient to move his tongue in different directions. These functional movements are similar to those performed during the making of the functional impression and will be discussed in the next topic. After completing the technique, it is necessary to verify that the mold does not present any problems. An incorrect anatomical impression generates an incorrect anatomical plaster cast, which generates an incorrect individual tray. Therefore, it is important to make a correct anatomical impression.

Table 1 shows the materials that can be used to make anatomical impressions and their disinfection protocols.

Functional impression technique (selective pressure technique)

The functional impression technique (selective pressure technique) described below is based on information from the literature (Boucher, 1944; Hardy and Kapur, 1958; Collett, 1970; Smith et al., 1979; Storer and McCabe, 1981; Olsson et al., 1982; Tamaki, 1983; Keller et al., 1984; Council on Dental Therapeutics and Council on

Table 1 – Materials that can be used to make anatomical impressions and their disinfection protocols

Impression material	Disinfection protocol	Plaster must be poured into the mold ...
Impression compound	The mold should be immersed in 2% glutaraldehyde or 2% chlorhexidine for 10 min	immediately after its disinfection
Alginate (irreversible hydrocolloid)	Disinfection should be performed by spraying 1% hypochlorite, 2% glutaraldehyde, or 2% chlorhexidine over the impression. Subsequently, the impression should remain inside a closed container for 10 min	immediately after its disinfection
Condensation silicone	The mold should be immersed in 1% hypochlorite, 2% glutaraldehyde, or 2–4% chlorhexidine for 10 min	within 1 hour
Addition silicone	The mold should be immersed in 1% hypochlorite, 2% glutaraldehyde, or 2–4% chlorhexidine for 10 min	within 1 week

Note: For the addition silicone, it is important to wait at least 1 hour before pouring the plaster into the mold, due to the release of hydrogen gas.

Prosthetic Services and Dental Laboratory Relations, 1985; Bergman, 1989; Ansari, 1997; Chaffee et al., 1999; Gennari Filho et al., 2002; Petropoulos and Rashedi, 2003; Rashedi and Petropoulos, 2003; Duncan et al., 2004; Zarb et al., 2004; Jeannin and Millet, 2006; Telles, 2009; Goiato et al., 2011a, b, 2012, 2013, 2014; Solomon, 2011a, b; Anusavice et al., 2013; Carlsson et al., 2013; Chain, 2013; Gennari Filho, 2013; Yilmaz and Özçelik, 2014; Arora et al., 2015; Carr and Brown, 2015; Habibzadeh et al., 2016; Özkan, 2017; The Academy of Prosthodontics, 2017; Jayaraman et al., 2018; Melo Neto et al., 2020; Moreno et al., 2020; www.zhermack.com/en/product/zetalabor/ [accessed on May 1, 2022]; www.zhermack.com/en/product/zetaplus/ [accessed on May 1, 2022]), and it is performed in 3 or 2 steps depending on the edentulous arch:

- Maxilla: I) Border molding; II) Final impression; and III) Posterior palatal seal ("post damming") (Figure 1A–D).
- Mandible: I) Border molding; II) Final impression (Figure 1B–D).

I) Border molding technique

1) Plaster casts are obtained through preliminary impressions using, for example, an irreversible hydrocolloid.

Before making the individual (custom) acrylic trays*, reliefs are made with wax over retentive areas of the plaster casts (cast partial relief method). The technician can also use wax to relieve areas of the plaster casts that are flaccid in the patient's

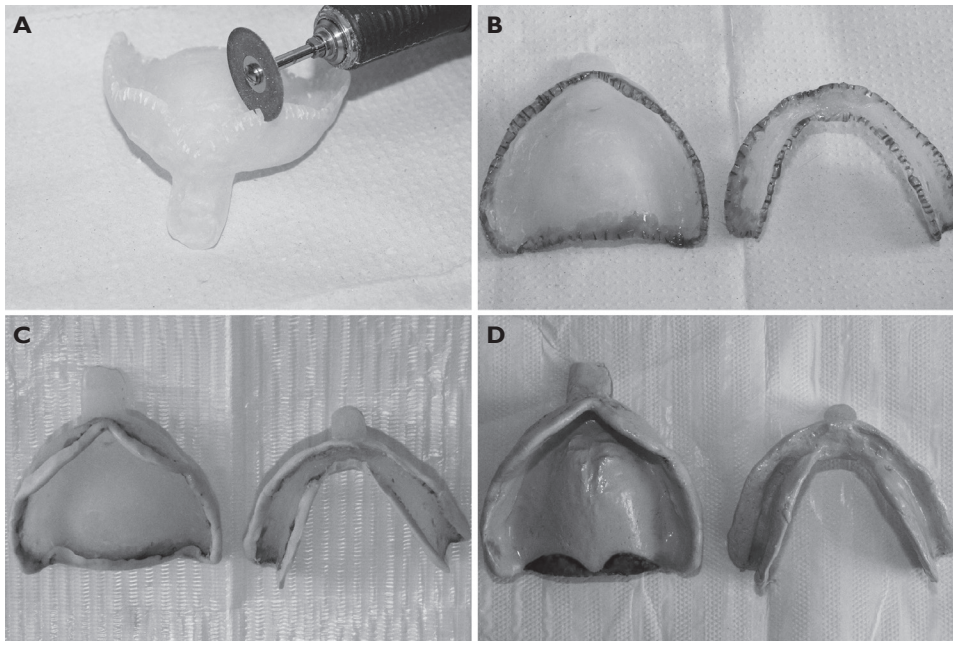

Figure 1 – A) Creation of mechanical retentions; B) adhesive applied to the edges of the trays; C) border molding performed; D) final.

mouth (cast partial relief method)**. However, for this to happen, the dentist must inform the prosthesis laboratory where these flaccid areas are be located. It is noteworthy that these reliefs are created to provide spaces between the flaccid mucosa and the individual tray; which will later be filled with an impression material, during the making of the final impression, without exerting pressure on the flaccid areas.

Note 1: For border molding, it is possible to transfer the wax, which was used to relieve areas of the plaster cast, to the individual tray. Thus, the border molding is carried out with wax reliefs on the inner surface of the tray. This method aims to ensure that, during the border molding, the spaces created between the tray and the mucosa are maintained. Subsequently, before taking the final impression, the wax on the inner surface of the individual tray is removed.

Despite this, it is perfectly possible to carry out the border molding technique without the presence of wax on the inner surface of the individual tray. The technique reported below contemplates this situation.

According to Telles (2009)
*"Regardless of the material used to make the individual tray, it must be rigid to avoid deformation of the impression material supported on it. The most used materials to

make individual trays are: (1) self-curing acrylic resin; (2) thermosetting acrylic resin; (3) light-curing composite resin; and (4) polystyrene sheet".

**"Some authors recommend that relief be performed on retentive areas of the plaster cast to facilitate the removal of the tray from the plaster cast. Others believe it is important to relieve certain regions of the plaster cast where the mucosa has some degree of flaccidity or resilience (e.g., palatine rugae region)".

**"The relief area should be as small as possible, and never cover the entire support area of the plaster cast, as in this case, the area of greatest compression of the tray (overextended edges) would be transferred to the base of the vestibule (and base of the floor of the mouth, for the mandible) (cast partial relief method). It is only possible to relieve the entire support area of plaster cast, if "stops" are created on the inner surface of the individual tray (cast total relief method). Thus, these "stops" can keep the tray in position over the ridge, after the wax relief has been completely removed".

Note: "Stops" can be made with acrylic resin or silicone putty.

2) To make the functional impression, the patient's oral tissue must be healthy and clean.
3) After the dentist receives the individual trays from the prosthetic laboratory, it is necessary to disinfect them before inserting them into the patient's mouth. This disinfection can be performed by immersing individual trays in 2% glutaraldehyde, 1–5% hypochlorite, or 2–4% chlorhexidine for 10 minutes (the higher the concentration of the solution, the greater the antimicrobial effect). Spraying 70% alcohol on the trays and waiting a few minutes, can also be a disinfection method.
4) Impressions of the maxillary and mandibular ridges are taken when the patient is seated in the dental chair. For molding the maxillary ridge – the dentist should be standing and positioned behind the patient. The dentist's elbow should be at the same height as the patient's labial commissure. For molding the mandibular ridge – the dentist should be standing and positioned facing the patient. The height of the patient's labial commissure should be in a position above the dentist's elbow so that the dentist can keep his spine upright. All these precautions allow the dentist to perform these impression procedures in an ergonomically correct position (upright spine).
   Before introducing the individual maxillary or mandibular tray into the patient's mouth, it is important to check its edges. If the edges of the tray are sharp, they must be adjusted so that they are rounded. The inner surface of the trays must also be checked, and if there are any sharp acrylic parts, they must be removed. Verification of the edges of the trays can be done by touch and by the dentist's vision.
5) Typically, the prosthetic laboratory pre-adjusts the individual trays based on the anatomical plaster casts. However, clinically, the dentist must check if the edges of the individual trays are overextended:

- Adjustment of the maxillary individual tray

Initially, the tray must be positioned over the patient's ridge. It is then held in position with one of the dentist's hands (middle finger resting on the tray in the palatal vault region), while the patient's upper lip and cheeks are gently moved towards the floor with the dentist's other hand (functional movements). If during movement of the upper lip or cheek towards the floor, the dentist notices that the individual tray is being displaced from its position, this indicates that the tray edge is overextended. Thus, the height of the edge of the individual tray must be reduced so that the edge of the tray is 2 mm from the base of the vestibule. When movements of the upper lip and cheeks are performed and the tray is not displaced from its position, it means that the edge of the tray is not overextended.

After adjusting the front and side edges of the individual tray, it is necessary to adjust the back edge of the tray. The dentist must locate and mark, with a copying pencil, on the mucosa, the posterior limit of the future upper complete denture. The posterior edge of the upper individual tray must end at the limit marked with the copying pencil. It is possible to facilitate the location of the posterior limit of the future maxillary prosthesis, through some methods: I) *Anatomical location*: with a copying pencil, a line is marked on the patient's oral tissue connecting the hamular notch on both sides, passing over the fovea palatinae (the fovea palatinae are located in the region of the soft palate); II) *"Ah" line*: ask the patient to say "ah", and then use the copying pencil to mark the "ah" line. This line must connect the hamular notch on both sides*** (the "ah" line is located in the soft palate region); and III) *"Valsalva maneuver"*****. The difference in colour between the hard and soft palate can help the dentist locate the posterior limit of the future denture (hard palate – pale pink, and soft palate – vivid pink). It is important to emphasize that the posterior limit of the future upper complete denture must be located on the soft palate ("line of fovea palatinae" or "ah" line).

After marking the line on the buccal tissue of the posterior limit of the upper complete denture, the dentist must verify that the individual tray is respecting this limit. Regarding the marked line, if the tray is overextended or underextended, it must be adjusted.

According to Zarb et al. (2004)
***Vibrating line (or "ah" line)
"The vibrating line is an imaginary line drawn across the palate that marks the beginning of motion in the soft palate when an individual says "ah". It extends from one hamular notch to the other. At the midline, it usually passes about 2 mm in front of the fovea palatinae. These are indentations near the midline of the palate formed by a coalescence of several mucous gland ducts. They are always in soft tissue, which makes them an ideal guide for the location of the posterior border of the denture".

"The vibrating line is not to be confused with the junction of the hard and soft palate because the vibrating line is always on the soft palate. It is not a well-defined line and should be described as an area rather than a line. The distal end of the denture should extend at least to the vibrating line. In most instances it should end 1 to 2 mm posterior to the vibrating line".

Important note regarding the vibrating line (or "ah" line)
"The vibrating line of the soft palate, normally used as a guide to the ideal posterior border of the denture, usually is located slightly anterior to the foveae palatinae. However, it may be on or slightly posterior to the foveae palatinae. The slight devia deviation from these markings is estimated by having the patient say "ah" and thus vibrate the soft palate".

****Valsalva maneuver
"The locations of the right and left hamular (pterygomaxillary) notches are marked with an indelible pencil. On the median line of the anterior part of the soft palate are two indentations formed by the coalescence of ducts known as the foveae palatinae. The shape of these depressions varies from round or oval to oblong. The dentist can make them more readily discernible by having the patient hold his nose and attempt to blow through it (Valsalva maneuver). This will accentuate the foveae palatinae and vibrating line".

- Adjustment of the mandibular individual tray

For the mandibular ridge, the same process of adjusting the tray is performed, the difference is that the movements of the patient's lower lip and cheeks are performed in the opposite direction to the floor. During this process, the index and middle fingers of the dentist's hand must gently hold the tray in position over the mandibular ridge, while the dentist's other hand performs the functional movements of the patient's lip or cheek (one finger on each premolar region of the tray). Subsequently, the patient must be asked to move his tongue upward, sideways and forward (functional movements). During this process, the index fingers of the dentist's hands must hold the tray gently over the patient's ridge (one finger on each premolar region of the tray). This will simulate the functional movements of the mylohyoid muscle. If the dentist perceives that the tray is being displaced from its position during functional movements, the height of the tray edge, in the tested region, must be reduced. The edges of the tray must have a distance of 2 mm from the base of the vestibule, and 2 mm from the floor of the mouth.

It is noteworthy that, when the maxillary or mandibular tray is on the ridge, the visual assessment can be performed to complement the adjustment of the lateral and frontal regions of the tray. Visually, it is possible to check the approximate distance between the edge of the tray and the base of the vestibule, and whether the tray is correctly contouring the buccal, labial, and lingual frenula.

The posterior extension of the future mandibular prosthesis must extend approximately one half to 2/3 over the retromolar pad.

Note: The buccal flange of the future denture must cover the buccal shelf. Buccal shelf is the bone area between the extraction sites of the molars and the external oblique line, and it forms the primary support for the mandibular denture as it is made primarily of cortical bone. Thus, the individual lower tray must cover this area on both sides of the mandibular arch.

6) Mechanical retentions with a carborundum disk can be performed along the entire length of the edges of each individual tray (Figure 1A). This creates mechanical retentions for the laboratory condensation silicone putty.
7) Apply an adhesive (Universal Tray Adhesive, Zhermack) to the inside and outside of the edges of the individual trays (Figure 1B). The authors of the present article recommend that initially a small amount of adhesive be dispensed into a sterilized dappen pot and then, using a disposable microbrush, the adhesive be applied to the edges of the trays. This prevents cross contamination. Then, wait 5 minutes for the adhesive solvent to evaporate, before adding the silicone putty to the edges of the tray.
8) Laboratory condensation silicone putty (Zetalabor or Titanium, Zhermack) must be handled according to the manufacturer's recommendations and added to all edges of the tray. After that, the individual tray must be positioned over the patient's ridge.

For the border molding technique using laboratory condensation silicone putty, it is possible to mold in a single step or in more than one step. Despite this, according to Smith et al. (1979):

"A material which will allow simultaneous moldings of all borders has two general advantages: (1) the number of insertions of the trays for maxillary and mandibular border molding could be reduced to two, a great time and motion advantage; and (2) development of all borders simultaneously avoids propagation of errors caused by a mistake in one section affecting the border contours in another section".

9) The functional movements mentioned above must be performed according to the arch of interest (Figure 1C).

According to Chafee et al. (1999)
"The objective of border molding is to "customize" the impression tray to establish the maximal extension and accuracy of the peripheral seal with no functional impingement of the tissues".

According to The Academy of Prosthodontics (2017)
"Border seal: the contact of the denture border with the underlying or adjacent tissues to prevent the passage of air or other substances".

According to Zarb et al. (2004) and Arora et al. (2015)
"Border molding is the process by which the shape of the border of the tray is made to conform accurately to the contours of the buccal and labial vestibules. This essential requirement of the tray's fit ensures an optimal peripheral seal. It begins with manipulation of the border tissue against a moldable impression material that is properly supported and controlled by the tray. The amount of support supplied by the tray and the amount of force exerted through the tissues vary according to the resistance or viscosity of the impression material".

According to Arora et al. (2015)
The peripheral seal is important to prevent air from entering between the complete denture and the mucosa, providing retention.

10) After the material hardens, stability and retention tests are performed for each individual tray.

- For the stability test:
  The tray is placed over the ridge and the index fingers are positioned over the premolar areas of the tray (one finger over each premolar area). The dentist must alternately compress these regions to verify that the tray is correctly seated on the ridge. The tray must not alternately move during this process.
- Vertical and horizontal retention tests:
  Vertical test: For the maxilla or mandible, pull the tray handle vertically downwards (maxilla) or upwards (mandible) respectively.
  Horizontal test: For the maxilla or mandible, pull tray handle horizontally in buccal direction.

Note 1: Normally, only the maxillary tray, after border molding, shows retention with the edentulous ridge.

Note 2: The height of the anterior handles of the individual trays should be 10 mm to simulate the height of the central incisors. This allows the operator to have a more realistic tactile perception of the retention level of the future denture.

Note 3: Based on the maxilla, after correct border molding, it is generally common for the tray to have adequate vertical retention with the ridge. However, the horizontal retention of the individual tray may be less than the vertical one at this time. Despite this, it is possible to increase the horizontal retention of the tray with the ridge later, in the "post damming" phase.

11) After removing the individual tray from the patient's mouth, check the

impression along the entire length of the tray edges. Along the entire length of the edges of the tray, the silicone putty must be well adhered. The width of the silicone edges should be 2–2.5 mm. In addition, it is recommended to inspect the posterior limits of the impressions to verify that they are correct. If there is a problem, it must be resolved.

Note: If any part of the impression shows adhesive failure with the tray, this situation must be corrected. Therefore, the dentist must remove the problematic part of the impression and repeat the previous steps for the affected area to correct it.

II) Final impression (also called "corrective impression")

12) It is recommended to drill the anterior palatal region of the individual tray to create a hole (Ø 1 mm or 1.5 mm). Thus, during the making of the final impression, the impression material leaks through the hole, reducing the hydraulic pressure on the palate.
13) Zinc oxide eugenol paste must be handled according to the manufacturer's recommendations.
14) The maxillary or mandibular tray loaded with impression paste is positioned over the patient's ridge. The previously reported functional movements must be performed. These movements should only be stopped when the material has hardened.
15) Retention and stability tests of individual trays must be performed. Normally, retention of the lower mold with the mandibular ridge does not occur, due to bone resorption.

Important notes:

- After obtaining the impression, if a small negative bubble is identified on its surface, it is possible to fill it with wax. When the negative bubble is medium in size, it is interesting to insert the mold over the edentulous ridge immediately after inserting the wax inside the bubble. Thus, the still softened wax copies the region of interest (Figure 2). This procedure can be performed in conjunction with the "post damming" step. However, when the negative bubble is very large, it is necessary to load the entire surface of the mold with paste for the reline process.
- After making the final impression, if parts of the internal surface of the tray are not covered with the paste (pressure areas), the tray must be relieved in these regions with a spherical dental drill. In this circumstance it is also interesting to relieve flaccid areas. Then it is necessary to load the entire surface of the mold with paste for the reline process (Figure 3).
- Once the impressions are finished, it is interesting to check whether the posterior limits of the edges are correct. If there is a problem, it must be resolved.
- Before carrying out the next step, it is important to wash the molds with water and clean the patient's mouth.

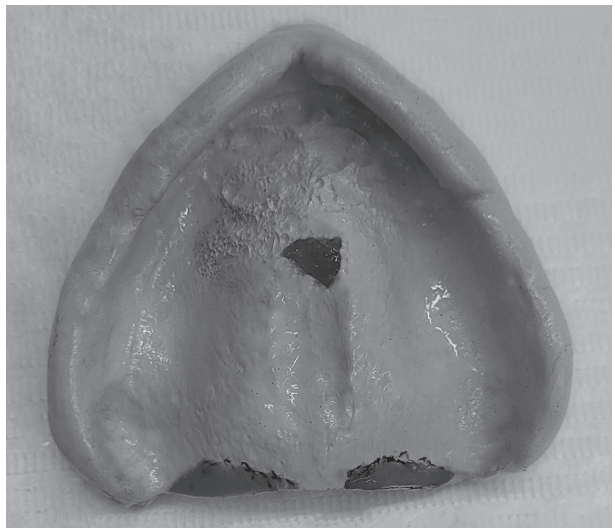

Figure 2 – Result obtained after inserting wax into a medium-sized bubble, and then inserting the mold over the patient's edentulous ridge. Note: The final impression correctly copied the patient's palatine torus region.

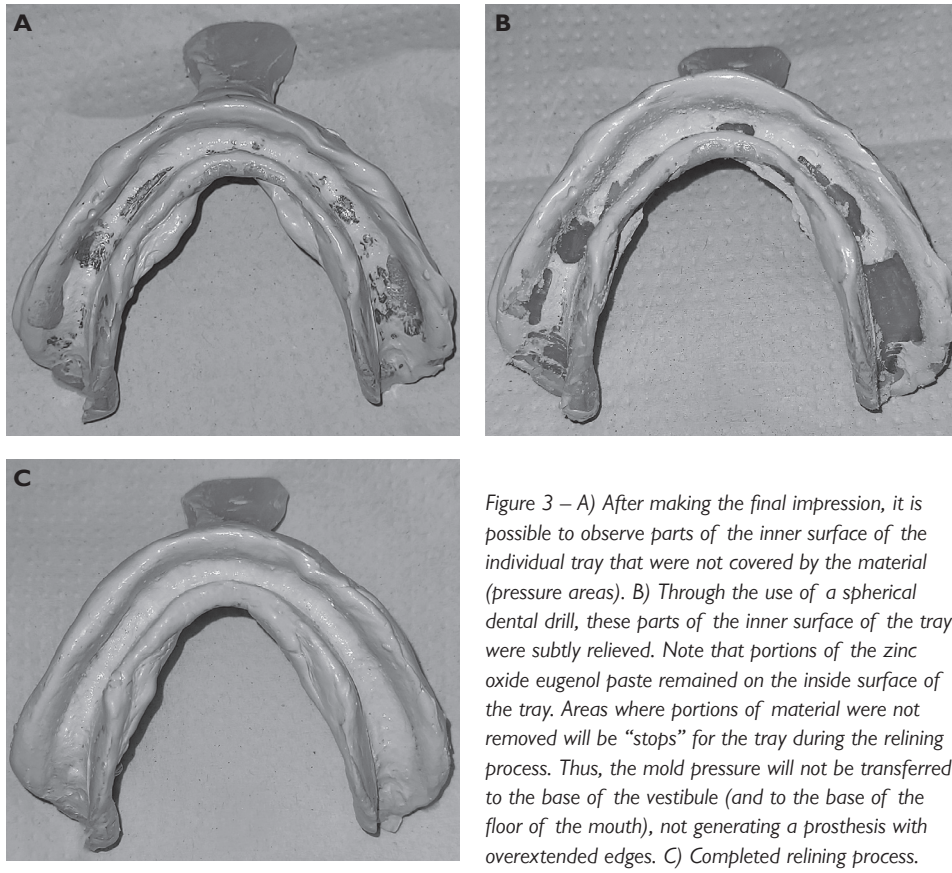

Figure 3 – A) After making the final impression, it is possible to observe parts of the inner surface of the individual tray that were not covered by the material (pressure areas). B) Through the use of a spherical dental drill, these parts of the inner surface of the tray were subtly relieved. Note that portions of the zinc oxide eugenol paste remained on the inside surface of the tray. Areas where portions of material were not removed will be "stops" for the tray during the relining process. Thus, the mold pressure will not be transferred to the base of the vestibule (and to the base of the floor of the mouth), not generating a prosthesis with overextended edges. C) Completed relining process.

III) Posterior palatal seal ("post damming")
According to Jeannin and Millet (2006)
"The soft palate can move in a superior position, which may result in air leakage in the posterior area of a complete denture. To prevent dislodgement of the denture, the soft palate must be impressed in its superior position when making the definitive impression. By creating this contact, air is prevented from passing under the denture during speech or respiration".

According to The Academy of Prosthodontics (2017)
"Posterior palatal seal area: the soft tissue area limited posteriorly by the distal demarcation of the movable and nonmovable tissues of the soft palate and anteriorly by the junction of the hard and soft palates on which pressure, within physiologic limits, can be placed; this seal can be applied by a removable complete denture to aid in its retention".

According to Hardy and Kapur (1958) and Rashedi and Petropoulos (2003)
"The development of the posterior palatal seal on the denture has the following advantages: (1) it provides retention, (2) it provides a close contact of the denture base with the mucous membrane which prevents food from getting under the denture; and (3) it supplies a thickened area that provides added strength across the denture".

16) Between the junction of the hard and soft palates and the "ah" line, there is a vibrating region located in the soft palate. During speech, this region vibrates due to the action of two muscles (tensor veli palatini and levator veli palatini). With the aid of a copying pencil, this region must be contoured on the patient's oral tissue. Thus, after inserting and removing the mold from the patient's mouth, the marking is transferred to it. A thin film of heated wax is applied over the demarcated region over the mold. After correctly covering the area of interest of the mold with wax, it is interesting to heat the wax again so that it becomes softer. Immediately after this procedure, the mold must be inserted and correctly fitted over the ridge. During the time that the mold is positioned on the ridge, the patient should be asked to say "ah".
17) After the wax has hardened, the previously reported retention and stability tests must be performed with the mold. Then, the tray is removed from the patient's mouth, and the dentist must check the impression. If the dentist verifies that the wax has flowed over the region of the posterior nasal spine or palatine raphe of the impression, it is necessary to remove it from that region and repeat the technique. The "post damming" has the function of increasing impression retention with the ridge, mainly in relation to horizontal retention. Thus, if after this procedure the retention of the final impression with the ridge is reduced, there was probably a failure in the technique, and it must be repeated.
18) The impression obtained must have clear details of the anatomical characteristics

of the ridge and adequate thickness of the impression materials (Figures 1D and 2). The width of the edges of the mold should be 2–2.5 mm. In addition, the mold must not have bubbles and fractures that could negatively influence the making of the functional plaster cast.

19) Wash the impressions and soak them in 2% glutaraldehyde, or 2–4% chlorhexidine for 10 min. Later, before pouring the plaster into the mold, it is important to wash the impression again to remove the disinfectant.
20) A wax strip must be attached ~2 mm below the outer edges of the mold, and a vertical wall of wax should be firmly attached to this wax strip, forming a wax box. Then, type III or IV plaster is poured into the mold.

Notes:
Some professionals prefer that the cast be made with type III plaster for the acrylization process. This occurs because type IV plaster is very hard and, therefore, after acrylization of the complete denture, it is difficult to separate the cast from the acrylic base. This difficulty in separating these materials can lead to human error, causing damage to the complete denture.

Based on the study by Habibzadeh et al. (2016), it is possible to pour the plaster into the mold, composed only of the zinc oxide and eugenol, within 7 hours. Within this time interval, this material maintains its dimensional stability. However, based on the reported technique, laboratory condensation silicone putty is also used. For this elastomer, it is necessary that the plaster be poured into the mold within 1 hour, to avoid alterations in its dimensional stability. Therefore, for the reported technique, the plaster must be poured into the mold, composed of condensation silicone and zinc oxide and eugenol, within 1 hour.

Based on the reported functional impression technique, the impression created with the previously reported materials simulates the hardness of the future complete denture. According to the authors of this article, this hardness of the mold gives the dentist, during tests of retention and stability of the mold, a more adequate tactile perception of how the retention and stability of the future prosthesis will be, which will be rigid. *The functional mold represents the acrylic base of the future complete denture*.

Discussion

The border molding technique, reported in this review, can also be used to make the functional impression to fabricate removable partial dentures, immediate complete dentures, obturator prostheses, overdentures, and Branemark protocol dentures (Gennari Filho et al., 2002).

As previously reported, the laboratory condensation silicone putty indicated for this technique is Zetalabor (Zhermack – 80 Shore A) or Titanium (Zhermack – 90 Shore A) (www.zhermack.com/en/product/zetalabor/ [accessed on May 1, 2022]). Zetalabor or Titanium silicone has a Shore A hardness greater than clinically

used condensation silicones (Zetaplus Soft [putty] – 60 Shore A, and Zetaplus [putty] – 70 Shore A; Zhermack) (www.zhermack.com/en/product/zetalabor/ [accessed on May 1, 2022]; www.zhermack.com/en/product/zetaplus/ [accessed on May 1, 2022]). Thus, the purpose of using a laboratory silicone putty, for the border molding, is to simulate the hardness of the acrylic base of a complete denture.

For border molding, the advantages of using a laboratory condensation silicone putty compared to an impression compound include: 1 – the silicone does not need preheating like impression compound (temperature of ~50 °C) (Tamaki, 1983; Özkan, 2017), avoiding burning the patient's mouth (Gennari Filho et al., 2002); 2 – *Gain clinical time*, because when using silicone putty, the dentist does not need to handle water heating equipment and wait for the water to heat up (Gennari Filho et al., 2002); 3 – it is easier to correct mold failures when silicone putty is used; 4 – it is easier to remove excess material after border molding (Gennari Filho et al., 2002); 5 – the plaster must be poured into the functional impression, composed of zinc oxide and eugenol and a laboratory condensation silicone putty, within 1 hour. On the other hand, when using compound in this case for border molding, the plaster must be poured into the impression within 10 min; and 6 – it is easier to avoid cross-contamination when a silicone putty is used.

Regarding cross-contamination, one of the problems with using compound for border molding, is the container used to receive the hot water (Gennari Filho et al., 2002). Typically, dental students and dentists pour hot water into PVC dental bowls. These PVC bowls normally cannot be sterilized because they become deformed. Thus, after using these PVC bowls, their disinfection may be inefficient, which favours cross-contamination (Gennari Filho et al., 2002). Furthermore, even if the dentist uses a device to heat the compound, the problem would be the same, due to the need to disinfect this device, which can also be inefficient (Gennari Filho et al., 2002). This situation is even more worrying today due to the COVID-19 pandemic (Melo Neto et al., 2020).

It is also important to note that a laboratory condensation silicone putty is less expensive than other materials used for border molding such as addition silicone and polyether, and it does not have an unpleasant odour like polysulfide (Petropoulos and Rashedi, 2003; Mehra et al., 2014).

Conclusion

The border molding technique reviewed in this study is comfortable and safe for the patient.

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Removable Partial Denture – Functional Impression Techniques: Review

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Abstract: The objective of this article is to concisely review the main clinical techniques used to make the functional impression to manufacture a removable partial denture. Through this review, the dentist can develop his clinical knowledge.

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Introduction

Removable partial denture is the treatment of choice for those patients who need to replace missing teeth for aesthetic and functional reasons, but cannot receive implants due to general health problems, financial limitations, or fear of surgery (Costa et al., 2009).

The functional impression to fabricate a removable partial denture is typically taken over the posterior free end of the dental arch (Kennedy classes I and II); and over the entire dental arch, when few teeth remain in the patient's arch (Todescan et al., 1996; Carreiro and Batista, 2014; Sakar, 2016). Functional impression can be made using one of these techniques: 1) "*Individual tray technique*" – In this technique, using an individual tray that covers the entire arch of the patient, the anatomical and functional impressions are taken simultaneously. In this technique, the copy of the teeth represents the anatomical impression, and the copy of the mucosa represents the functional impression. The master cast obtained by this technique is used to manufacture the metallic framework, and all the acrylic bases (saddles) of the future denture; 2) "*Altered cast technique*" – This technique is performed in a clinical session subsequent to the clinical session that aims to evaluate the metallic framework; and, 3 and 4) "*Open mouth technique*" and "*Closed mouth technique*" – One of these techniques can be used in the clinical session for aesthetic and functional evaluation of wax-attached acrylic teeth, immediately after this procedure. In these last three techniques, an individual tray attached to the metallic framework, which only covers the posterior free end of the dental arch, is used (e.g., Kennedy's class II). In Kennedy class I cases, the two posterior free end areas are covered by individual trays attached to the framework. These last three techniques only aim to obtain the definitive acrylic base of the prosthesis, which will be located on the posterior free end, or on the free ends, of the dental arch (Kennedy class II and I cases) (Applegate, 1937; de Fiori, 1993; Todescan et al., 1996; Carreiro and Batista, 2014; Sakar, 2016).

The objective of this article is to concisely review the main clinical techniques used to make the functional impression to manufacture a removable partial denture. Through this review, the dentist can develop his clinical knowledge.

Review

Depending on the functional impression technique chosen, it is performed at a certain time within the clinical sequence to fabricate a removable partial denture. All information reported below is based on the literature (Applegate, 1937; Hidels, 1952; Christensen, 1962; Holmes, 1965; Leupold and Kratochvil, 1965; Blatterfein et al., 1980; de Fiori, 1993; Todescan et al., 1996; Cucci and Vergani, 1997; Kliemann and Oliveira, 1998; Pegoraro, 1998; Gennari Filho et al., 2002; Zarb et al., 2004; Costa et al., 2009; Goiato et al., 2013; Carreiro and Batista, 2014; Yilmaz and Özçelik, 2014; Carr and Brown, 2015; Sakar, 2016; The Academy of Prosthodontics, 2017; www.zhermack.com/en/product/zetalabor/ [accessed on August 12, 2023]; www.zhermack.com/en/product/zetaplus/ [accessed on August 12, 2023]).

Clinical sequence to fabricate a removable partial denture

1) Clinical and radiographic examination.
2) Mouth preparations (periodontal treatment, restorations using composite resin, etc.).
3) Molding to obtain the study casts.
4) Assembly of the study casts on the semi-adjustable articulator to study the clinical case.
5) Analysis of the study casts using a dental surveyor.
6) Planning.
7) Abutment teeth preparation (rest seats; guide-planes; preparation of adequate retentive areas, using composite resin, for the retentive arm of the clasp; etc.).
8) Molding to obtain the master cast using a metal tray of stock and alginate, or condensation or addition silicone (putty and fluid) (anatomic form impression); or *individual tray technique*, if necessary.
9) Metal framework try-in.
10) *Functional impression (altered cast technique)*, if necessary, when the *individual tray technique* has not been previously performed.
11) Wax rims phase – (i) Use of the facebow to mount the upper master plaster cast (or altered master plaster cast) on the articulator; (ii) maxillomandibular registration; and (iii) finalization of the assembly of the master plaster casts (or altered master plaster casts) on the articulator. At this stage, the selection of artificial teeth is also carried out (size, width, shape, shade, and material).
12) Aesthetic and functional try-in of the wax-attached acrylic teeth, and then (in the same clinical session), *the open mouth* or *closed mouth technique* is performed, if necessary (*when the altered cast technique* or *individual tray technique* has not been previously performed). In this step, the shade selection of the artificial gingiva is also carried out.
13) Delivery of the removable partial denture to the patient, after the laboratory stage of acrylization; occlusal adjustment; and oral hygiene instructions.
14) Clinical controls.

Note 1: When the functional impression is taken, it can be made in only one of these steps: 8, 10 or 12.

Note 2: In Kennedy class III and IV cases, when there is no need to make the functional impression, the previously reported clinical sequence is performed without this type of impression. Thus, the master plaster cast will be generated by the anatomical impression, where the metallic framework and the definitive acrylic bases (saddles) of the future prosthesis will be made.

Anatomical impression can be made with alginate using a metal stock tray (step 8). It is recommended that the stock tray always be made of metal, as the plastic tray can generate deformations to the mold after it is removed from the patient's mouth.

Furthermore, it is recommended that the metallic tray be previously customized with wax to increase the extension of its edges. This will provide a complete copy of all areas of the dental arch that will be important to fabricate the removable partial denture.

The anatomical impression can also be made using the condensation or addition silicone (putty and fluid). It is contraindicated to pre-customize the edges of the metal stock tray when using one of these silicones. Two techniques can be used with condensation silicone or addition silicone (putty and fluid): I) For the first technique (one-step technique), silicone putty is added into the metal stock tray, and then silicone fluid is added over the first material. Thus, these two material consistencies are used at the same time to make the anatomical impression; and II) The second technique (relining technique, also known as the two-step technique) consists of a relining procedure. Thus, initially, a mold is obtained with the silicone putty, which is subsequently relieved, and then it is relined with the fluid silicone.

In situations of tooth mobility, and when a silicone will be used to make the anatomical impression, it is more interesting to use the relining technique. This will help prevent tooth movement due to the fluid consistency of the silicone (two-step technique). It is worth remembering that for the one-step technique, the silicone putty can generate tooth movement more easily.

Note 3: The individual tray technique is the only technique in which anatomical and functional impressions are taken simultaneously.

For the other techniques reported in this article, anatomical and functional impressions are taken at different times. In this case, the anatomical impression will be used to generate the master plaster cast, where the framework will be made; and the *altered cast technique*, *open mouth technique* or *closed mouth technique* will be used to alter the master plaster cast, creating the altered master plaster cast. The altered part of the altered master plaster cast will be used to create the acrylic base of the future removable partial denture.

Note 4: In step 11, in a situation of rehabilitation of only the partially edentulous mandibular arch, it would not be necessary to use the facebow again to mount the upper plaster cast on the articulator; because this would have been done before (step 4).

Note 5: The anatomical impression and the individual tray technique generate the master plaster cast (step 8).

Altered cast technique generates the altered master plaster cast (step 10).

The *open mouth technique* or *closed mouth technique* (step 12) can also generate the altered master plaster cast to complete the fabrication process of the removable partial denture (using a muffle). However, this is not a mandatory situation, as the acrylization stage using a muffle can only be carried out with the mold attached to the framework (without the master plaster cast).

Note 6: When the edentulous space of the posterior free end is short (e.g., Kennedy class II), only the anatomical impression is enough “to fabricate all parts

of the removable partial denture". Thus, it is not necessary to make the functional impression.

Dentist position during impression techniques

All techniques described below should be performed with the patient comfortably seated in the dental chair. The dentist's position in relation to the patient depends on the patient's edentulous arch that will be molded:

- Maxilla – The dentist should be standing and positioned behind the patient. The dentist's elbow should be at the same height as the patient's labial commissure.
- Mandible – The dentist should be standing and positioned facing the patient. The height of the patient's labial commissure should be in a position above the dentist's elbow so that the dentist can keep his spine upright.

Respecting these positions, the dentist can perform these procedures with his spine erect.

Border molding

The Glossary of Prosthodontic Terms (The Academy of Prosthodontics, 2017) defines the "border molding" as: "1. the shaping of impression material along the border areas of an impression tray by functional or manual manipulation of the soft tissue adjacent to the borders to duplicate the contour and size of the vestibule; 2. determining the extension of a prosthesis by using tissue function or manual manipulation of the tissues to shape the border areas of an impression material".

The border molding technique can be performed using materials such as a *low-fusing* compound or a silicone putty. With regard to silicone putty, the authors of the present study indicate the use of a laboratory condensation silicone putty (Zetalabor or Titanium; Zhermack). These laboratory silicones putties (Zetalabor/Titanium; Zhermack) have a higher Shore A hardness than clinical condensation silicone putties (Zetaplus = 70 Shore A/Zetaplus Soft = 60 Shore A; Zhermack). The use of a high-hardness laboratory condensation silicone putty aims to simulate the hardness of the edges of the future prosthesis.

The border molding technique with a laboratory silicone putty can be carried out by following the steps: 1) After adjusting the edges of the individual tray, mechanical retentions are created on the edges of this tray; and 2) The adhesive is applied to the edges of the individual tray so that the silicone putty is glued to them.

Individual tray technique

This technique is generally indicated for cases where few teeth remain in the patient's dental arch. Despite this, it can be used for all Kennedy class I, II, III and IV situations.

The authors of this study suggest that the border molding be performed with a laboratory condensation silicone putty.

Technique steps

This technique is similar to the functional impression technique for making a complete denture:

1) The study plaster cast obtained from the impression of the patient's dental arch, using a stock tray and alginate, can be used to manufacture the individual tray. The wax relief is performed on the plaster cast, and then the individual acrylic resin tray is made on the entire surface of the plaster dental arch.

For regions where teeth are located, the individual tray created on the plaster cast is fully relieved by 2 mm, while for the edentulous regions, this individual tray can be partially relieved. In this case, partial relief with wax on the plaster cast is performed only on retentive and flaccid edentulous areas, according to the patient's oral condition. Thus, for flaccid areas of the patient's ridge, the dentist must inform the dental laboratory where these areas are located on the plaster cast, so that the prosthetic technician can perform the necessary reliefs before manufacturing the individual tray.

For partially relieved individual upper tray (edentulous areas of the maxilla), there is the possibility of creating "stops" on its inner part for the teeth. However, for this dental arch, it is possible to take the impression without "stops" for the teeth, as the hard palate acts as a "stop". On the other hand, for a partially relieved lower individual tray (edentulous areas of the mandible), it is recommended to create "stops" on its inner part for the teeth.

When an individual tray is fully relieved (dental and edentulous regions of the maxilla or mandible), "stops" must be created for both the teeth and the edentulous areas to take the impression. In this case, the "stops" have the function of preventing overextension of the edges of the tray. The "stops" can be made with acrylic resin or silicone putty. When a silicone putty will be used, the adhesive must be previously applied to the individual tray regions of interest.

When "stops" are created, they must be made before the next step.

2) It is important to adjust the height of the edges of the individual trays. The edges of a tray should be 2 mm from the base of the vestibule (and from the base of the floor of the mouth, based on the mandible), except for the posterior edge. For the maxilla, the posterior edge of the tray must connect the hamular notch on both sides, passing over the palatine fovea (or the "ah" line), or over the junction between the hard and soft palate. It is important to note that the posterior limit of the future prosthesis will depend on the type of major connector. For the palatal plate, the posterior limit of the major connector must be the "ah" line, or the posterior end line of the hard palate (line between hard and soft palate). The remaining types of major connectors must be located on the hard palate only. For the mandible, the tray must extend approximately one-half to 2/3 over the retromolar pad, based on the posterior free end area of the ridge.

Visually, for the anterior region of the ridge (maxilla or mandible), it is possible to verify approximately the distance from the edge of the tray to the base of the vestibule. However, the visual aspect may not be as reliable for adjusting the height of the individual tray edges. Furthermore, visual assessment is limited to the anterior areas of the maxilla and mandible. Thus, it is important to adjust the lateral and frontal edges of the trays, performing functional movements that will be reported below:

- Maxilla – One of the dentist's hands must hold the tray in position over the dental arch, while the dentist's other hand moves the patient's cheek or upper lip toward the floor. If the dentist notices that during these movements the tray is dislocating from its position, then the edges of the tray are overextended. In this situation, the height of the edges of the acrylic tray must be reduced and, subsequently, the tray must be tested again. This process must be repeated until the tray does not move from its position during movements of these oral structures.
- Mandible – The lower tray adjustment process is similar to the method reported for the maxilla. The difference is that the movements of the patient's lower lip and cheeks are performed upwards. In addition, it is recommended that the patient move his tongue sideways and upwards. If the dentist notices that during these movements the tray is dislocating from its position, then the edges of the tray are overextended. In this situation, the height of the edges of the acrylic tray must be reduced and, subsequently, the tray must be tested again. This process must be repeated until the tray does not move from its position during movements of these oral structures.

3) Border molding is performed using a *low-fusing compound* or a laboratory silicone putty. When a silicone putty will be used, mechanical retentions are previously created on the edges of the tray, and an adhesive is applied over them. During the border molding, the same functional movements reported previously must be performed. These movements have the function of copying the base region of the vestibule and determining the correct extension of the edges of the acrylic base of the future prosthesis.

4) The adhesive must be applied to the entire internal area and edges of the individual tray.

5) Use a fluid elastomer to make the final impression (i.e., polyether, condensation silicone, polysulfide, or addition silicone). During the final molding, the same functional movements reported above must be performed.

6) The plaster cast obtained using this technique is called the master cast.

Note: The final impression may also be called "corrective impression".

Altered cast technique (or applegate technique)

"Altered cast removable partial denture impression" is defined by the Glossary of Prosthetic Terms (The Academy of Prosthodontics, 2017) as: "a negative likeness of a portion or portions of the edentulous denture bearing area made independent

of and after the initial impression of the natural teeth; this technique uses an impression tray(s) attached to the framework of the removable partial denture, or its likeness". Furthermore, the term "altered cast" is defined by the Glossary of Prosthetic Terms (The Academy of Prosthodontics, 2017) as: "a final cast that is revised in part before processing a denture base".

This technique is indicated for molding the free end (Kennedy class II), or free ends, of the arch (Kennedy class I). The disadvantage of this technique is that it requires one more clinical session compared to the other three techniques initially reported.

The authors of this study suggest that the border molding be performed with laboratory condensation silicone putty.

Technique steps

1) In another clinical session, after trying and adjusting the metallic framework, this technique is performed. It is important to highlight that before the clinical session to make the functional, the prosthesis laboratory makes an acrylic resin tray attached to the framework (only for the free end area). Therefore, for Kennedy class I, the functional impression is taken over two edentulous areas (two free ends), and for Kennedy class II, over one edentulous area (one free end). The posterior edentulous area at the free end of the dental arch is a region that will provide mucosal support for the denture. The tray made by the dental laboratory is relieved (1 mm) to provide space for the impression material.

Costa et al. (2009) reported important information about removable partial dentures with dental and mucosal support: "On one hand, the abutment tooth presents a limited movement of around 0.1 mm; on the other hand, the mucosa, which varies in compressibility from 0.4 to 4 mm, has an average resilience of 1.3 mm. This means that the mucosa confers a freedom of movement to the saddle approximately 13-fold higher than that allowed by the dental organ in its alveolus".

The functional impression is important to prevent the saddle of the future prosthesis from excessively compressing the mucosa of the free end, during mandibular rest or mastication. Excessive compression of the mucosa can cause failure of the blood supply, mucosal damage, discomfort, and bone resorption. In addition, it is important to report that the lack of adequate contact between the saddle and the posterior free end mucosa generates an overload on the abutment tooth, leading to periodontal problems (e.g., bone resorption). Thus, the functional impression is also important for a more favourable distribution of masticatory stress, and reduction of the leverage effect on the abutment tooth, generating greater comfort and oral health for the patient.

2) The edges of the tray should be 2 mm from the base of the vestibule (and from the base of the floor of the mouth based on the mandible), except for the

posterior edge. For the maxilla, the tray must cover the tuber maxillae (the limit of the posterior edge of the tray is the hamular notch); and for the mandible, the tray must extend approximately one-half to 2/3 over the retromolar pad.

3) Border molding is performed using a *low-fusing compound* or a laboratory silicone putty. Remembering that to use silicone, it is important to create mechanical retentions on the edges of the tray, and then apply the adhesive over them. Material is added to the edges of the tray, and the framework is fitted over the patient's teeth. It is worth mentioning that it is important to make sure that the framework is well fitted over the patient's teeth. During this process, the dentist must hold the framework with one of his hands and perform the previously reported functional movements with his other hand. In addition, for the lower arch, the patient is asked to move his tongue sideways, upwards and forwards while the dentist keeps the framework stabilized in position.

4) Final impression can be made with zinc oxide eugenol paste or a fluid elastomer (i.e., polyether, condensation silicone, polysulfide, or addition silicone). Remembering that to use an elastomer it is necessary to previously apply the adhesive on the inner surface of the tray. During this process, the dentist must hold the framework with one of his hands, and the previously reported functional movements must be performed. During border molding and final molding, the patient remains with his mouth open. In addition, the dentist must not apply pressure with his fingers on the acrylic tray.

5) The free end of the master plaster cast is cut and removed (e.g., Kennedy class II), the framework is fitted over this cast, and the plaster is poured over the mold. Subsequently, the altered master plaster cast is obtained.

Note: Functional impression of an edentulous area not located at the posterior free end of the arch is not advantageous using this technique. This is because in the laboratory, the technician would have to cut the plaster master cast into several pieces to remove the edentulous areas. This could later make it difficult to create the altered master cast. Furthermore, in the acrylization step using a muffle, parts of the altered master plaster cast could separate, resulting in failure of the prosthesis fabrication.

Closed mouth technique/open mouth technique

These techniques are indicated for molding the free end (Kennedy class II) or free ends of the arch (Kennedy class I). Immediately after the aesthetic and functional try-in of the wax-attached acrylic teeth, one of these techniques can be used. For these techniques, the provisional acrylic base (saddle) serves as an individual tray.

Closed mouth technique

Technique steps

1) For this technique, an occlusal adjustment of the acrylic teeth is required. For this, carbon paper is used to detect premature contacts of acrylic teeth. Subsequently, premature contacts must be worn down.

2) It is important to wear down the inside surface of the acrylic base to provide space (1 mm) for the impression material. An evidencing paste can be used to help with this process. Normally, it is common that the dental laboratory does not perform a previous relief of the internal part of the acrylic base or does not perform a sufficient relief for this clinical phase.

3) There are two possibilities for adjusting the edges of the acrylic base:

I) When border molding is not performed – After fitting the framework over the patient's teeth, the dentist must gently move the patient's cheeks to check the extent of the edges of the acrylic base. The edges of the acrylic base must not impede movements of the patient's cheeks; or the movements of the patient's cheeks must not displace the acrylic base from its position. This is also important when the patient moves his tongue. A distance from the edge of the acrylic base to the base of the vestibule (and to the floor of the mouth, based on the mandible) of 1 mm may be a satisfactory distance. Obviously, the edges of the acrylic base cannot impede functional movements, therefore, their wear in height can exceed 1 mm in certain areas when this happens (i.e., 1.5 or 2 mm). The posterior limit of the acrylic bases of the maxilla and mandible is the same previously reported for the altered cast technique.

II) When border molding is performed – It can only be done with a silicone putty, and this technique is performed with the patient's mouth open. A distance from the edge of the acrylic base to the base of the vestibule (and to the floor of the mouth, based on the mandible) of 2 mm is recommended. The posterior limit of the acrylic bases of the maxilla and mandible is the same previously reported for the altered cast technique. The technical procedures for border molding are similar to those previously reported for the altered cast technique.

4) Apply Vaseline to buccal and lingual/palatal surfaces of acrylic teeth. Vaseline is only necessary if the zinc oxide eugenol paste is to be used later. If a fluid elastomer is used, it is not necessary to apply Vaseline.

5) Final impression can be made with zinc oxide eugenol paste or a fluid elastomer. Remembering that to use an elastomer it is necessary to previously apply the adhesive on the inner surface of the acrylic base. Load the acrylic base with an impression material. Then, the metal framework must fit correctly over the patient's teeth. The patient with his mouth still open must move his tongue sideways, upwards, and forwards, while the dentist holds the metallic framework in position. Subsequently, the patient must gently occlude his teeth (note: the dentist must verify that the occlusion of the patient's teeth is correct), and the same functional movements previously reported must be performed by the dentist.

6) The free end of the master plaster cast is cut and removed, the framework is fitted over this cast, and the plaster is poured over the mold. Subsequently, the altered master plaster cast is obtained. However, there is another way to end the process without creating the altered master plaster cast. Thus, after this technique, the plaster can be poured directly onto the free end mold, without the framework

being fitted over the teeth of the master cast. Subsequently, this set is included in a muffle (without the master cast) to complete the fabrication of the removable partial denture. It is important to point out that before completing the manufacture of the prosthesis using the muffle, it is necessary to correct the waxing of the gingival region, as it is common for it to be damaged during the technique.

Open mouth technique

Technique steps

For this technique, a previous occlusal adjustment with carbon paper is not necessary. This technique will follow the same principles as the previous technique (closed mouth technique); however, the patient's mouth will always remain open during this technique. The border molding is also optional.

Immediately after performing the open mouth technique, before removing the impression from the patient's mouth, it is important that the patient correctly occlude his teeth to prove the success of the procedure.

Note: Border molding is optional for open mouth and closed mouth techniques. For these techniques, it is not recommended to use a low-fusing compound due to the need to heat it, which can melt the wax that holds the artificial teeth.

Based on all the techniques reported in this review, when border molding is performed, the width of the mold edges should be 2 to 2.5 mm. Furthermore, after making the final impression, the width of the edges should also be within the range of 2 to 2.5 mm.

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Polypharmacy and Drug Interactions in the COVID-19 Pandemic

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Abstract: The COVID-19 pandemic generated a great impact on health systems. We compared evolution, polypharmacy, and potential drug-drug interactions (P-DDIs) in COVID-19 and non-COVID-19 hospitalizations during first wave of pandemic. Prescriptions for hospitalized patients ≥ 18 years (COVID-19 and non-COVID-19 rooms) between April and September 2020 were included. The computerized medical decision support system SIMDA and the physician order entry system Hdc.DrApp.la were used. Patients in COVID-19 rooms were divided into detectable and non-detectable, according to real-time reverse transcription polymerase chain reaction (RT-PCR). Number of drugs, prescribed on day 1, after day 1, and total; polypharmacy, excessive polypharmacy, and P-DDIs were compared. 1,623 admissions were evaluated: 881 COVID-19, 538 detectable and 343 non-detectable, and 742 non-COVID-19. Mortality was 15% in COVID-19 and 13% in non-COVID-19 (RR [non-COVID-19 vs. COVID-19]:

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0.84 [95% CI] [0.66–1.07]). In COVID-19, mortality was 19% in detectable and 9% in non-detectable (RR: 2.07 [1.42–3.00]). Average number of drugs was 4.54/patient (SD ± 3.06) in COVID-19 and 5.92/patient (±3.24) in non-COVID-19 ($p<0.001$) on day 1 and 5.57/patient (±3.93) in COVID-19 and 9.17/patient (±5.27) in non-COVID-19 ($p<0.001$) throughout the hospitalization. 45% received polypharmacy in COVID-19 and 62% in non-COVID-19 (RR: 1.38 [1.25–1.51]) and excessive polypharmacy 7% in COVID-19 and 14% in non-COVID-19 (RR: 2.09 [1.54–2.83]). The frequency of total P-DDIs was 0.31/patient (±0.67) in COVID-19 and 0.40/patient (±0.94) in non-COVID-19 ($p=0.022$). Hospitalizations in the COVID-19 setting are associated with less use of drugs, less polypharmacy and less P-DDIs. Detectable patients had higher mortality.

Introduction

On March 11, 2020, the World Health Organization (WHO) declared a new world pandemic (WHO, 2020), reaching our centre (Hospital de Clínicas "José de San Martín" – HCJSM) on March 15, 2020 (CDC COVID-19 Response Team, 2020; Ludueña et al., 2020). The COVID-19 pandemic had a significant impact on all health systems. In our hospital, special rooms were assigned to care for these patients, which functioned in parallel with hospitalization rooms for non-COVID-19 patients. Health personnel found themselves faced with caring for those affected by a pandemic while continuing to care for patients with the usual pathologies that are treated in the Department of Medicine. The drugs proposed for treatment in the first stage of the pandemic generated great doubts not only regarding their efficacy but also their potential adverse effects (Gandhi et al., 2020; Roden et al., 2020).

Polypharmacy and potential drug-drug interactions (P-DDIs) were under study in our hospital at that time, with a computerized medical decision support system (CMDSS) SIMDA in association with the physician order entry system (POES) Hdc.DrApp.la (Barcia et al., 2023). The emergence of the Pandemic gave us a unique opportunity to evaluate the impact of a new disease, new drugs and new P-DDIs.

The drugs that have been used in COVID-19 patients, include antiviral drugs (remdesivir, molnupiravir, favipiravir, ivermectin), steroids (dexamethasone), monoclonal antibodies (anti-spike protein including casirivimab and imdevimab or sotrovimab), immunomodulatory drugs (anti-interleukin-6 tocilizumab or the janus kinase inhibitor baricitinib), anticoagulants (heparin, enoxaparin) and antibiotics (including macrolides like azithromycin with potential antiviral and immunomodulatory effects, doxycycline, ceftriaxone). All these drugs have been the subject of academic discussion about their efficacy, safety, and in particular the risk of interactions and risk/benefit balance. Most of them have been associated with important adverse reactions such as arrhythmias, prolongation of the QT interval, or neurological toxicity, among others (Gandhi et al., 2020; Roden et al., 2020).

In this study, we compared the evolution, polypharmacy, and P-DDIs between hospitalizations in COVID-19 wards and hospitalizations in non-COVID-19 wards

in the Department of Internal Medicine of the HCJSM during the first wave of the COVID-19 pandemic in Buenos Aires, Argentina, between April 4, 2020 and September 3, 2020. This period was included in the implementation phase of our work: the CDMSS SIMDA was used in both groups (Barcia et al., 2023). We also analyzed the impact of the administration of convalescent plasma in patients with COVID-19.

Material and Methods

Setting

The Hospital de Clínicas "José de San Martín" is a teaching hospital dependent on the Facultad de Medicina of the Universidad de Buenos Aires, located in the Ciudad Autónoma de Buenos Aires. The HCJSM has more than 3,200 employees and receives more than 400,000 external consultations per year. The HCJSM does not have a unified electronic medical record system between the different departments and services, but there are customized developments in each area with occasional points of contact. The Department of Internal Medicine was in charge of its 8 usual rooms and 3 rooms were added due to the COVID-19 pandemic: 11 rooms in total. The rooms were divided into COVID-19 rooms and non-COVID-19 rooms in accordance with the requirements of each moment. The medical prescriptions were made by Internal Medicine resident physicians, with personalized access to the Hdc.DrApp.la POES after signing a consent with authorization for the use of information for the study. All the resident physicians voluntarily adhered to the use of the systems. Internal Medicine physicians supervised the prescriptions in the Hdc.DrApp.la POES.

Selection criteria

All prescriptions from all patients 18 years of age or older admitted to the wards of the Department of Internal Medicine of the HCJSM during the period detailed above were evaluated with respect to the following inclusion and exclusion criteria:

Inclusion criteria: prescriptions for patients 18 years of age or older who were hospitalized at the start of the study, or who required hospitalization during the phase of the study.

Exclusion criteria: prescriptions for patients who were hospitalized more than 14 days before the start date of each phase.

Elimination criteria: prescriptions for patients who were hospitalized for readmission following identical and preestablished therapeutic schemes (example: chemotherapy), and without complications during hospitalization that would justify other treatments. In the case of readmission, only the first hospitalization was recorded. Patients who remained hospitalized beyond 60 days after the closing date and patients with discordant data between the different systems that could not be resolved were also eliminated.

Data collection

The study was conducted between April 4, 2020, and September 3, 2020. This period constitutes intervention phase of a phased study that compares before/after the implementation of the CMDSS SIMDA (Barcia et al., 2023). In this phase, the CMDSS SIMDA was available, which detects P-DDIs automatically and adjusts drug dosage according to renal function. To determine the P-DDIs, DrugBank was used (Wishart et al., 2018). Glomerular filtration rate was calculated using the CPK-Epi formula (Levey et al., 2009) and the adjusted doses were calculated based on standardized creatinine clearance formulas (Karsch-Völk et al., 2013).

Resident physicians were in charge of filling prescriptions: informed consent was obtained from all participants. The prescriptions were classified into those of the first day and prescriptions after the first day. P-DDIs were analyzed for the first-day prescriptions. The hospital record data was obtained from the "Camas" computer system, exclusive to the HCJSM. The data from POES Hdc.DrApp.la were compared with those from the Camas system. Drugs administered orally, parenterally, inhaled, transdermally, or intrathecally were included. Fluid and electrolyte infusions, topical application drugs, and oral, enteral, or parenteral nutrition schemes were excluded. Supplementary oxygen administration (yes/no) during hospitalization was included. The pharmacological treatments on day 1 were divided into 4 groups: usual medication, which is that which the patient received prior to admission and continues during hospitalization; current medication, which is the one that was added due to the problem that led to hospitalization; thromboprophylaxis and insulin therapy. The following combinations were registered as 1 single drug: trimethoprim-sulfamethoxazole (cotrimoxazole), ampicillin-sulbactam, amoxicillin-clavulanic acid, levodopa-carbidopa, piperacillin-tazobactam, and valsartan-sacubritil. The different insulin formulations were registered as a single drug. Fixed combinations of: antihypertensives (except valsartan-sacubritil), bronchodilators, drugs for benign prostatic disease, and drugs for digestive disorders were recorded as 2 or more drugs. Polypharmacy was defined as the prescription of 5 or more drugs; excessive polypharmacy such as the prescription of 10 or more drugs (Jyrkkä et al., 2009; Leelakanok et al., 2017; Masnoon et al., 2017).

In the COVID-19 wards, patients suspected of having this disease or diagnosed with this disease were admitted to isolation rooms with 1 or 2 beds, according to the medical and epidemiological condition of each patient. The diagnosis of COVID-19 infection was made by nasopharyngeal swab (NPS) with the determination of the viral genome through a real-time reverse-transcription polymerase chain reaction (RT-PCR), according to the protocol of Centers for Disease Control and Prevention (CDC, Atlanta, USA) (CDC, 2019). The results of the RT-PCR studies were compared with the data registered in the Sistema Integrado de Información Sanitaria Argentina (SISA). According to the result, each patient in the COVID-19 group was assigned to the detectable or non-detectable subgroup. The situation of

hospitalization and isolation were redefined according to this result, in accordance with the clinical and epidemiological conditions of each case.

The convalescent plasma transfusion was carried out in the context of a HCJSM protocol, designed to systematize the transfusion from June 1, 2020. Patients included were ≥ 18 years old, diagnosed with COVID-19 by RT-PCR (detectable) in NPS, who had to sign an informed consent, and with at least 1 of the following severity criteria: oxygen saturation < 93% with fraction of inspired oxygen (FiO_2) of 21%, arterial pressure of oxygen PaO_2/FiO_2 ratio < 300, progression of radiological infiltrates greater than 50% in the last 24–48 hours, septic shock or multiple organ dysfunction. Terminally ill patients (life expectancy < 6 months) were excluded. Treatment with convalescent plasma had to be established in the first 14 days from the onset of symptoms, in 2 transfusions of 200 to 300 ml, separated by 48 hours, according to the criteria of the treating medical team.

With the information generated by the HDC.DrApp POES and the confirmatory data of each hospitalization of the Camas system, an Excel spreadsheet was generated. For this form, the information of each patient and the consistency between the systems were verified. Once the Excel spreadsheet was completed, another blinded statistical analysis was performed, without the identity of the patients.

Variables

Age, length of hospitalization, mortality, referral to critical area, and mortality in patients who were transferred to critical areas were compared between groups. The number of drugs in all the modalities analyzed, the percentage of patients with polypharmacy and with excessive polypharmacy, and the number of P-DDIs in studied groups were also compared. The same variables were compared between patients 65 years or older in relation to patients younger than 65 years. In the COVID-19 group, the same comparisons were done between COVID-19 detectable and COVID-19 non-detectable. The evolution among those who received convalescent plasma transfusion was also analyzed.

Ethics

The study and its subsequent adjustments were approved by the Department of Internal Medicine, the Ethics Committee, the Department of Teaching and Research, and the Management of the HCJSM. The identity and confidentiality of the data of each patient were preserved. No animals were used in the study. All procedures with people were carried out in accordance with the ethical standards of the regulations for studies, both national and international, and with the Declaration of Helsinki revised in 2013.

This trial was registered with ClinicalTrial.gov (NCT03901820) and Registro Nacional de Investigaciones en Salud, RENIS (IS003175).

Statistical analysis

Normal variables were expressed as mean (±SD). Univariate differences between qualitative data were evaluated with the chi-square test, Yates' correction, or Fisher's exact test. The differences between the quantitative data were explored with ANOVA and post hoc tests. Statistica 6.0 and MedCalc 2009 programs were used.

Results

Patient demographics

1,675 hospitalizations were registered in the evaluated period. After applying the inclusion, exclusion, and elimination criteria, 1,623 hospitalizations of 1,491 patients entered the study, with 132 readmissions (Figure 1). Among the 1,623 hospitalizations, 38 were reassigned during hospitalization between to COVID-19 and non-COVID-19 wards. Patients who were in the COVID-19 and non-COVID-19 wards during the same hospitalization were included in the COVID-19 group.

The patients in the COVID-19 group were significantly younger ($p<0.001$), hospitalized for less time ($p<0.001$), referred to critical areas more frequently, and had higher mortality among those referred to critical areas than the non-COVID-19 group (Table 1). The higher mortality of the COVID-19 group did not reach statistical significance. Those over 65 years of age had significantly higher mortality in both groups. In the COVID-19 group, but not in the non-COVID-19 group, those ≥ 65 years also had significantly longer hospital stays, more referrals to critical areas, and higher mortality among those referred to critical areas.

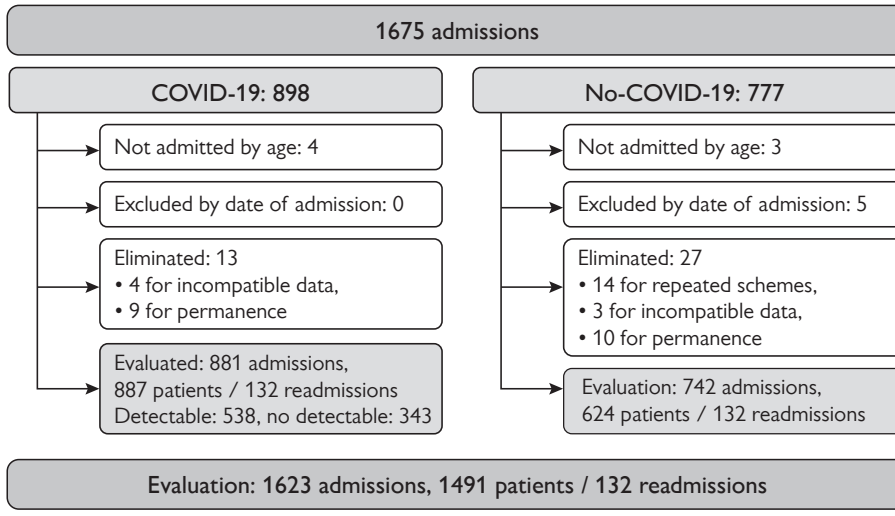

Figure 1 – Population evaluated.

Table 1 – Characteristics of the evaluated population and its evolution, according to mortality and referral to critical area, with comparison COVID-19 vs. non-COVID-19 and by age between <65 vs. ≥65 years

Variable	COVID-19				Non-COVID-19				Non-COVID-19 vs. COVID-19
	total	<65 years	≥65 years	statistics	total	<65 years	≥65 years	statistics	statistics
N=1,623 (%)	881 (54.28)	519 (58.91)	362 (41.09)		742 (45.72)	386 (52.02)	356 (47.98)		
Female N (%)	448 (50.85)	259 (49.90)	189 (52.21)		357 (48.11)	168 (43.52)	189 (53.09)		
Age media ± SD	57.57 ± 20.81	43.24 ± 12.83	78.86 ± 8.53	p<0.001	61.25 ± 19.42	46.23 ± 13.61	77.63 ± 8.48	p<0.001	p<0.001
Length of stay days ± SD	13.24 ± 16.86	10.53 ± 15.11	17.26 ± 18.47	p<0.001	15.90 ± 20.81	15.19 ± 16.95	16.68 ± 24.31	p=0.330	p=0.004
Mortality (%)	136 (15.44)	18 (3.47)	118 (32.60)	RR 7.62 (4.70–12.35)	96 (12.94)	25 (6.48)	71 (19.94)	RR 3.30 (2.12–5.13)	RR 0.84 (0.66–1.07)
Referral to critical area (%)	66 (7.49)	25 (4.82)	41 (11.33)	RR 1.91 (1.17–3.09)	28 (3.77)	14 (3.63)	14 (3.93)	RR 1.16 (0.56–2.42)	RR 0.50 (0.33–0.78)
Mortality in referral to critical area (%)	31 (46.97)	6 (24.00)	25 (60.98)	RR 4.84 (2.00–11.72)	8 (28.57)	4 (28.57)	4 (28.57)	RR 1.16 (0.29–4.63)	RR 0.31 (0.14–0.66)

SD – standard deviation; RR – relative risk

Prescription characteristics

COVID-19 patients had significantly fewer drugs on day 1, both total and usual, current ($p<0.001$), thromboprophylaxis, and insulin ($p=0.024$). They also had significantly less drugs added after day 1, fewer total drugs during hospitalization, less polypharmacy, less excessive polypharmacy, less total P-DDIs, and less severe P-DDIs. In the comparison by age of the patients, those ≥ 65 years old presented significantly more usual drugs on day 1 in both groups. In the COVID-19 group, but not in the non-COVID-19 group, patients ≥ 65 years also received more total drugs on day 1, with more thromboprophylaxis and more insulin on day 1. They also presented significantly higher values of drugs added after day 1, total drugs during hospitalization, polypharmacy, excessive polypharmacy, total P-DDIs, and moderate P-DDIs. Among patients in the non-COVID-19 group, those < 65 years received significantly more current drugs on day 1 than those ≥ 65 years. In COVID, in 11 hospitalizations (1.12%) there were no pharmacological indications on the first day and in 428 hospitalizations (48.58%) no new medication was added after the first day (Table 2).

Drugs characteristics and P-DDIs

Among the drugs used for COVID-19, the most frequent indications (% compared to the COVID-19 group) were: dexamethasone 274 (31.10%), ceftriaxone 267 (30.30%), clarithromycin 204 (23.15%), oseltamivir 71 (8.05%), azithromycin 45 (5.10%), amoxicillin-clavulanic 41 (4.65%), hydroxychloroquine 33 (3.74%), ritonavir-lopinavir 17 (1.92%) and darunavir 1 (0.11%). On the other hand, among COVID-19 patients, 639 (72.53%) received enoxaparin, 526 (59.70%) received paracetamol. Oxygen therapy in the COVID-19 population was significantly higher than in the non-COVID-19 population and in both groups it was higher among those patients aged 65 or over (Table 3).

The most frequent P-DDIs were analyzed, showing that clarithromycin stands out among the associated drugs in the potential P-DDIs in the COVID-19 group (Table 4).

COVID-group disaggregated analysis

In the COVID-19 group, the 881 patients were divided into 538 detectable and 343 non-detectable, according to the RT-PCR result. Of the 14 readmissions in the COVID-19 group, 6 were detectable in both, 3 non-detectable in both, and 5 detectable once and non-detectable once. COVID-detectable patients were older and had higher amounts of length of hospitalization, mortality, and referral to the critical area. Among the detectable patients hospitalized in the COVID-19 wards, those ≥ 65 years of age had significantly longer hospitalizations, and higher mortality, risk for critical area referral, and mortality (among those who were referred to critical areas). Among the patients admitted to the non-detectable COVID-19 wards, those ≥ 65 years old, the percentage of women was significantly higher, they had significantly longer hospitalizations, higher mortality, and a higher risk of being referred to the

Table 2 – Drugs per patient on day 1, divided into usual medication, current medication, thromboprophylaxis, and insulin; drugs added after day 1, total drugs during hospitalization, polypharmacy, excessive polypharmacy, P-DDIs on day 1, total and according to severity. In all these cases, COVID-19 and non-COVID-19 hospitalizations are compared and by age between <65 vs. ≥65 years

Parameter	COVID-19			
	total	<65 years	≥65 years	statistics
N=1,623 (%)	881 (54.28)	519 (58.91)	362 (41.09)	
Oxygen therapy (%)	206 (23.38)	86 (16.57)	120 (33.15)	p=0.025
Day 1 total drugs ± SD	4.54 ± 3.06	3.53 ± 2.57	6.03 ± 3.13	p<0.001
Day 1 usual drugs ± SD	1.78 ± 2.26	1.14 ± 1.80	2.72 ± 2.53	p<0.001
Day 1 current drugs ± SD	1.94 ± 1.51	1.71 ± 1.45	2.29 ± 1.55	p<0.001
Day 1 thromboprophylaxis (%)	600 (68.10)	294 (56.64)	306 (84.53)	p=0.027
Day 1 insulin (%)	117 (13.28)	53 (10.21)	64 (17.67)	p=0.021
Drugs added after day 1 ± SD	1.04 ± 2.29	0.88 ± 2.16	1.27 ± 2.45	p=0.012
Total drugs in hospitalization ± SD	5.57 ± 3.93	4.41 ± 3.54	7.30 ± 3.87	p<0.001
Polypharmacy (%)	397 (45.06)	161 (31.02)	236 (65.19)	RR 1.70 (1.44–2.01)
Excessive polypharmacy (%)	59 (6.70)	15 (2.89)	44 (12.15)	RR 3.41 (1.92–6.06)
P-DDIs/patient total SD	0.31 ± 0.67	0.19 ± 0.55	0.48 ± 0.79	p<0.001
P-DDIs/patient moderate ± SD	0.17 ± 0.51	0.07 ± 0.34	0.31 ± 0.67	p<0.001
P-DDIs/patient severe ± SD	0.06 ± 0.30	0.05 ± 0.30	0.08 ± 0.30	p=0.098
P-DDIs/patient mild ± SD	0.08 ± 0.30	0.07 ± 0.28	0.09 ± 0.32	p=0.326

P-DDIs – potential drug-drug interactions; SD – standard deviation; RR – relative risk

critical area, without significant differences in mortality among those who were referred to critical areas (Table 5).

Detectable patients had a significantly higher oxygen requirement, more thromboprophylaxis, more drugs added after day 1, and more total drugs during hospitalization, with no significant differences in the other variables analyzed (Table 6). Among detectable hospitalized patients in the COVID-19 wards, those 65 years and older received significantly more oxygen therapy, more total drugs on day 1, both current and usual, and thromboprophylaxis; more drugs added after day 1, more polypharmacy, more excessive polypharmacy, more total P-DDIs, and more moderate P-DDIs than those younger than 65 years. Insulin indication on day 1, severe P-DDIs, and mild P-DDIs were not significantly higher in those 65 years or older than in those younger than 65 years.

Non-COVID-19				Non-COVID-19 vs. COVID-19
total	**<65 years**	**≥65 years**	**statistics**	**statistics**
742 (45.72)	386 (52.02)	356 (47.98)		
35 (4.72)	8 (2.07)	27 (7.58)	p=0.018	p<0.001
5.92 ± 3.24	5.91 ± 3.32	5.92 ± 3.16	p=0.941	p<0.001
2.42 ± 2.31	2.12 ± 2.28	2.74 ± 2.30	p<0.001	p<0.001
2.51 ± 2.22	2.86 ± 2.30	2.13 ± 2.06	p<0.001	p<0.001
577 (77.76)	291 (75.58)	286 (80.11)	p=0.836	p=0.024
157 (21.15)	63 (16.36)	94 (26.33)	p=0.017	p=0.024
3.25 ± 4.10	3.49 ± 4.58	3.00 ± 3.49	p=0.102	p<0.001
9.17 ± 5.27	9.40 ± 5.73	8.92 ± 4.71	p=0.220	p<0.001
460 (61.99)	240 (62.18)	220 (61.80)	RR 1.07 (0.92–1.23)	RR 1.38 (1.25–1.51)
104 (14.02)	55 (14.25)	49 (13.76)	RR 1.04 (0.72–1.50)	RR 2.09 (1.54–2.84)
0.40 ± 0.94	0.36 ± 0.97	0.44 ± 0.91	p=0.243	p=0.022
0.18 ± 0.53	0.16 ± 0.50	0.21 ± 0.55	p=0.154	p=0.565
0.11 ± 0.41	0.12 ± 0.44	0.10 ± 0.37	p=0.604	p=0.006
0.11 ± 0.38	0.09 ± 0.36	0.13 ± 0.41	p=0.145	p=0.082

Among the non-detectable patients admitted to the COVID-19 wards, those 65 years of age or older received significantly more oxygen therapy, more total drugs on day 1, more usual drugs on day 1, more current drugs on day 1, more thromboprophylaxis on day 1, more insulin on day 1, more total drugs during hospitalization, more polypharmacy, more excessive polypharmacy, more total P-DDIs, and more moderate P-DDIs than in those under 65 years of age. There were no significant differences in drugs added after day 1, severe P-DDIs and mild P-DDIs were not significantly higher among those 65 years and older compared with those younger than 65 years (Table 6).

Among the 538 detectable patients admitted to COVID-19 wards, 114 received convalescent plasma transfusion. No differences in mortality could be observed between those who received convalescent plasma (23/114: 20.17%) compared to those who did not (81/424: 19.10%) (RR [relative risk]: 1.05, 95% CI [confidence

Table 3 – The 20 most used drugs in each group are presented

	COVID-19 (N=881)		Non-COVID-19 (N=742)	
	drugs	**N (%)**	**drugs**	**N (%)**
1	Enoxaparin	639 (72.53)	Enoxaparin	502 (67.65)
2	Paracetamol	526 (59.70)	Omeprazole	275 (37.06)
3	Ceftriaxone	263 (29.85)	Insulin	158 (21.29)
4	Dexamethasone	253 (28.71)	Atorvastatin	131 (17.65)
5	Clarithromycin	212 (24.06)	Paracetamol	131 (17.65)
6	Omeprazole	207 (23.49)	Enalapril	127 (17.11)
7	Insulin	150 (17.02)	ASA	120 (16.17)
8	Enalapril	132 (14.98)	Piperacillin TZB	92 (12.39)
9	Piperacillin TZB	115 (13.05)	Tramadol	92 (12.39)
10	Clonazepam	98 (11.12)	Meprednisone	83 (11.18)
11	ASA	97 (11.01)	Ceftriaxone	79 (10.64)
12	Quetiapine	85 (9.64)	Cotrimoxazole	76 (10.24)
13	Levothyroxine	84 (9.53)	Amlodipine	71 (9.56)
14	Morphine	72 (8.17)	Bisoprolol	71 (9.56)
15	Amlodipine	69 (7.83)	Dexamethasone	69 (9.29)
16	Tramadol	67 (7.60)	Levothyroxine	69 (9.29)
17	Lactulose	66 (7.49)	Metoclopramide	69 (9.29)
18	Bisoprolol	64 (7.26)	Allopurinol	61 (8.22)
19	Atorvastatin	60 (6.81)	Furosemide	61 (8.22)
20	Carvedilol	60 (6.81)	Ranitidine	52 (7.00)
20	Meprednisone	60 (6.81)		

ASA – acetylsalicylic acid; TZB – tazobactam

interval]: 0.69–1.59). In the subanalysis that compares the evolution according to the date convalescent plasma was administered in relation to the date of onset of symptoms, no significant differences were observed in mortality among those who received it within the first 3 days of the onset of symtoms (8/38: 21.05%), between the 4th and 7th day of the onset of symptoms (6/24: 25.00%) or after the 7th day of the onset of symptoms (9/52: 17.30%).

Discussion

This work shows important data on mortality, drug prescription pattern and interactions linked to the pandemic. Its design started in a previous period, and with originally different purposes (evaluating the effects of the implementation of an interaction detection software) makes it a unique material to be able to compare how the epidemiological situation modified the use of drugs, and to detect factors demographic factors associated with increased risk, such as belonging to age groups. The present work also shows higher mortality in patients with detectable virus.

In our study, younger patients diagnosed with COVID-19 were hospitalized for shorter periods (10.53 ± 15.11 vs. 17.26 ± 18.47, $p<0.001$; Table 1), were more

Table 4 – Potential drug-drug interactions (P-DDIs) more frequent in each group

COVID-19 (N=881)				Non-COVID-19 (N=742)			
Drugs	**P-DDIs**	**N**	**%**	**Drugs**	**P-DDIs**	**N**	**%**
Clarithromycin-Dexamethasone	altered metabolism by CYPs	71	8.06	Metoclopramide-Morphine	constipation	15	2.02
ASA-Enoxaparin	risk of bleeding	66	7.49	Amlodipine-Paracetamol	hypertension	14	1.89
Enalapril-Paracetamol	hypertension	32	3.63	Clonazepam-Paracetamol	less effective benzodiazepines	12	1.62
Clonazepam-Paracetamol	less effective benzodiazepines	31	3.52	Alprazolam-Paracetamol	less effective benzodiazepines	10	1.35
Clarithromycin-insulin	hypoglycemia	30	3.41	Atorvastatin-Paracetamol	altered metabolism by CYPs	9	1.21
Levothyroxine-Paracetamol	less effective Levothyroxine	26	2.95	Levothyroxine-Paracetamol	less effective Levothyroxine	8	1.08
ASA-insulin	hyperglycemia	24	2.72	Losartan-Paracetamol	hypertension	7	0.94
Losartan-Paracetamol	hypertension	21	2.38	Enalapril-Paracetamol	hypertension	7	0.94
Carvedilol-Paracetamol	altered metabolism by CYPs	20	2.27	Carvedilol-Paracetamol	altered metabolism by CYPs	7	0.94
Atorvastatin-Paracetamol	altered metabolism by CYPs	20	2.27	Amiodarone-Bisoprolol	bradycardia	7	0.94

ASA – acetylsalicylic acid; CYP – cytochrome p450; P-DDIs – potential drug-drug interactions

frequently referred to critical areas, and had higher mortality among those referred to critical areas than patients in non-COVID-19 wards. Since patients with confirmed COVID-19 and suspected COVID-19 patients were admitted to the COVID-19 wards, not all of them were detectable: detectable were those with the highest mortality: 19.33%. In relation to another study carried out at the same time in our country, organized by the Sociedad Argentina de Medicine, in 37 centers, with 4,776 patients admitted to Medical Clinic Services, ≥18 years old with confirmed COVID-19, our detectable COVID population was older (61 vs. 56.9 years, although the study did not adjust for other factors), was hospitalized for a longer time (15 days vs. 8 days), had greater referral to the critical area (19.7 vs. 14.8%)

Table 5 – Evolution of COVID-19 hospitalizations according to detectable and non-detectable, total and by age <65 o ≥65 years

Parameter	Detectable				Non-detectable				Detectable vs. non-detectable
	total	<65 years	≥65 years	statistics	total	<65 years	≥65 years	statistics	statistics
N=881 (%)	538 (61.06)	288 (53.53)	250 (46.46)		343 (38.94)	231 (67.34)	112 (32.65)		
Female N (%)	262 (48.70)	130 (44.98)	132 (53.01)	p=0.894	186 (54.23)	90 (39.13)	96 (84.96)	p=0.027	
Age media ± SD	60.79 ± 19.83	45.21 ± 12.30	78.87 ± 8.07		53.50 ± 21.39	41.02 ± 12.75	78.89 ± 9.69		p<0.001
Length of stay days	15.24 ± 15.21	12.57 ± 12.33	18.34 ± 17.50	p<0.001	10.18 ± 18.72	7.93 ± 17.63	14.72 ± 20.07	p<0.001	p<0.001
Mortality (%)	104 (19.33)	10 (3.46)	94 (37.75)	RR 10.91 (5.81–20.48)	32 (9.33)	7 (3.04)	25 (22.12)	RR 7.27 (3.24–16.30)	RR 2.07 (1.42–3.00)
Referral to critical area	51 (9.47)	19 (6.59)	32 (12.8)	RR 1.94 (1.12–3.33)	15 (4.37)	6 (2.59)	9 (8.03)	3.09 (1.12–8.47)	RR 2.16 (1.23–3.79)
Mortality in referral to critical area (%)	26 (50.99)	3 (15.78)	23 (71.87)	RR 4.55 (1.57–13.15)	5 (33.33)	3 (50.00)	2 (22.22)	RR 0.44 (0.10–1.91)	RR 1.52 (0.71–3.28)

SD – standard deviaton; RR – relative risk

and higher mortality (19.33 vs. 12.3%) (Boietti et al., 2021). In another study from a single center in Buenos Aires city with 417 patients, the average age was 43 years and mortality 3.8% (Melendi et al., 2020). The correlation between age and unfavorable evolution is consistent in studies and meta-analyses (Bonanad et al., 2020; Boietti et al., 2021). Our patients 65 years of age or older had higher mortality in both the COVID-19 and non-COVID-19 groups, reaching the highest percentage among detectable COVIDs: 37.75%.

Another important parameter was the drug use pattern during the COVID-19 pandemic such as lower drug use, less polypharmacy, and less P-DDIs in COVID-19 hospitalizations. These data were noticeable, taking into account the number of specific treatments for coronavirus proposed with dissimilar therapeutic evidence. At the beginning of the pandemic, there was no approved treatment for COVID-19 disease. In the absence of a specific antiviral treatment, the WHO prioritized drugs to be investigated in clinical trials based on *in vitro* efficacy. Recommendations were made to treat these infections with different antiviral drugs that had been tested on other coronaviruses. Lopinavir-ritonavir had demonstrated inhibitory activity *in vitro* during the severe acute respiratory syndrome coronavirus (SARS-CoV-1) outbreak. This combination was already available in our country and was approved by the Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT) for use in patients with HIV infection (Ministerio de Salud Argentina, 2020a). The treatment established in our hospital was adapted to the recommendations of the Ministerio de Salud de la Nación Argentina. These recommendations were updated, based on available evidence and ongoing clinical trials (Ministerio de Salud Argentina, 2020b, c, 2021). The treatment regimen proposed at the begining of our study was as follows: for mild infection without pneumonia: no treatment; for mild infection with pneumonia according to pneumonia severity score, CURB-65: 0–1 (Lim et al., 2003): consider lopinavir/ritonavir; for severe respiratory infection (CURB-65: >2 pts) in >60 years, with comorbidities (arterial hypertension, diabetes mellitus, heart disease, chronic obstructive pulmonary disease (COPD), chronic renal failure, immunocompromised): lopinavir/ritonavir or darunavir/ritonavir + hydroxychloroquine (Singh et al., 2020). In addition, antibiotic treatment with ampicillin-sulbactam or ceftriaxone + azithromycin + oseltamivir was recommended. These treatment guidelines were published on March 20, 2020, and remained current through May 30, 2020 (Ministerio de Salud Argentina, 2021). Due to the limited evidence of therapeutic efficacy on COVID-19, the epidemiological dynamics of SARS-CoV-2, and due to the little or no favorable clinical impact of treatments with lopinavir-ritonavir, darunavir/ritonavir, hydroxychloroquine and oseltamivir, added to poor tolerance and adverse effects, these drugs were no longer used (Ministerio de Salud Argentina, 2021): this can be observed in the low rate of prescriptions observed for these drugs in our population: oseltamivir 71 (8.05%), hydroxychloroquine 33 (3.74%), ritonavir-lopinavir 17 (1.92%) and darunavir 1 (0.11%). On the other hand, in the COVID-19

Table 6 – Oxygen therapy, drugs per patient on day 1, divided into usual medication, current medication, thromboprophylaxis, and insulin; drugs added after day 1, total drugs during hospitalization, polypharmacy, excessive polypharmacy, P-DDIs on day 1, total and according to severity in detectable and non-detectable patients, total and by age <65 o ≥65 years

Parameter	Detectable			
	total	<65 years	≥65 years	statistics
N=881 (%)	538 (61.06)	288 (53.53)	250 (46.46)	
Oxygen therapy (%)	172 (31.9)	72 (24.91)	100 (40.16)	p<0.001
Day 1 total drugs ± SD	4.68 ± 2.89	3.72 ± 2.40	5.80 ± 3.02	p<0.001
Day 1 usual drugs ± SD	1.83 ± 2.27	1.19 ± 1.84	2.57 ± 2.49	p<0.001
Day 1 current drugs ± SD	1.95 ± 1.43	1.74 ± 1.44	2.19 ± 1.38	p<0.001
Day 1 thromboprophylaxis (%)	407 (75.65)	192 (66.66)	215 (86.00)	p<0.001
Day 1 insulin (%)	79 (14.68)	35 (12.15)	44 (17.60)	p=0.391
Drugs added after day 1 ± SD	1.17 ± 2.30	0.91 ± 1.95	1.48 ± 2.62	p=0.003
Total drugs in hospitalization ± SD	6.19 ± 6.11	5.23 ± 5.21	7.31 ± 6.86	p<0.001
Polypharmacy (%)	257 (47.77)	100 (34.60)	157 (63.05)	RR 1.82 (1.51–2.19)
Excessive polypharmacy (%)	34 (6.32)	6 (2.08)	28 (11.24)	RR 5.42 (2.28–12.87)
P-DDIs/patient total ± SD	0.32 ± 0.68	0.21 ± 0.58	0.45 ± 0.76	p<0.001
P-DDIs/patient moderate ± SD	0.18 ± 0.53	0.09 ± 0.39	0.28 ± 0.64	p<0.001
P-DDIs/patient severe ± SD	0.06 ± 0.31	0.05 ± 0.34	0.08 ± 0.27	p=0.364
P-DDIs/patient mild ± SD	0.08 ± 0.29	0.07 ± 0.28	0.09 ± 0.31	p=0.453

P-DDIs – potential drug-drug interactions; SD – standard deviation; RR – relative risk

population the use of antibiotics ceftriaxone in 267 patients (30.30%), clarithromycin in 204 (23.15%), azithromycinin 45 (5.10%), amoxicillin-clavulanate in 41 (4.65%) was high, which were prescribed more than in the non-COVID-19 population. Treatment with these antibiotics was maintained in cases of suspected bacterial superinfection throughout the period evaluated. In the Boietti et al. (2021) study, 27.9% received antibiotics, while in another study from a single center in Buenos Aires city with 417 patients, 39.6% received oral antibiotics and 29.3% intravenous antibiotics (Melendi et al., 2020).

Beyond antiviral or antibacterial treatment, the use of oxygen therapy, thromboprophylaxis, treatment with corticosteroids and symptomatic treatment with paracetamol should be highlighted. In the Boietti et al. (2021) study, 36.7% received supplemental oxygen therapy. Of the patients with O_2 supplementation, 25.5% (n=448) required intensive care unit (ICU) admission, and of these, 170 (45.7%) received mechanical ventilatory assistance (Boietti et al., 2021). Our

Non-detectable				Detectable vs. non-detectable
total	**<65 years**	**≥65 years**	**statistics**	**statistics**
343 (38.94)	231 (67.34)	112 (32.65)		
35 (10.20)	15 (6.52)	20 (17.70)	p<0.001	p<0.001
4.29 ± 3.30	3.21 ± 2.69	6.50 ± 3.36	p<0.001	p=0.066
1.69 ± 2.25	1.06 ± 1.71	2.96 ± 2.65	p<0.001	p=0.345
1.93 ± 1.64	1.63 ± 1.44	2.54 ± 1.85	p<0.001	p=0.863
193 (56.26)	102 (44.15)	91 (81.25)	p<0.001	p<0.001
38 (11.07)	18 (7.79)	20 (17.85)	p=0.006	p=0.063
0.60 ± 1.99	0.61 ± 2.12	0.58 ± 1.73	p=0.915	p=0.001
4.85 ± 5.73	3.51 ± 5.15	7.35 ± 5.21	p=0.003	p<0.001
140 (40.82)	56 (24.35)	84 (74.34)	RR 3.05 (2.37–3.93)	p=0.637
25 (7.29)	8 (3.48)	17 (15.04)	RR 4.33 (1.92–9.72)	p=0.732
0.29 ± 0.66	0.16 ± 0.49	0.54 ± 0.86	p<0.001	p=0.463
0.15 ± 0.49	0.05 ± 0.28	0.35 ± 0.72	p<0.001	p=0.402
0.06 ± 0.29	0.04 ± 0.24	0.09 ± 0.37	p=0.178	p=0.815
0.08 ± 0.30	0.07 ± 0.25	0.11 ± 0.39	p=0.236	p=0.974

detectable population received oxygen therapy in 31.9% of all cases or only in detectable cases.

Thromboprophylaxis, especially with enoxaparin, was common in both groups: 72.53% in COVID-19 and 67.65% in non-COVID. In a systematic review and meta-analysis of 33 studies (31 observational, 2 randomized clinical trials compared), heparins, in addition to low molecular weight ones, showed efficacy in reducing mortality in hospitalized patients with COVID-19, both at doses of thromboprophylaxis (hazard ratio – HR: 0.63, 95% CI 0.57–0.69) and anticoagulant dose (HR: 0.56, 95% CI 0.47–0.66), although with higher risk of bleeding with anticoagulant dose (odds ratio – OR: 2.01, 95% CI 1.14–3.53) in comparison with doses of thromboprophylaxis (Giossi et al., 2021).

Among the corticosteroids, the most used in COVID-19 was dexamethasone: 253 (28.71%), while in non-COVID-19 9.29% received dexamethose. In the Boietti et al. (2021) study, 29.7% received corticosteroids. In another study with 417

patients, 20.6% received corticosteroids (Melendi et al., 2020). This strategy of using dexamethasone in COVID-19 was consolidated from the preliminary publication of the RECOVERY Collaborative Group et al. (2021) study, in which an improvement in the prognosis of patients requiring oxygen therapy was observed if dexamethasone was used, this benefit was extensive for other corticosteroids among patients requiring oxygen therapy (van Paassen et al., 2020; Pasin et al., 2021). Paracetamol was a drug widely used for symptomatic treatment in the COVID-19 population (59.7%) and much less used in the non-COVID-19 population (17.65%).

Even so, and despite the considerations mentioned about potentially antiviral therapies and other therapies linked to the pandemic, such as the use of oxygen therapy, anticoagulants, and corticosteroids, the use of drugs throughout the entire hospitalization was significantly lower in COVID-19 than in non-COVID: 5.57 drugs/patient vs. 9.17 drugs/patient. This is a consequence of fewer drugs on day 1, both regular and current, and fewer drugs added after day 1. These differences are probably justified by the fact that it is a younger population and with a single hospitalization clinical condition. We also observed significantly less polypharmacy in (COVID-19 45.06%) than in non-COVID-19 (61.99%) and also less excessive polypharmacy: 6.70 and 14.02% respectively. In a systematic review of articles on COVID-19 published between November 2019 and September 2020, 7 articles with 10,519 detectable COVID-19 patients were included: 4,818 of them had polypharmacy (Iloanusi et al., 2021). In 5 of these 7 articles, polypharmacy was associated with unfavorable outcome. The presence of polypharmacy was significantly associated with detectable COVID, death among reactive COVID-19 males, greater kidney damage, and a higher frequency of adverse drug effects. The use of antipsychotics was associated with increased morbidity and mortality, both in men and women (Iloanusi et al., 2021).

Although the original trial was oriented towards classical pharmacological therapy, some characteristics of the study (design, methodology, data recording) allowed an excellent opportunity to evaluate other therapeutic strategies used during the pandemic (first phase of pandemics), such as the case of convalescent plasma. COVID-19 convalescent plasma is plasma collected from donors recovered from acute COVID-19 infection, with high levels of neutralizing antibodies against the SARS-CoV-2 virus, conferring immunity through direct binding and inactivation of the SARS-CoV-2 virus by neutralizing anti-SARS-CoV-2 antibodies, antibody-dependent complement activation, cytotoxicity, and phagocytosis. In addition to improving clearance, antibodies may also decrease disease severity and facilitate recovery, by modulating the exaggerated immune response and cytokine storm associated with severe disease and multiorgan dysfunction (Rojas et al., 2020). The administration of convalescent plasma generated considerable expectations in the first wave of the COVID-19 pandemic, whose effectiveness had to be demonstrated (Mucha and Quraishy, 2020). In a Cochrane review of a total of 5,443 participants in 20 studies, it did not show conclusive evidence to support the

efficacy of convalescent plasma in reducing mortality, improving clinical symptoms, or shortening hospital stay (Singh and Gupta, 2021). Other systematic reviews reach conflicting conclusions (Barreira et al., 2021; Janiaud et al., 2021; Kloypan et al., 2021). In our study, we did not observe a benefit in reducing mortality in the 114 patients who received convalescent plasma. These results are consistent with those observed in a multicenter clinical trial conducted in our country with 333 patients with similar characteristics (Simonovich et al., 2021). Based on the information emerging from these observations, convalescent plasma treatment appears to be of greatest benefit if administered early in the course of the disease, with high neutralizing antibody titres, in patients without respiratory compromise, according to another multicenter clinical trial, with 160 patients also carried out in our country (Libster et al., 2021).

The antiviral drugs used in the COVID-19 context, together with dexamethasone, hydroxychloroquine and antibacterials, raised alarms due to the probability of severe P-DDIs (Kumar and Trivedi, 2021). We observed a relatively low P-DDIs rate, both in the COVID-19 and non-COVID-19 population: 0.31/patient and 0.40/patient, respectively. This may be due, in part, to the fact that the drugs that presented the most P-DDIs were used comparatively little, with the exception of clarithromycin and dexamethasone, and, on the other hand, to the fact that the prescriptions were made with the SCSDM SIMDA that detected P-DDIs. We did not find articles that explored the frequency of P-DDIs in this type of hospitalization.

Limitations

The present work presents limitations to be taken into account, such as: (1) it was carried out in a single healthcare center, linked to a relatively homogeneous population that may not represent population groups from other regions; (2) analyzes the therapeutics used in the institution, which may present differences with other health institutions, given that multiple recommendations were generated during the pandemic that changed very quickly and with great intercenter heterogeneity, (3) physicians could modify prescriptions or write new ones on the system-generated paper forms: if those prescriptions were not later added as regular medications, they were not entered into the study; (4) we did not analyze parenteral hydration plans or the addition of electrolytes to these plans: this led to, for example, evaluating potassium intakes by mouth but not those made intravenously.

Conclusion

Hospitalizations completed during the COVID-19 pandemic (first phase) were associated with a particular prescription pattern, characterized by a lower number of drug use, with the consequent lower prevalence of polypharmacy and therefore lower risk of interactions (P-DDIs). Even so, the separation of patients in specific rooms showed that the group assigned as COVID-19, and in particular those with detectable virus, presented a higher mortality.

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Association of COVID-19 Infection and Acute Mesenteric Ischemia

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Abstract: COVID-19 is an infectious disease that is considered to be a thromboinflammatory disorder. The study was aimed to determine the prevalence of COVID-19 in patients with acute mesenteric ischemia (AMI) and the outcomes of surgical treatment in relation to COVID-19. A total of 140 patients were included in this multicentric study divided into two groups: the test group (n=65) consisted of cases of AMI detected during the COVID-19 pandemic and the control group (n=65) consisted of cases of AMI detected before the pandemic. Test group patients were classified as COVID-positive (COVID+), or COVID-negative (COVID–) if they tested positive, respectively negative test for COVID-19 on admission. Primary outcomes were: prevalence of COVID-19 infection among test group patients, association between COVID-19 infection and inoperability, and between COVID-19 and treatment outcome. Secondary outcomes were association between each blood parameter and inoperability and treatment outcome. There were no statistically significant differences between inoperability and COVID-19 positivity on admission, overall mortality between the control group and the test group and overall mortality between COVID+ and COVID– patients, as well as among those patients that have been surgically treated ($p>0.05$). There were statistically significant differences between serum amylase levels ($p=0.034$), and serum LDH levels ($p=0.0382$) and inoperability, between serum LDH levels and postoperative mortality ($p=0.0151$), and overall mortality ($p=0.00163$). High level of LDH and serum pancreatic amylase are associated with a higher rate of inoperability and a higher postoperative and overall mortality rate. COVID-19 does not seem to independently influence the treatment outcome of AMI.

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Introduction

Acute mesenteric ischemia (AMI) is a rare disease caused by insufficient intestinal blood flow, being involved in 0.1 to 1 per 1,000 hospital admissions, mostly affecting elderly patients. Early diagnosis and treatment are crucial to successful treatment and prevention of bowel necrosis as it has a high mortality of 60–80%. Thromboembolism of the superior mesenteric artery (SMA) is the most common cause of AMI (Clair and Beach, 2016). Patients with AMI present with acute abdominal pain as well as vomiting, diarrhoea, abdominal distension and blood in the stool, and abdominal tenderness which is minimal in the early stages but progresses to diffuse peritonitis as intestinal necrosis develops (Carver et al., 2016). Laboratory tests in AMI include white blood cell count, lactate, D-dimer and metabolic acidosis. Definitive diagnosis is achieved with CT (computed tomography) angiography. Treatment options include endovascular repair and open surgical therapy in the form of surgical embolectomy or bowel resection. COVID-19 is an infectious disease of the respiratory system caused by the SARS-nCoV2 virus, with significant vascular manifestations. The vascular endothelium plays an important role in the inflammatory response triggered by COVID-19 (Thomas and Scully, 2022). A complex interplay between the endothelium, the immune system and the coagulation cascade arises, which leads to the development of thrombosis. The endothelium is subject to several prothrombotic changes including glycocalyx shedding, loss of cytoprotective signalling and antithrombotic effectors. COVID-19 has been associated with a variety of events caused by abnormal coagulation, such as venous thromboembolism, pulmonary embolism, deep vein thrombosis, and arterial thromboses. COVID-19 is therefore considered to be a thromboinflammatory disorder (Mathieu et al., 2021).

In light of this evidence, the hypothesis of acute mesenterial ischemia as a complication of infection with the SARS-nCoV-2 virus is plausible.

The objective of this study was to evaluate the prevalence of COVID-19 in patients with AMI during the pandemic as well as the outcomes of surgical treatment in relation to COVID-19.

Material and Methods

This retrospective cohort multicentric study was done on patients treated in three public tertiary care hospitals in Skopje, North Macedonia. One hundred and forty patients admitted to hospital under suspicion of AMI were accepted in this study. Data was gathered anonymously using electronic medical records of the hospitals the patients were treated in. Inclusion criteria were patients with radiological and/or per operative evidence of AMI. Patients were excluded if AMI was excluded during treatment. They were divided into two groups with respect to the time period they were detected, either during or before the pandemic. The test group (n=65) consisted of cases detected during the COVID-19 pandemic. The first registered COVID-19 case in North Macedonia was detected on 26 February 2020 (Chen et al., 2022). Patient data was gathered until 30 November 2021, which amounts to

a time period of 644 days. The control group (n=65) consisted of cases detected during an equal time period of 644 days before the pandemic, i.e., between 28 April 2018 and 31 January 2020. Test group patients were classified as COVID-positive (COVID+) if they tested positive for COVID-19 on admission with a rapid antigen test and a subsequent PCR, or COVID-negative (COVID–) if they had a negative rapid antigen test on admission.

All patients were evaluated with respect to operability and treatment outcome. Patient operability was evaluated with two categories: operable, which designates patients that were subjected to intestinal resection of any kind; and inoperable, which designates patients that were not subjected to surgical treatment due to terminal disease stage. Treatment outcome was similarly evaluated: patients that were successfully treated and discharged were classified as survivors, and patients that were treated surgically without success or patients that were considered inoperable on admission were classified as non-survivors. Postoperative mortality is defined as the mortality rate of patients that have undergone surgery. Overall mortality is defined as the mortality rate of patients that have deceased both after surgery as well as due to terminal stage. The values of the following blood parameters on admission were noted: D-dimers, C-reactive protein (CRP), lactate dehydrogenase (LDH), and pancreatic amylase. Primary outcomes were: prevalence of COVID-19 infection among test group patients with AMI. Association between COVID-19 infection and inoperability, and between COVID-19 and treatment outcome. Secondary outcomes were: association between each blood parameter and inoperability, association between each blood parameter and treatment outcome.

Statistical analysis

Statistical analysis was performed by Mann Whitney U-test for continuous variables. Fisher's exact test and odds ratio with 95% confidence interval was used for frequencies. The level of statistical significance was $p<0.05$. RStudio was used for the statistical analysis.

Results

During both time periods, 140 patients were hospitalized under the suspicion of AMI. Ten patients were excluded due to exclusion of AMI as a diagnosis, which resulted in a final count of 130 patients. The control group and the test group contained 65 patients each. General patient characteristics can be found in Table 1. In the test group, 7 patients (10.78%) were COVID+. Five of them were surgically treated, of which 2 successfully recovered and 3 did not survive. Two patients were considered terminal. There were no statistically significant differences between inoperability on admission and COVID-19 positivity on admission ($p>0.05$). In the control group, 19 patients (29.23%) were considered inoperable. In the test group, 16 patients (24.62%) were considered inoperable. There was no difference between

Table 1 – General patient characteristics

	Control group (n=65)		Test group (n=65)
	COVID+ (n=7)	COVID– (n=58)	
Age	60.85 ± 8.13	68.64 ± 12.1	69.85 ± 13
Sex (m/f)	4/3	32/26	36/29
Inoperable	**2**	**14**	**19**
Treatable	**5**	**44**	**46**
Recovered	2	26	29
Deceased	3	18	17

m – male; f – female

the incidence of AMI during and before the pandemic due to the identical size of the control and the test groups. There were no statistically significant differences in overall mortality between the control group and the test group (56.9% vs. 61%; p>0.05). There was no statistically significant difference in overall mortality between COVID+ and COVID– patients (71.43% vs. 55.17%; p>0.05), as well as among those patients that have been surgically treated (40% vs. 59%; p>0.05). There were statistically significant differences between serum amylase levels on admission and inoperability on admission (p=0.034; Figure 1). There were statistically significant differences between serum LDH levels on admission and inoperability on admission (p=0.0382; Figure 1) as well as between serum LDH levels on admission and postoperative mortality (p=0.0151), as well as LDH levels on admission and overall mortality (p=0.00163). No statistically significant differences were noted between D-dimers and inoperability on admission (p>0.05; Figure 1), between D-dimers and overall (p>0.05) as well as postoperative mortality (p>0.05). Likewise, CRP influenced neither inoperability on admission (p>0.05; Figure 1) nor overall (p>0.05) nor postoperative mortality (p>0.05). Among the COVID+ patients, there were no statistically significant differences in CRP, D-dimer, LDH and amylase levels between the survivors, the patients that died after surgical treatment and the inoperable patients (p>0.05).

Discussion

AMI is a well-documented complication of COVID-19 (Helms et al., 2020; Serban et al., 2021; Chen et al., 2022; Gupta et al., 2022). Risk factors for AMI include conditions which favour thrombus formation such as atrial fibrillation, heart failure, recent myocardial infarction, cardiac thrombi, mitral valve disease, atherosclerosis, portal hypertension, and history of thromboembolic events (Bala et al., 2022). In addition to these factors, infection with the SARS-nCoV2 virus triggers a variety of mechanisms which promote thrombus formation in accordance with Virchow's triad, which consists of endothelial damage, turbulent blood flow and circulating

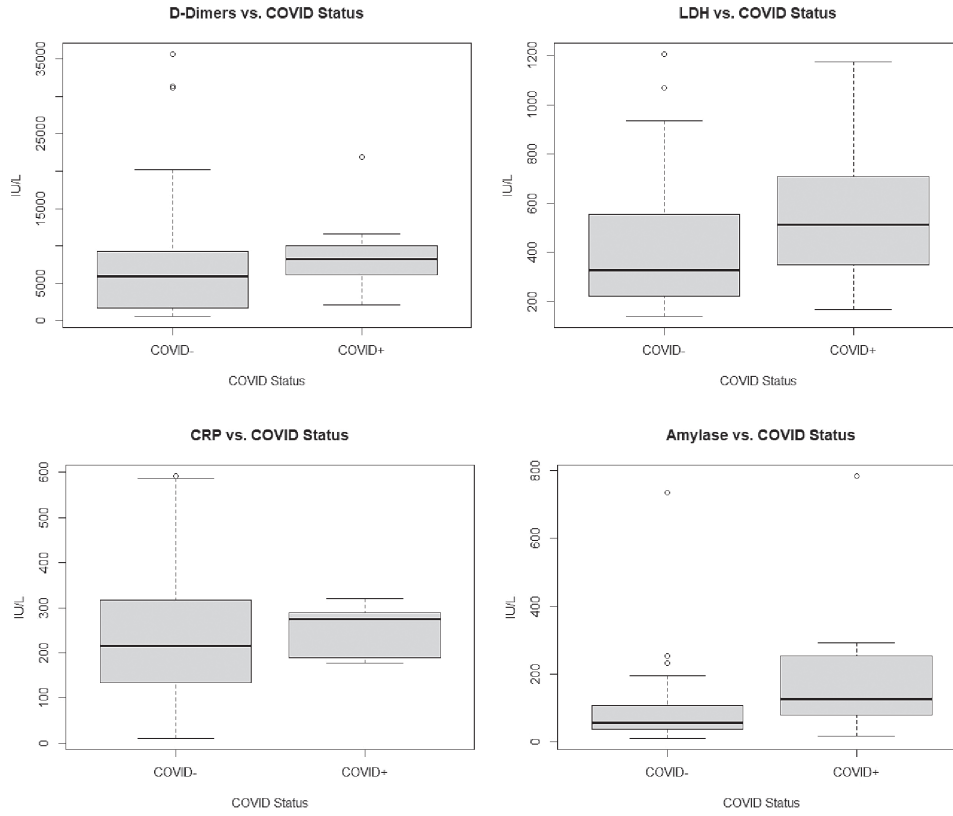

Figure 1 – Comparison of blood parameter levels.

factors that favour coagulation (Bobescu et al., 2021). There are multiple lines of evidence supporting the hypothesis of COVID-19-induced AMI. Platelet activation has been documented in COVID-19, in which the binding of SARS-nCoV2 to angiotensin-converting enzyme 2 (ACE2) has been implicated (Zhang et al., 2020). Endothelial dysfunction could be caused by viral entry in pericytes, where the expression of the ACE2 receptor is high (Becker, 2020). Endotheliosis is another mechanism of endothelial injury, given that viral particles and inclusion bodies as well as inflammatory cells within capillaries have been observed in patients with COVID-19. In one study was demonstrated endotheliosis of the submucosal vessels of the small intestine, providing evidence that COVID-19 coagulopathy may be directly implicated in the pathogenesis of AMI (Varga et al., 2020). Additionally, infection with SARS-nCoV2 can also lead to activation of the complement system, which can independently cause endothelial injury as well as induce exocytosis of P-selectin and von Willebrand factor multimers from endothelial cells that promote platelet adhesion (Noris et al., 2020). In our study, the prevalence of COVID-19

among patients with AMI is 10.78%. We believe this percentage in reality to be higher, considering that patients were classified as COVID+ only if they received positive COVID testing results on admission, without taking into account prior convalescence. Concerning the blood parameters, our results provided some curious findings. In patients with COVID-19, D-dimers have a good predictive value for the occurrence of arterial or venous thrombosis (Betoule et al., 2020) and also correlate with the severity of disease, development of acute respiratory distress syndrome (ARDS), and mortality (Nauka et al., 2020). In our study, D-dimer levels on their own did not seem to be indicative of a poorer prognosis of AMI in COVID+ patients. On the other hand, LDH levels on admission were associated with higher rate of inoperability on admission, higher postoperative and overall mortality; and amylase levels which were associated with higher rate of inoperability. Additionally, these blood parameters seemed to influence the treatment outcome independently of COVID-19. A study by Nachmias-Peiser et al. (2022) analysed mortality in patients with suspected AMI. In this study, LDH was found to be a significant risk factor for mortality in the AMI group as well as in the non-AMI group (Nachmias-Peiser et al., 2022). In our study, we think that the relation between LDH levels and both postoperative and overall mortality may be due to the delay in the procurement of medical care, with a possible correlation between LDH levels and the duration of ischemia, given that LDH is an indicator of injury severity (Guzmán-de la Garza et al., 2013). Evidence of increasing LDH levels in AMI over time has already been demonstrated in experimental models (Roth et al., 1989; Cakir et al., 2019). Of course, these findings require further confirmation in a prospective setting. COVID-19 can initially present with gastrointestinal symptoms (Redd et al., 2020). Given that the diagnosis of AMI requires a high degree of suspicion, we think that it is of utmost importance that COVID-19 patients do not be evaluated only for respiratory symptoms but also for symptoms hinting at potential involvement of the gastrointestinal system. El Moheb et al. (2020) reported a significant incidence of gastrointestinal complications in COVID-19 ARDS patients compared to non-COVID-19 ARDS patients, including bowel ischemia. A strength of this study is that it observes the incidence of AMI in relation to COVID-19 during a longer period of time. In this regard, our study expands the current body of research pertaining to AMI and COVID-19 which consists mostly of case reports. Another strength of this study is that it derives patients from all three public tertiary care hospitals in North Macedonia that treat this pathology. Tertiary health care in North Macedonia is highly centralized, which means that the overwhelming majority of patients evoking clinical suspicion of AMI would be transported to one of the three hospitals that have been included in this study. Consequently, the cases treated in these hospitals are an indicator of the incidence of AMI in the entire country. Limitations of this study include its retrospective design and the lack of prior COVID convalescence data. A more accurate estimate of the prevalence of COVID-19 in patients with AMI could be done with additional evaluation of prior convalescence. The strength of

the associations between COVID-19 and treatment outcomes, and the associations between the blood parameters and treatment outcomes could be more precisely determined with a case-control study.

Conclusion

COVID-19 prevalence in AMI is 10.78%. High level of LDH is associated with a higher rate of inoperability and a higher postoperative and overall mortality rate. High level of serum amylase is associated with a higher rate of inoperability. COVID-19 does not seem to independently influence the treatment outcome of AMI.

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Evaluation of Retinal Nerve Fibre Layer Thickness and Choroidal Thickness in Parkinson Disease Patients

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Abstract: To evaluate the retinal nerve fibre layer (RNFL) thickness and choroidal thickness (CT) in Parkinson disease (PD) patients. A comparative cross-sectional, hospital-based study. 39 PD and 39 controls were recruited, who were gender and age matched. Subjects that fulfilled the inclusion criteria underwent optical coherence tomography for evaluation of RNFL thickness and choroidal thickness (CT). There was significant reduction of RNFL thickness in average (adjusted mean 88.87 μm vs. 94.82 μm, P=0.001), superior (adjusted mean 110.08 μm vs. 119.10 μm, P=0.002) and temporal (adjusted mean 63.77 μm vs. 70.36 μm, P=0.004) in PD compared to controls. The central subfoveal CT was significantly thinner in PD compared to controls (adjusted mean 271.13 μm vs. 285.10 μm, P=0.003). In PD group, there was significant weak negative correlation between the duration of PD with average RNFL thickness (r=–0.354, P=0.027), moderate negative correlation between the

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duration of PD with central subfoveal CT (r=–0.493, P=0.001), and weak negative correlation between the stage of PD with central subfoveal CT (r=–0.380, P=0.017). PD group had significant thinner average, superior and temporal RNFL thickness and CT compared to controls.

Introduction

Parkinson disease (PD) is a complex disease and the second most common neurodegenerative disorder after Alzheimer disease (Kalia and Lang, 2015). It was first described by James Parkinson in his "Essay on the shaking palsy" in 1817 (Parkinson, 2002). It affects one to two per 1,000 of the population and affects about 1% of people over 60 years of age (Tysnes and Storstein, 2017). PD prevalence increases with age and up to five to ten folds in those over 60 years old (Simon et al., 2020). The number of PD patients worldwide doubled from 2.5 million in 1990 to 6.1 million in 2016, and this number is estimated to double again to 12.9 million by 2040 (Dorsey, 2018; Dorsey and Bloem, 2018). The Malaysian Parkinson's Disease Association estimated about 15,000 to 20,000 PD patients in Malaysia (Bexci and Subramani, 2018).

The pathogenesis of PD is degeneration of dopaminergic neurons in substantia nigra and loss of their axons in the nigrostriatal pathway, with presence of α-synuclein containing Lewy bodies as the pathological feature (MacMahon et al., 2021). PD is primarily a motor syndrome with four cardinal signs of resting tremor, rigidity, bradykinesia, and postural instability (Jankovic, 2008). The diagnosis of PD is based on the motor symptoms and can be further categorised into Hoehn and Yahr severity staging based on the sides of involvement and severity of motor symptoms.

On the other hand, non-motor symptoms are frequently present and may even be the dominating features, including sleeping disorders, autonomic disorders, psychiatric disorders, cognitive impairment, and sensory disorders (Jankovic, 2008; Postuma et al., 2015). Visual symptoms are also part of the non-motor manifestations, including reduced contrast sensitivity, reduced colour vision, convergence insufficiency, and reading difficulty (Nowacka et al., 2014). Abd Hamid et al. (2021) observed a statistically significant reduction of visual acuity in PD patients compared to normal controls. Dopamine dysfunction in PD will affect the retina as dopaminergic amacrine cells and specific types of dopamine receptors such as D1R, D4R and D2 are found in the retina. The pathognomonic α-synuclein containing Lewy bodies are also found in the retina (Indrieri et al., 2020).

Multiple studies have been done to measure the changes in the retina in PD but have shown different results. The studies that measured the retinal nerve fibre layer (RNFL) thickness in PD patients reported contrasting findings. A meta-analysis of 32 studies on RNFL thickness in PD patients observed that most of those studies demonstrated significant thinning in certain parts of RNFL in PD group compared to normal controls, including studies by Garcia-Martin et al. (2014b), Kaur et al. (2015),

Moschos and Chatziralli (2018), Huang et al. (2021), and etc. In contrast, Tsironi et al. (2012) and Nowacka et al. (2015) did not observe any significant differences of RNFL thickness in PD group and normal controls.

Meanwhile, the choroid is the middle ocular vascular layer in between the outer sclera and inner retina. It plays an important role in the oxygenation and nutrition of the inner retina, thermal regulation of the retina, elimination of retinal waste material, and secretion of growth factors (Nickla and Wallman, 2010). Thus, the retinal functions are critically dependent on both the structural and functional health of the choroid. It has been found that the choroid is affected in many ocular diseases including Alzheimer disease, a common disorder that has been categorised together with PD under neurodegenerative disease (Bayhan et al., 2015). With the development of technology, optical coherence tomography (OCT) allows a non-invasive *in vivo* imaging of all retinal layers and choroid. Spectral-domain OCT (SD-OCT) such as Cirrus HD-OCT 5000 has been shown to be used to measure choroidal thickness (CT) (Manjunath et al., 2010).

To date, the few available studies on CT in PD have shown contrasting results. Moschos and Chatziralli (2018) and Eraslan et al. (2016) reported significant reduction of CT in PD, but Oktem et al. (2019) demonstrated the opposite finding with significant increment of CT in PD. Meanwhile, Robbins et al. (2021) did not observe any significant difference of CT in PD and normal controls.

Currently, PD is a clinical diagnosis and there is no test to make a conclusive diagnosis (Lim et al., 2012). This study is to evaluate the changes in RNFL thickness and CT in PD patients compared to normal controls. Measurement of RNFL thickness and CT might provide potential non-invasive parameters to support the diagnosis of PD and monitor the disease progression.

Material and Methods

This comparative cross-sectional study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (USM/JEPeM/20100507) and was conducted in accordance with the Declaration of Helsinki for human research.

Selection of patients

Recruitment of PD patients was conducted in the Neurology Clinic, Hospital Universiti Sains Malaysia. The sample size was calculated by PS (Power and Sample) Software version 3.1.6 by referring to studies by Satue et al. (2013) and Moschos and Chatziralli (2018). A total of 39 PD patients were recruited. Those patients were known cases of PD on treatment that met the diagnostic criteria of PD and were under Neurology Clinic follow-up. Only those who were able to communicate and undergo examinations and tests were selected. The control group consists of 39 individuals aged 50 years and older who presented to the Ophthalmology Clinic, Hospital Universiti Sains Malaysia. Only PD patients and control subjects without impaired media opacity including corneal scar, significant cataract, and

vitreous opacity that affect the quality of OCT images were included in this study. Subjects who had pre-existing optic neuropathy, retinopathy, maculopathy, history of trauma or previous ocular surgery, and systemic disease such as cerebral vascular accident, intracranial lesion, neurological and demyelinating diseases were excluded. All participants who consented to take part in the study underwent visual acuity assessment, thorough ocular examinations, and fundus evaluation with slit lamp biomicroscope (Topcon Corp, Japan). Intraocular pressure measurement was performed to rule out ocular pathology such as glaucoma or ocular hypertension, which would have precluded participation in the study. All participants were then subjected to OCT (Zeiss Cirrus HD-OCT 5000) examination for RNFL thickness and CT for the right eye.

Optical coherence tomography (OCT)

OCT examinations for RNFL thickness and CT of the right eye were performed using the Zeiss Cirrus HD-OCT 5000 machine. Both tests were performed by a single well-trained operator (optometrist). Only the test or repeated test that yielded a signal strength of ≥ 6/10 was taken for interpretations to ensure the accuracy of the results. Measurements of the average, superior, inferior, nasal, and temporal RNFL thickness will be automatically generated by the machine. As for the measurement of CT, the setting of the OCT would be changed to HD 1-line 100× beforehand, then the foveal centre would be focused, and a single high-definition raster scan would be generated. The central subfoveal CT was then carefully measured manually using the Cirrus linear measurement tool from the outer portion of the hyperreflective line corresponding to the retinal pigment epithelium to the inner surface of the sclera.

Statistical analysis

Data analysis was performed using the IBM SPSS statistics version 27.0 (IBM Corp., Armonk, Chicago, IL, USA). Descriptive analysis was used for the mean values and standard deviation (SD). For demographic data, they will be tested for comparison of age, race and gender. The Student's *t*-test and Pearson's chi-square test were used to analyse the demographic data. Independent *t*-test was used to compare

Table 1 – Evans strength of correlation

Coefficient value (r)	Strength of correlation
0.00–0.19	very weak
0.20–0.39	weak
0.40–0.59	moderate
0.60–0.79	strong
0.80–1.00	very strong

the means of RNFL thickness and central subfoveal CT. All P-values of < 0.05 were considered statistically significant. Analysis of covariance (ANCOVA) was used to control potential confounding factors, namely age, gender and underlying medical illness. Pearson correlation analysis was used to determine the correlation coefficient (r) of the association of the duration and stage of PD with the average RNFL thickness and CT. General guidelines for assigning strength of correlation by Evans (1996) will be used (Table 1). A value > 0 indicates a positive association whereas a value < 0 indicates a negative association. P-value < 0.05 was considered as statistically significant.

Results

The distribution of demographic data is shown in Table 2. There was a total of 78 participants, which were comprised of 39 PD patients and 39 controls. Among them, 51 were male, while 27 were female. PD group comprised of 27 males and 12 females, while the control group had 24 males and 15 females. The age of the participants ranged from 50 to 73 years old, with a mean age of 62.5 ± 5.2 years for PD patients and 63.4 ± 6.5 years for controls. PD and control groups were gender and age matched as the discrepancy was not statistically significant (P=0.475 for gender, P=0.519 for age). 74 of the participants were Malay and 4 were Chinese. Among the 39 PD patients, the mean duration of PD was 4.7 years ± 1.6 while the mean stage of PD was 2.9 ± 0.6.

Table 2 – Demographic data of PD patients and controls

	PD (n=39)	Controls (n=39)	P-value*
Mean age (year) – mean (SD)	62.5 (5.2)	63.4 (6.5)	0.519[a]
Gender – n (%)			0.475[b]
Male	27 (69.2)	24 (61.5)	
Female	12 (30.8)	15 (38.5)	
Race – n (%)			0.040[b]
Malay	39 (100.0)	35 (89.7%)	
Chinese	0 (0.0)	4 (10.3%)	
Medical illness – n (%)			0.346[b]
No medical illness	10 (25.6)	13 (33.3)	
Diabetes mellitus	8 (20.5)	9 (23.1)	
Hypertension	14 (35.9)	7 (17.9)	
Multiple medical illness	7 (17.9)	10 (25.6)	
Mean duration of PD (year) – mean (SD)	4.7 (1.6)	–	–
Mean stage of PD – mean (SD)	2.9 (0.6)	–	–

[a]independent *t*-test; [b]Pearson's chi-square test; *P<0.05 significant; PD – Parkinson disease; SD – standard deviation

Table 3 – Comparison of mean RNFL thickness of right eye between PD patients and controls

RNFL	PD (n=39)		Control (n=39)		Adj. mean differences (95% CI)	df	P-value*
	mean (SD)[a]	adj. mean[b] (95% CI)	mean (SD)[a]	adj. mean[b] (95% CI)			
Average (μm)	89.36 (9.00)	88.87 (86.36, 91.38)	94.33 (11.00)	94.82 (92.32, 97.33)	–5.95 (–9.51, –2.40)	73.1	**0.001**
Superior (μm)	110.56 (12.88)	110.08 (106.07, 114.09)	118.62 (13.82)	119.10 (115.09, 123.11)	–9.03 (–14.72, –3.34)	75.6	**0.002**
Inferior (μm)	116.77 (10.52)	115.81 (111.38, 120.25)	120.44 (25.83)	121.39 (116.96, 125.83)	–5.58 (–11.87, 0.72)	50.2	0.081
Temporal (μm)	64.41 (11.84)	63.77 (60.64, 66.90)	69.72 (11.73)	70.36 (67.23, 73.49)	–6.59 (–11.03, –2.15)	76.0	**0.004**
Nasal (μm)	65.95 (14.41)	66.05 (62.42, 69.68)	68.72 (10.51)	68.62 (64.99, 72.25)	–2.57 (–7.72, 2.58)	69.5	0.323

[a]independent *t*-test; [b]adjusted mean using ANCOVA after controlling for age, gender and underlying medical illness; *P<0.05 significant; RNFL – retinal nerve fibre layer; PD – Parkinson disease; CI – confidence interval; SD – standard deviation; adj. – adjusted

The mean RNFL thickness of PD patients and controls is reflected in Table 3. We observed a lower mean RNFL thickness in the average and all four quadrants in the PD group when compared to the control group. After controlling for potential confounders which are age, gender, and underlying medical illness using ANCOVA, there was a decrease in the mean RNFL thickness in the average and all four quadrants. However, a statistically significant reduction was only noticed in the average (adjusted mean 88.87 μm; 95% CI [confidence interval] = 86.36, 91.38 vs. 94.82 μm; 95% CI = 92.32, 97.33; P=0.001), superior (adjusted mean 110.08 μm; 95% CI = 106.07, 114.09 vs. 119.10 μm; 95% CI = 115.09, 123.11; P=0.002) and temporal (adjusted mean 63.77 μm; 95% CI = 60.64, 66.90 vs. 70.36 μm; 95% CI = 67.23, 73.49; P=0.004) quadrants but not in the inferior and nasal quadrants in the PD group as compared to the controls.

The comparison of the mean central subfoveal CT between PD patients and controls is shown in Table 4. The central subfoveal CT was thinner in PD group as compared to the controls. After controlling potential confounders which are age, gender and underlying medical illness using ANCOVA, there was a significant reduction in the central subfoveal CT in PD group as compared to the controls (adjusted mean 271.13 μm; 95% CI = 264.67, 277.60 vs. 285.10 μm; 95% CI = 278.63, 291.56; P=0.003).

Table 5 shows the correlation between the duration of PD with average RNFL thickness in PD patients. There was a statistically significant weak negative correlation between them (r=–0.354, P=0.027).

Table 4 – Comparison of mean central subfoveal CT of right eye between PD patients and controls

CT	PD (n=39)		Control (n=39)		Adj. mean differences (95% CI)	df	P-value*
	mean (SD)[a]	adj. mean[b] (95% CI)	mean (SD)[a]	adj. mean[b] (95% CI)			
Central subfoveal CT (μm)	272.13 (14.82)	271.13 (264.67, 277.60)	284.13 (27.99)	285.10 (278.63, 291.56)	–13.96 (–23.13, –4.79)	57.8	**0.003**

[a]independent *t*-test; [b]adjusted mean using ANCOVA after controlling for age, gender and underlying medical illness; *P<0.05 significant; CT – choroidal thickness; PD – Parkinson disease; CI – confidence interval; SD – standard deviation; adj. – adjusted

Table 5 – Correlation between duration of PD with average RNFL thickness in PD patients

Parameter	Pearson's correlation (r)	P-value*
Average RNFL thickness (μm)	–0.354	**0.027**

*P<0.05 significant; RNFL – retinal nerve fibre layer; PD – Parkinson disease

Table 6 – Correlation between stage of PD with average RNFL thickness in PD patients

Parameter	Pearson's correlation (r)	P-value*
Average RNFL thickness (μm)	–0.253	0.120

*P<0.05 significant; RNFL – retinal nerve fibre layer; PD – Parkinson disease

Table 7 – Correlation between duration of PD with central subfoveal CT in PD patients

Parameter	Pearson's correlation (r)	P-value*
Central subfoveal CT (μm)	–0.493	**0.001**

*P<0.05 significant; PD – Parkinson disease; CT – choroidal thickness

Table 8 – Correlation between stage of PD with central subfoveal CT in PD patients

Parameter	Pearson's correlation (r)	P-value*
Central subfoveal CT (μm)	–0.380	**0.017**

*P<0.05 significant; PD – Parkinson disease; CT – choroidal thickness

Table 6 shows the correlation between the stage of PD with average RNFL thickness in PD patients. There was a weak negative correlation, however it was not statistically significant (r=–0.253, P=0.120).

Table 7 shows the correlation between the duration of PD with central subfoveal CT in PD patients. There was a statistically significant moderate negative correlation between them (r=–0.493, P=0.001).

Table 8 shows the correlation between the stage of PD with central subfoveal CT in PD patients. There was a statistically significant weak negative correlation between them (r=–0.380, P=0.017).

Discussion

PD is characterised by motor symptoms of tremor, rigidity, bradykinesia, and postural instability, however, visual disturbance such as impairment of visual acuity, reduction of contrast sensitivity and reading difficulty are also frequently reported among PD patients (Bodis-Wollner, 2013; Weil et al., 2016). Studies have shown that the prevalence of having at least one visual symptom among PD patients was 77.3% to 82% (Urwyler et al., 2014; Borm et al., 2020). The pathogenesis of PD is the loss of dopaminergic neurons in the substantia nigra and depletion of their axons in the nigrostriatal pathway, with the presence of Lewy bodies as the pathognomonic histopathological sign (MacMahon et al., 2021). Dopaminergic cells and dopamine receptors are also found in the retina (Indrieri et al., 2020). α-synuclein is a protein that forms a main part of Lewy bodies, and it is harmful to the retina (Indrieri et al., 2020; MacMahon et al., 2021). An autopsy study has found that this protein accumulated in the retina of all nine PD patients and none of the six controls (Ortuño-Lizarán et al., 2018). On the other hand, the choroid which forms the ocular vascular layer has been found to be abnormal in many ocular diseases, including another common neurodegenerative disorder, the Alzheimer disease (Bayhan et al., 2015). However, to date, the few available studies on the CT in PD patients are contradictory (Eraslan et al., 2016; Moschos and Chatziralli, 2018; Satue et al., 2018; Brown et al., 2021; Robbins et al., 2021).

The demographic profile of our study showed the mean age of our PD patients as 62.5 ± 5.2 years and 63.4 ± 6.5 years for controls. Both groups were age-matched as there was no significant difference between their mean ages. Most of the participants in this study were Malays, as our study was done in Kelantan. According to the Department of Statistics Malaysia, 96.0% of the estimated population of Kelantan in 2021 was Malays (Department of Statistics Malaysia, 2021). No significant discrepancies in gender and underlying medical illness between PD patients and controls. Within the PD group, the number of men was over two times that of women (male = 27, female = 12), which was higher than the PD prevalence with a male to female ratio of 1.48 reported in a meta-analysis (Moisan et al., 2016). This disparity occurred by chance, there was no bias in selection. Age, gender and underlying medical illness had been included in the analysis by ANCOVA as potential confounding factors.

In terms of RNFL thickness, our result showed reduction in all four quadrants in PD patients as compared to controls. The reduction was statistically significant in the average RNFL thickness with a mean difference of –5.95 μm (P=0.001), superior RNFL quadrant with a mean difference of –9.03 μm (P=0.002) and temporal RFNL quadrant with a mean difference of –6.59 (P=0.004) after controlling the potential confounding factors. Our result is comparable to a meta-analysis of 32 studies on RNFL in PD patients, where most of those studies reported thinning in the average and some RNFL quadrants, and only three studies showed reduction of the average and all four RNFL quadrants. After pooling and re-analysing the data, the pooled mean difference revealed reduction in the average and all four RNFL quadrants in PD patients compared to controls, with the amplitude of reduction in the inferior more than the superior, and more in the temporal than the nasal quadrant. By comparing to a meta-analysis of 24 studies on Alzheimer disease which reported RNFL thinning in an opposing order (greater reduction in superior than inferior and nasal than temporal), they postulated the possibility of using this pattern of RNFL thinning to differentiate PD from other neurodegenerative disorders (Chan et al., 2019; Huang et al., 2021).

Another meta-analysis published in 2014 that included some earlier studies (13 studies in total, five overlapped with the studies in the formerly mentioned meta-analysis) also showed similar results with an identical pattern of RNFL thinning after analysing the pooled data, namely the reduction was more in the inferior than the superior, and more in the temporal than the nasal quadrant. They attributed this greater reduction in the temporal quadrant to involvement of the papillomacular bundle, which is typically susceptible to neurodegenerative diseases (Yu et al., 2019). To date, most of the available studies demonstrated RNFL thinning in PD patients. Garcia-Martin et al. (2014b) reported significant thinning in the average, superior, inferior, and temporal quadrants. Studies by Moschos and Chatziralli (2018) and Kaur et al. (2015) both found significant thinning in average, superior and temporal quadrants. A more recent study by Abd Hamid et al. (2021) showed significant thinning in average, superior and inferior quadrants. Satue et al. (2013) found significant thinning only in the inferior quadrant. The study by Pilat et al. (2016) is one of the few that reported significant thinning in average and all four quadrants. These studies postulated that progressive retinal dopaminergic cell loss causes atrophy of their axons, and subsequently leading to corresponding RNFL thinning. Garcia-Martin et al. (2014a) demonstrated a more prominent mean RNFL thinning correlated with a thinner mean ganglion cell layer. On the other hand, Tsironi et al. (2012) found no significant difference between PD patients and controls. They attributed this dissimilarity to differences in study population, sample size, disease stage and imaging device (Tsironi et al., 2012). Nowacka et al. (2015) also reported similar results.

Our results showed a significant weak negative correlation between the duration of PD with average RNFL thickness in PD patients (r=–0.354, P=0.027). There was

also a weak negative correlation between the Hoehn and Yahr severity stage of PD with the average RNFL thickness but not statistically significant (r=–0.253, P=0.120). Our results are comparable to study by Sengupta et al. (2018) that found a significant correlation between disease duration and RNFL thickness and attributed this finding to the simultaneous neurodegeneration in both the brain and retina. However, like our results, they also did not observe any significant correlation between severity and RNFL thickness. They related this discrepancy to their small sample size of 34 PD patients (Sengupta et al., 2018). Similarly, Atum and Demiryürek (2021) also did not find any significant correlation between Hoehn and Yahr severity staging average RNFL. Contrary to our result, Garcia-Martin et al. (2014b) reported a significant negative correlation between the severity stage of PD with certain parts of RNFL thickness but not with the duration of disease. Meanwhile, Cubo et al. (2014) did not find any significant association between severity stage and RNFL thickness in PD patients. Anyway, several studies have demonstrated significant reduction of RNFL thickness when they followed up PD patients over two, three and five years respectively to signify the progressive anatomical changes together with disease progression (Satue et al., 2017; Atum and Demiryürek, 2021; Kamata et al., 2022).

In terms of central subfoveal CT, our results showed statistically significant reduction in PD patients compared to controls, with a mean difference of –13.96 μm (P=0.003) after controlling the potential confounding factors. Our result is consistent with the study by Moschos and Chatziralli (2018) that attributed their finding to the synergistic outcome of vascular abnormalities and neurodegeneration that might be potentially responsible for clinical risk and disease progression. Eraslan et al. (2016) also revealed similar result and attributed it to the disease-related hypoperfusion due to choroidal blood flow abnormalities, the hypotensive effect of dopamine agonist and reduction of metabolic activity secondary to retinal ganglion cell loss. Kamata et al. (2022) observed a comparable result and related it to autonomic dysfunction in PD leading to the reduction of choroidal blood flow. In contrast to our study, Oktem et al. (2019) demonstrated a significant thickening of central subfoveal CT in PD patients compared to controls and related it to the increment of perivascular connective tissue and enlargement of perivascular spaces in PD patients. Satue et al. (2018) also observed thicker central subfoveal choroid in PD patients, but not statistically significant. Meanwhile, Robbins et al. (2021) did not find any significant changes in the central subfoveal CT between PD patients and controls. However, they observed significant differences between the two groups in terms of other choroidal parameters, including total choroidal area, luminal area, and choroidal vascularity index. They postulated choroidal vascularity abnormalities occurred without changes in CT (Robbins et al., 2021).

The correlation analysis between the duration of PD and central subfoveal CT in PD patients revealed a statistically significant moderate negative correlation between them (r=–0.493, P=0.001). Similarly, there was also a statistically significant weak negative correlation between the Hoehn and Yahr severity stage of PD and central

subfoveal CT in PD patients (r=–0.380, P=0.017). Eraslan et al. (2016) observed a significant negative correlation between duration of PD with CT. However, there was no correlation between the severity and CT (Eraslan et al., 2016). Contrary to our study, Oktem et al. (2019) found no correlation between the duration of PD and CT.

Limitations and recommendations

While conducting our study, we identified several limitations. First, this was a cross-sectional study and there was no follow-up to compare the changes in all the studied parameters over time. We suggest a longitudinal study with repeated OCT on the same patients to be done in the future to evaluate the possible progress in RNFL thickness and CT in the long term, probably over a minimum of two years, as Atum and Demiryürek (2021) who followed up subjects every six months only started to observe a significant reduction in RNFL thickness at two years duration.

Second, our patient selection was limited by the motor dysfunction in some PD patients. Hoehn and Yahr staging classifies PD into five severity stages. However, we were only able to include those in stage two to four. The characteristic motor symptoms in PD had hindered us from capturing a proper OCT image in some PD patients, especially those in stage five, as they could not maintain the steady positioning and focusing that were very much required while performing the OCT. We suggest including patients with severe stages to be included in future studies, probably with more advanced and patient-friendly devices to better study the changes in RNFL thickness and CT.

Third, we conducted our study in only one centre in Kelantan, with Malays contributing to 96.0% of the local population. This might cause population bias and affect the accuracy of the results. We suggest a multi-centred study with a larger sample size and include various ethics to better study the effect of PD on the studied parameters.

Conclusion

Our study showed that PD patients have a statistically significant reduction of RNFL thickness in the average, superior, and temporal quadrants when compared to controls. There was also a significant reduction in central subfoveal CT in PD patients compared to controls. Thus, we believe RNFL thickness and CT might be useful non-invasive parameters to help to support the diagnosis of PD and monitor the disease progression.

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A Practical and Applicable New Index as an Indicator of Inflammation in the Diagnosis of Erectile Dysfunction: C-reactive Protein-to-Albumin Ratio

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Abstract: Current evidence suggests that the significant underlying pathophysiological mechanism in erectile dysfunction (ED) is endothelial dysfunction. It is clinically essential to monitor ED because inflammatory processes lead to dysfunctional endothelium and the progression of atherosclerosis. The current retrospective analysis assessed the registers of 90 patients with ED complaints (ED group) and 78 healthy people without ED complaints (control group) who were being managed at the urology units of the surgical outpatient clinic. The international index of erectile function-5 (IIEF-5) evaluated the ED. C-reactive protein (CRP)/albumin ratio (CAR) value was determined by manually dividing serum CRP value by the albumin value in patients whose CRP value was between 0 and 5 mg/l. The average CAR was 0.45 ± 0.37 (ED group) versus 0.22 ± 0.1 in the control group (p=0.0001). IIEF-5 results were negatively correlated with CAR values (r=−0.299; p=0.0001). The strongest cut-off of CAR for predicting ED was 0.025, with 81.8% sensitivity and 75% specificity (p=0.0001). The ED group showed higher levels of CAR and CRP than the control group. CAR can be used as a practical, easy-to-calculate, and cost-effective index in diagnosing ED patients.

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Introduction

Erectile dysfunction (ED) denotes that a person cannot provide full penile erection or maintain it for fulfilling sexual intercourse (Sooriyamoorthy and Leslie, 2022). ED was generally considered a psychogenic disorder in the past; however, it is now known that more than 80% of the cases have an organic etiology (Yafi et al., 2016). The causes of ED can be multifactorial. However, vascular causes are among the top pathogenic factors because the penis has a significant vascular bed. The primary pathophysiological mechanism underlying vasculogenic ED is endothelial dysfunction (Vlachopoulos et al., 2008).

Acute phase reactants (APR) are inflammatory indicators that show substantial variations in serum levels in cases of infection, trauma, inflammatory diseases, and malignancy. The most important positive APR is C-reactive protein (CRP); the most significant negative APR is albumin.

Endothelial cells, which constitute the inner lining of penile arteries, regulate the vascular tone and penile blood flow according to stimuli. Nitric oxide (NO) released from endothelial cells is a vital neuromodulator for the standard induction and maintenance of erections. With the effect of NO, relaxation occurs in the corpus cavernosum and penile vascular smooth muscle. Thus, there is an increase in blood flow from the systemic circulation to the penis. In vasculogenic ED, nitric NO production from endothelial cells is reduced; thus, endothelial cells' regulatory role is inhibited. Inflammatory processes result in endothelial dysfunction and the progression of atherosclerosis (Devaraj et al., 2004; Bisoendial et al., 2007). Because endothelial dysfunction also causes atherosclerosis, the concomitance of ED and systemic atherosclerosis is common (Vlachopoulos et al., 2008). Therefore, CRP – one of the inflammatory markers – may help determine the prognosis of ED and cardiovascular diseases and monitor the treatment (Li et al., 2019; Rencuzogullari et al., 2019). Another molecule also synthesized by the liver, albumin can preserve the microvasculature and lower the rise in vascular permeability by its anti-inflammatory, antioxidant, and anti-apoptotic effects (Vincent et al., 2014). In the literature, low serum albumin levels have been accepted as a biomarker for predicting situations like acute coronary syndrome, heart failure, kidney failure, and stroke (Chien et al., 2017). These two molecules are actively involved in diagnosing and following vascular pathologies. Considering that the incidence of cardiovascular diseases is high among patients with ED, these two molecules can be used as early indicators of ED (Zhao et al., 2019). The current research evaluates the diagnostic effectiveness of albumin and CRP, which are considered inflammation markers in ED, and a new index, CRP/albumin ratio (CAR), among patients with ED.

Material and Methods

Study design

This cross-sectional retrospective research was carried out by evaluating registers of healthy individuals and patients with ED complaints who were being managed at

the urology units of the surgical outpatient clinic between July 2019 and May 2022. Ninety participants with ED complaints and 78 healthy participants without ED complaints who met the eligibility criteria were enrolled.

Participation criteria

The current study included sexually active and married patients aged between 40 and 70 and with the international index of erectile function-5 (IIEF-5) of less than 22 score. Individuals having a psychiatric disorder, history of penile or pelvic trauma or surgery, congestive cardiac condition, endocrine disruption other than diabetes, chronic liver/kidney disease, current inflammation and/or antibiotic drug use, patients with CRP > 10 mg/l, history of neurological disorders, high prostate-specific antigen (PSA) levels suggesting the need for prostate biopsy, urogenital system cancer, those using drugs that cause iatrogenic ED, and individuals below 34 years old or over 72 years old were excluded. As part of routine care, a comprehensive physical examination was performed for each patient presenting to the urology outpatient clinic, and a detailed anamnesis was taken from all patients and recorded. Height, weight, and waist circumference measurements were obtained for each patient and body mass index (BMI) (kg/m^2).

In the present study, a history of previous surgery, medication use, and smoking and alcohol use were recorded for each participant. A fasting sample was collected from each participant between 8 and 10 a.m. Fasting blood glucose, glycosylated hemoglobin (HbA1c), complete blood count, sedimentation, CRP, vitamin-B12, vitamin D, folate, prolactin, follicle-stimulating hormone, luteinizing hormone, total testosterone, oestradiol, insulin, thyroids, total PSA and free PSA levels were measured. Albumin levels ranged from 35 to 55 g/l, and CRP was between 0 and 5 mg/l. CAR value was estimated by dividing CRP by albumin. HbA1c level of ≥ 6.5 or currently on pharmacological treatment for diabetes defined diabetes mellitus (DM). Dyslipidaemia was described as having fasting blood cholesterol ≥ 200, high-density lipoprotein (HDL) < 40 mg/dl, and low-density lipoprotein (LDL) ≥ 130 mg/dl or being currently on pharmacological treatment for dyslipidaemia.

The IIEF-5 form with a list of five questions was used in the current study. Each question is scored from 1 to 5 in this form, with 5 being the best response. A summary score of 5–7 points indicate severe ED, 8–11 points indicate moderate ED, 12–16 points indicate moderate-mild ED, 17–21 points indicate mild ED, and 22–25 points indicate no ED.

Statistical analysis

Power calculation was carried out with G-Power 3.05 program for Windows. A sample of a reference study was considered for the power analysis. When the data about patient and control groups were measured, the investigation had a power level of 83 percent (post hoc power analysis). The Kolmogorov-Smirnov method determined the normality. The variance was analysed using the Mann-Whitney U

and independent *t*-test. Pearson's chi-squared test compared categorical variables, whereas Spearman's correlation investigated the correlations. Descriptive statistics were reported using mean and standard deviation for numerical data and frequency and proportion for the categorical data. A p-value below 0.05 was identified as the criterion for significance. SPSS v24.0 (IBM Co., Ohio, USA) was utilized for the analysis.

Results

The study included 168 men, 90 sexually active, and 78 healthy controls. The characteristics of the study participants did not differ significantly in their age, weight, height, and body mass index (p>0.05). In addition, of the study subjects did not differ in smoking (p=0.344), alcohol abuse (p=0.103), and comorbid diseases such as elevated blood pressure and coronary-artery disease (p=0.156 and p=0.099). Patients with similar demographic data were selected, while others were excluded from the study.

Mean serum CRP concentrations were more significant in the patient group (3.41 ± 1.98 mg/l) compared to the control group (1.61 ± 1.05 mg/l) (p=0.001). As seen in Figure 1, the CAR was 0.45 ± 0.37 in the patients and 0.22 ± 0.1 in the control (p=0.0001). The albumin levels were reduced in the ED group (39.4 ± 15.3 g/l) than those in the control group (45.5 ± 3.6 g/l) (p=0.008). In addition, the groups did not differ in laboratory parameters (p>0.05). Pearson correlation revealed that IIEF-5 was not correlated with albumin (p=0.133) but indicated a negative correlation with CRP (r=−0.335; p=0.0001) and CAR (r=−0.299; p=0.0001). As seen in Figure 2, the cut-off of CAR for predicting ED was 0.025, which had a sensitivity of 81.8% and a specificity of 75% (ROC

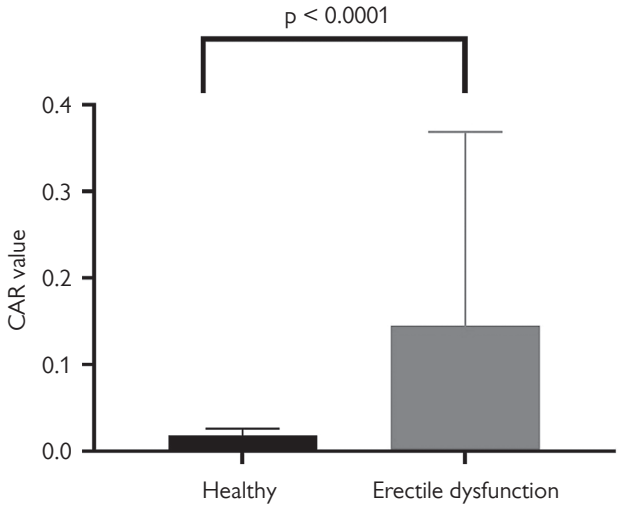

Figure 1 – C-reactive protein/albumin ratio (CAR) for comparison of groups.

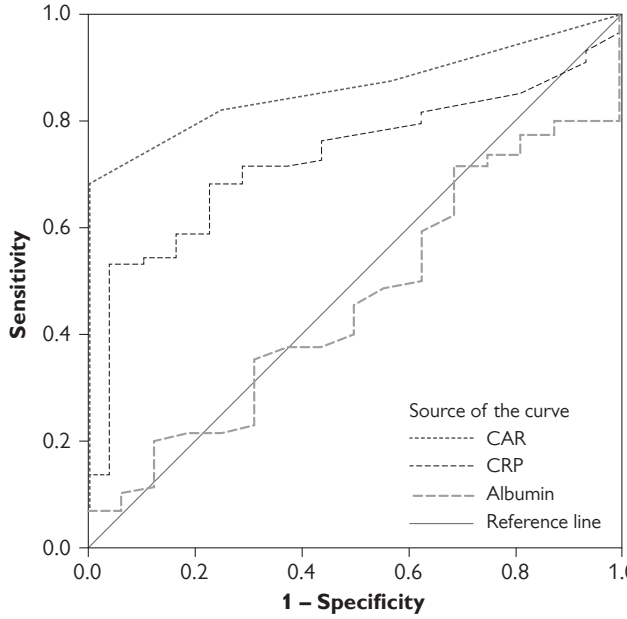

Figure 2 – The receiver operating characteristic (ROC) curve of C-reactive protein/albumin ratio (CAR) for predicting erectile dysfunction.

[receiver operating characteristic]: 0.862, 95% CI [confidence interval]: 0.801–0.923, p=0.0001). While CRP had a substantial potential for diagnosing ED in the ROC curve, the same was not valid for albumin (Table 1).

Discussion

It is necessary to distinguish whether the etiology of ED is primarily a psychological or an organic cause. Although the etiology of ED may include various factors, diagnostic tools are crucial for these patients (Sooriyamoorthy and Leslie, 2022). In this term, CAR can be used as a practical device due to its easy-to-calculate and cost-effective index in diagnosing ED.

For elderly patients, the leading cause of ED is organic diseases due to the vascular failure of penile arteries and veins caused by atherosclerosis (Stuckey et al., 2007). Vascular inflammation mediates a crucial function in the continuation

Table 1 – The ROC analysis for predicting erectile dysfunction

Variables	AUC	SE	P-value	95% CI	
				lower	upper
CAR	0.862	0.031	0.0001	0.801	0.923
CRP	0.729	0.043	0.0001	0.644	0.814
ALB	0.459	0.050	0.4280	0.361	0.557

CRP – C-reactive protein; CAR – CRP/albumin ratio; ALB – albumin; AUC – area under curve; CI – confidence interval; SE – standard error; ROC – receiver operating characteristic

and manifestation of atherosclerosis and endothelial dysfunction (Devaraj et al., 2004; Bisoendial et al., 2007; Vlachopoulos et al., 2008). It has been shown that atherosclerosis is not caused by passive vascular damage induced by the penetration of lipids but by an aggressive inflammatory mechanism (Guay, 2007). Inflammatory diseases play a role in initiating and developing atherosclerosis and cause a stable atherosclerotic plaque to become an unstable lesion (Ross, 1993). ED shares the same modifiable risk factors as cardiovascular risk factors, including hypertension, diabetes, dyslipidemia, smoking, obesity, metabolic syndrome, sedentary lifestyle (Sangiorgi et al., 2021).

Albumin is the most common protein in the plasma of mammals, which is synthesized only by the liver at a rate of 9–14 g per day in healthy persons (Vincent et al., 2014; Czub et al., 2018). Albumin has begun to be used to evaluate the mortality and morbidity of some diseases. The most critical negative acute phase reactant is albumin, and serum albumin levels decrease in the presence of chronic inflammatory conditions (Gulhar et al., 2022). Hypoalbuminemia is a robust prognostic marker in many disease states and in several processes. Apart from the usual prognostic markers in patients with cardiovascular disease, low serum albumin has independently emerged as a robust prognostic parameter in these patients (Arques, 2020). Previous studies found a correlation between hypoalbuminemia and ED in chronic viral liver diseases and in patients undergoing haemodialysis and continuous peritoneal dialysis (Toda et al., 2005; Martín-Díaz et al., 2006; Kim et al., 2015; Costa et al., 2018; Kusumawardhani et al., 2021). Few studies in the literature compare ED patients without chronic liver disease or chronic renal failure with healthy patients (Demir and Barlas, 2021; Balta and Mikhailidis, 2022). A positive correlation was found between hypoalbuminemia and ED in a study conducted on individuals with chronic kidney disease. This study showed that subjects with albumin lower than 3.5 g/dl had a higher frequency of ED (Costa et al., 2018). Another study conducted on subjects with chronic viral liver disease found a correlation between IIEF-5 scores and serum albumin levels; albumin was defined as an independent predictor of ED (Toda et al., 2005). In subjects undertaking continuous ambulatory peritoneal dialysis, the risk of experiencing sexual dysfunction, after adjusting for age, was 9.3 times higher in subjects having an albumin of < 3.5 g/dl compared with patients with an albumin level of 3.5–5 g/dl (Kusumawardhani et al., 2021). In a study conducted among individuals having liver disease due to chronic hepatitis B, serum albumin level was 4.1 ± 0.5 g/dl in patients with ED and 4.4 ± 0.3 g/dl in patients without ED. High albumin lowered the risk of ED occurrence (Kim et al., 2015). Demir and Barlas (2021) performed a comparative analysis of individuals with ED versus those who do not have ED and showed that albumin levels were considerably lower among individuals having ED versus the control group. However, the difference between the groups did not reach statistical significance (Demir and Barlas, 2021). In our study, mean albumin values were significantly lower in the ED group (39.4 ± 15.3 g/l) compared to the control group (45.5 ± 3.6 g/l) ($p=0.008$).

Pearson correlation analysis also showed that IIEF-5 scores were not correlated with albumin levels.

CRP is an APR synthesized by the liver following the release of proinflammatory cytokines, like IL-6, and activates the complement system by binding to the surface of tissue debris and bacteria, which induces phagocytosis. It is used as a sensitive marker to monitor the temporality of inflammatory conditions. Because it is an inflammatory component of atherosclerosis, CRP can be used to assess cardiovascular risk when analysed using more sensitive assays – high-sensitivity CRP (hs-CRP) tests – for measuring deficient CRP concentrations (Moutachakkir et al., 2017; Herwald and Egesten, 2021). CRP, a marker of fibrinolytic activity and atherosclerosis, is a known predictor for myocardial infarction and stroke in healthy individuals. Some studies have shown that CRP and hs-CRP can be valuable inflammatory markers for assessing ED risk (Zambon et al., 2010; Elzanaty et al., 2016; Shigehara et al., 2016; Li et al., 2019). In the study conducted by Demir and Barlas (2021) comparing individuals with ED and healthy subjects, the CRP was higher in those with ED than in control. Higher CRP levels were related to a higher risk of ED, and a close association was also found between CRP levels and IIEF (Demir and Barlas, 2021). In the present study, serum CRP was also markedly higher in the patient than in the control with a negative correlation of IIEF-5.

There is still a need for non-invasive parameters that can be easily measured, simple to use, inexpensive and can suggest a prognosis. CAR has recently been put into use, and an increasing number of studies are being conducted on it. It has been used as a prognostic and mortality indicator for cardiovascular diseases, gastric, pancreatic, and hepatocellular malignancies, diabetes mellitus, patients with sepsis, and patients hospitalized in intensive care (Saito et al., 2018; Rencuzogullari et al., 2019). Limited studies are available investigating CAR and ED interplay (Demir and Barlas, 2021; Balta and Mikhailidis, 2022). Demir and Barlas (2021) revealed that CAR was significantly greater in individuals with ED compared to the controls; higher CAR values were observed to be related to a higher ED risk. The IIEF-5 results were negatively correlated with the CAR levels, i.e., as the ED worsened, CAR values also increased. In Demir and Barlas (2021) study, the serum CRP level optimal for diagnosing ED was ≥ 2.70 mg/l (sensitivity – 53.5%; specificity – 61.7%). In contrast, the optimal value of CAR for ED detection was ≥ 0.55 (sensitivity – 56.6%; specificity – 59.6%) (Demir and Barlas, 2021). In the present study, the optimal cut-off value of CAR for predicting ED was 0.025 (sensitivity – 81.8%; specificity – 75%). These findings indicate that the CAR can strongly detect ED occurrence easily and non-expensively.

Some limitations require consideration for the current research, although our strong side includes having a relatively large population-based sample. The retrospective design of the study limits the follow-up and long-term outputs. We could not remove the effects of unknown or unassessed confounders and did not classify the type of ED (i.e., vasculogenic or psychologic), which could be sources of

bias. The results apply to specific settings of Turkey and should not be generalizable to other countries or ethnic groups.

Conclusion

CAR strongly increased in the ED. These results show that by determining CAR values, inflammatory markers can be used to assess the occurrence and determine the ED level of severity ED. Further studies with larger sample sizes should further explore the relation between albumin, CRP, CAR, and ED.

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Demonstration of the Rationale for Therapeutic Drug Monitoring of Isavuconazole: A Case Report with a Lung Transplant Recipient

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Key words: Isavuconazole – Antifungals – Pharmacokinetics – Therapeutic drug monitoring – Lung transplant recipient

Abstract: Mucormycosis is a rare invasive fungal disease diagnosed in immunocompromised patients, including those with diabetes or iron overload, and in patients treated for hematological malignancies or after transplantation. Isavuconazole is a triazole antifungal effective against Mucorales with good tolerability, but with potential for relatively high interindividual variability in pharmacokinetics. This report demonstrates the case of a lung transplant recipient treated with isavuconasole that exhibits a very long elimination half-life of 159 hours, and discusses the practical implications of this finding for dosage adjustment and need for therapeutic drug monitoring.

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Introduction

Lung transplant recipients are at significant risk of invasive mycotic infection (Pappas et al., 2010). Isavuconazole, a new broad-spectrum triazole antifungal, is the most recently approved agent for the treatment of systemic fungal infections (aspergillosis and mucormycosis) with the good tolerability of long-term isavuconazole prophylaxis and treatment in lung transplant recipients (Marty et al., 2016; Monforte et al., 2022).

Isavuconazole can be administered parenterally (as an intravenous infusion) or orally (as hard capsules). Following oral administration, isavuconazol exhibits very high absolute bioavailability of 98%, allowing interchangeable use of intravenous and oral dosing. Isavuconazole is highly bound to plasma proteins (> 99%) and its steady-state volume of distribution (Vd) of 450 L indicates extensive distribution. Majority of drug is metabolized via CYP3A enzymes and subsequently via glucuronosyltransferases. Metabolites are excreted both through the urine and faeces. Only less than 1% of the dose administered is excreted renally in unchanged form (EMA, 2015).

The recommended posology consists of loading dose of 200 mg every 8 hours for the fits 48 hours followed by maintenance dose of 200 mg once daily (EMA, 2015). There is still no clear consensus on the need for routine therapeutic drug monitoring (TDM) of isavuconazole in clinical practice. While some authors found no exposure-response relationship and thus concluded that there was no evidence for TDM (Desai et al., 2017), another study identified steady-state trough level of 5 mg/l as a threshold for toxicity (Furfaro et al., 2019) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has set the clinical breakpoint at 2 mg/l for *Aspergillus fumigatus* and *Aspergillus flavus* (EUCAST, 2020).

The aim of this report is to demonstrate the case of a lung transplant recipient treated with isavuconasole that exhibits a very long elimination half-life, and to discuss the practical implications of this finding for dosage adjustment and need for TDM.

Case report

This case report was approved by the local Ethics Committee under the No. EK-873/22. Written informed consent was obtained from patient before data collection and analysis. A 70-year-old female (height 160 cm, weight 50 kg) received a bilateral lung transplantation in May 2022 for chronic obstructive pulmonary disease. During the transplantation, the right upper lobe was resected due to the oversized graft. Antithymocyte globulin and perioperative plasmapheresis were used as induction, followed by maintenance immunosuppression regimen consisting of tacrolimus, mycophenolic acid and prednisolone. Voriconazole, azithromycin, trimethoprim-sulfamethoxazole and valganciclovir were used as a prophylactic anti-infective treatment. The patient was dismissed 5 weeks after transplantation in mid-June in a good clinical condition, with physiological values of pulmonary function tests.

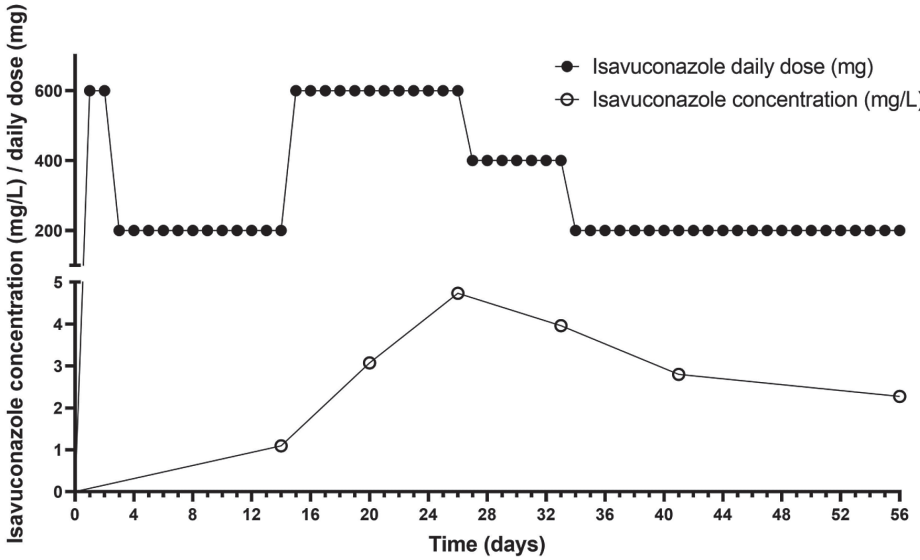

Figure 1 – Isavuconazole daily doses and plasma concentrations in the course of therapy.

In August, the patient was readmitted with severe type 1 respiratory failure with leukocytosis and elevation of C-reactive protein. Serum creatinine, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltransferase at admission were 130 μmol/l, 0.37 μkat/l, 0.22 μkat/l and 2.82 μkat/l, respectively. Thoracic CT (computed tomography) showed a newly formed decay cavity in the lower lobe of the right lung, suspicious of mycotic infection. Bronchoscopy with BAL (bronchoalveolar lavage) sampling was performed – microscopy, cultivation and PCR were positive for *Rhizopus microsporus*. Antifungal therapy with both intravenous (Ambisome®) and inhalation (Fungizone®) amphotericin B and intravenous isavuconazole (Cresemba®) was started. The duration of inhalation amphotericin B, intravenous amphotericin B and intravenous isavuconazole therapy was 31, 48 and 55 days, respectively. Subsequently, the patient continues with oral isavuconazole therapy until present day. The course of dosing and measured isavuconazole levels during intravenous therapy is shown in Figure 1. *Mucor* infection was regularly monitored by bronchoscopy at 1–3 monthly intervals, when cultivation was already negative.

The levels of isavuconazole were measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) operated in positive ion mode. The scan type used dynamic multiple reaction monitoring (MRM). We used an Agilent Technologies 1290 Infinity II LC system, including an autosampler, binary pumps and a thermostatted column compartment with 6470 Triple Quad (Agilent Technologies, Santa Clara, CA, USA). Our LC-MS/MS method in human plasma has been successfully validated.

Isavuconazole pharmacokinetic parameters in our patient were calculated based on isavukonazole dosing and its plasma concentration-time profile by maximum a posteriori estimation using the Bayesian approach within Monolix Suite software version 2021R1 (Lixoft SAS, Antony, France). One-compartmental model with first-order elimination best fits the concentration-time data. Model was parametrized in terms of Vd and clearance (CL). Initial (a priori) estimates were adopted from previous pharmacokinetic study in solid-organ transplant recipients (Vd = 338 ± 458 L, CL = 4.1 ± 2.7 L/h) (Wu et al., 2018). Elimination half-life (t1/2) was calculated as $t1/2 = \ln 2 \times Vd/CL$.

Calculated isavuconazole Vd, CL and t1/2 in our patient are 1014.9 L, 4.43 L/h and 158.8 h, respectively.

Discussion and Conclusion

The summary of product characteristics does not explicitly state the elimination half-life of isavuconazole, and pharmacokinetic data in the literature are also relatively sparse. Multiple-dose pharmacokinetic study of isavuconazole after intravenous and oral administration in healthy volunteers states mean half-life of 84.5–117 hours (Schmitt-Hoffmann et al., 2006). From the mean Vd and CL values in the solid-organ transplant recipients (Wu et al., 2018), a half-life of only 57 hours can be calculated. However, the high variability of Vd and CL in this population (coefficient of variation of 135% and 66%, respectively) suggests that the individual elimination half-life values must also oscillate considerably around the mean. This is also confirmed by the half-life value of almost 160 hours observed in our patient. Such a long half-life implies that after initiation of therapy, steady-state levels will not be reached until after about 30 days. However, in clinical practice, drug levels are usually measured much earlier after initiation of therapy, and unless the level measurement is accompanied by a pharmacokinetic simulation of the time course of drug levels, and unless the long half-life phenomenon is taken into account, the dose of isavuconazole may be hastily adjusted on the basis of the early measured level. This is also seen in our case (Figure 1), where the maintenance dose of 200 mg once daily was increased to threefold based on the subtherapeutic level measured on day 14 of therapy. However, this was not yet a steady-state level and with the dosage increased to 600 mg/day, this dose had to be reduced again on day 27 of therapy as the level approached the threshold for toxicity.

It is also worth mentioning the high Vd, which in our patient was approximately twice the average value reported in the *Summary of Product Characteristics* (EMA, 2015). In such patients, it may then be useful to prolong the administration of the loading dose (200 mg every 8 hours) from two to at least three days for rapid achievement of the pharmacokinetic/pharmacodynamic (PK/PD) target.

In conclusion, based on this case report, we would like to point out the need for TDM of isavuconazole, not only in the sense of measuring the level, but also its

interpretation by pharmacokinetic simulations according to the principles of best practice in TDM.

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Paratesticular Dedifferentiated Liposarcoma with Rhabdomyoblastic Differentiation: A Case Report and Review of the Literature

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Abstract: Liposarcomas of the paratesticular tissue is a rare pathological entity. The symptoms are similar to inguinal hernias or hydroceles. We present the case of an 84-year-old man with a rare paratesticular liposarcoma that manifested as painless right hemiscrotal swelling. Testicular tumour markers were negative. Imaging revealed a heterogeneous mass with a fat component. He underwent a radical orchiectomy on the left side to remove the associated mass. This revealed dedifferentiated liposarcoma (DDLS) with rhabdomyoblastic differentiation and MDM2 amplification. The surgical margins were negative, and the patient had a metastatic workup that included magnetic resonance imaging (MRI) of the abdomen and pelvis. Because of the disease's rarity, there is no clear agreement on radiotherapy and chemotherapy roles.

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Introduction

Most paratesticular solid lesions in adults are benign, although malignant tumours can be seen in 3% of cases (Rafailidis et al., 2021). The most common types of malign paratesticular tumours are rhabdomyosarcoma and liposarcoma (Cardenosa et al., 1990). A more common form of paratesticular liposarcoma is well-differentiated and dedifferentiated types (Li et al., 2022). Here, we present the clinical history and management of a case with primary paratesticular dedifferentiated liposarcoma in light of the literature.

Case report

An 84-year-old man was referred to our urology outpatient clinic in May 2022 after complaining of swelling in his left scrotum for two weeks. A painless, slow-growing, fixed mass in the left scrotum was not accompanied by any obvious promoting or alleviating factors. There are no additional symptoms or signs. According to the patient, there were no discernible personal conditions related to the current clinical manifestation. There was also no evidence of family history. The only positive finding on physical examination was a rigid mass in the left scrotum, about 5 cm in diameter (Figure 1).

The laboratory examinations, including testicular tumour markers, reveal no specific abnormalities (hemogram, urinalysis, ESR – erythrocyte sedimentation rate, brucella agglutination test, liver, and kidney function tests, and chest X-ray).

Scrotal ultrasound revealed a large left scrotal heterogeneous mass ultrasounds-predominantly hyperechoic in echotexture; however, these findings were not specific. However, contrast-enhanced magnetic resonance imaging (MRI) revealed

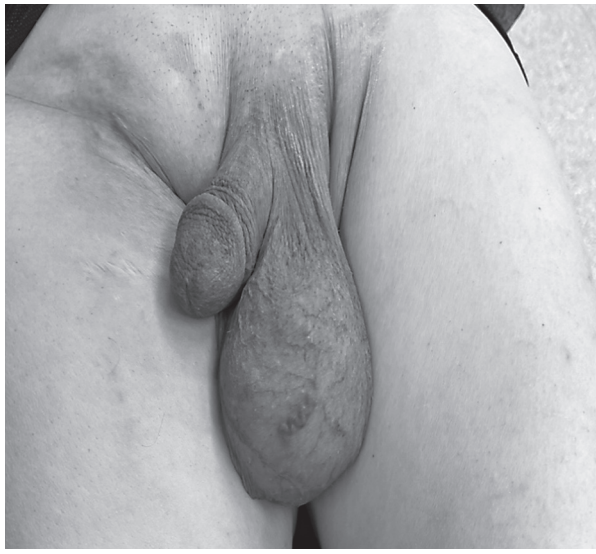

Figure 1 – Physical examination. A left scrotal mass measuring 6 cm was noted.

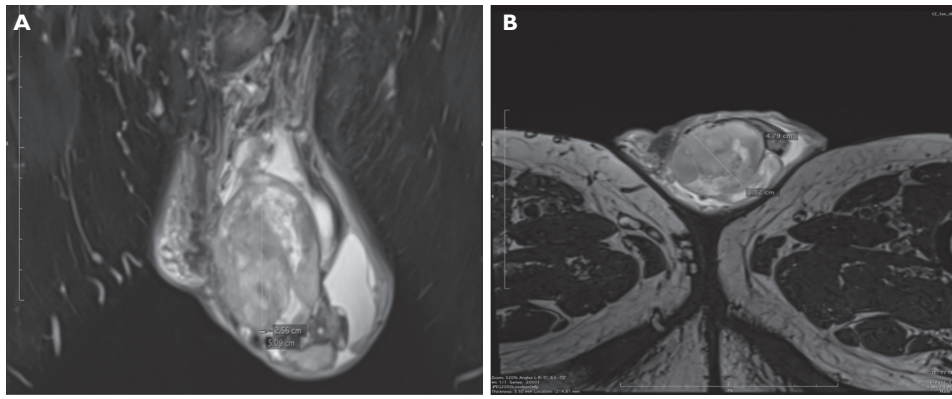

Figure 2 – Sagittal and transverse magnetic resonance images.

a 48×38×50 mm nonhomogeneous space-occupying lesion of the left testis. There was no lymphadenopathy or distant metastasis found (Figure 2).

Following the examinations, the patient underwent a radical left orchidectomy with wide local excision that included the paratesticular mass as well as the left testicle and all left inguinal canal contents up to the deep inguinal ring while sparing the left ilioinguinal nerve. A 5×4×6 cm enlarged left testicle was removed from the scrotum (Figure 3). There is no visible inflammatory adhesion to the organs.

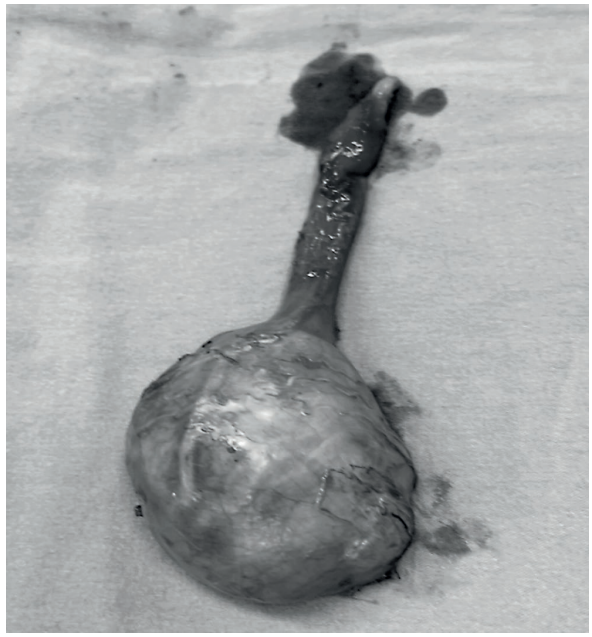

Figure 3 – Testis and cord after orchidectomy.

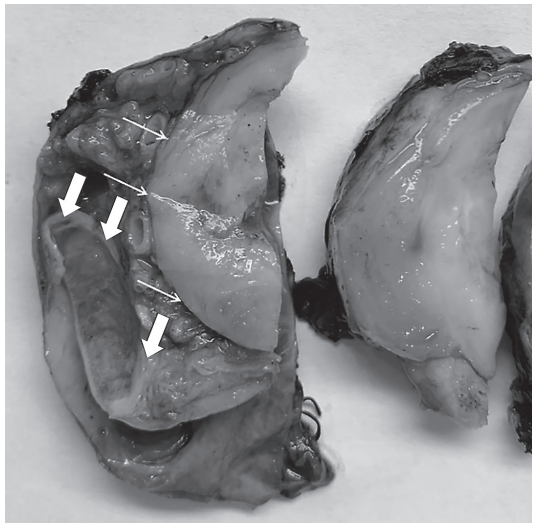

Figure 4 – Gross appearance of dedifferentiated liposarcoma (thin arrows) and testis (thick arrows).

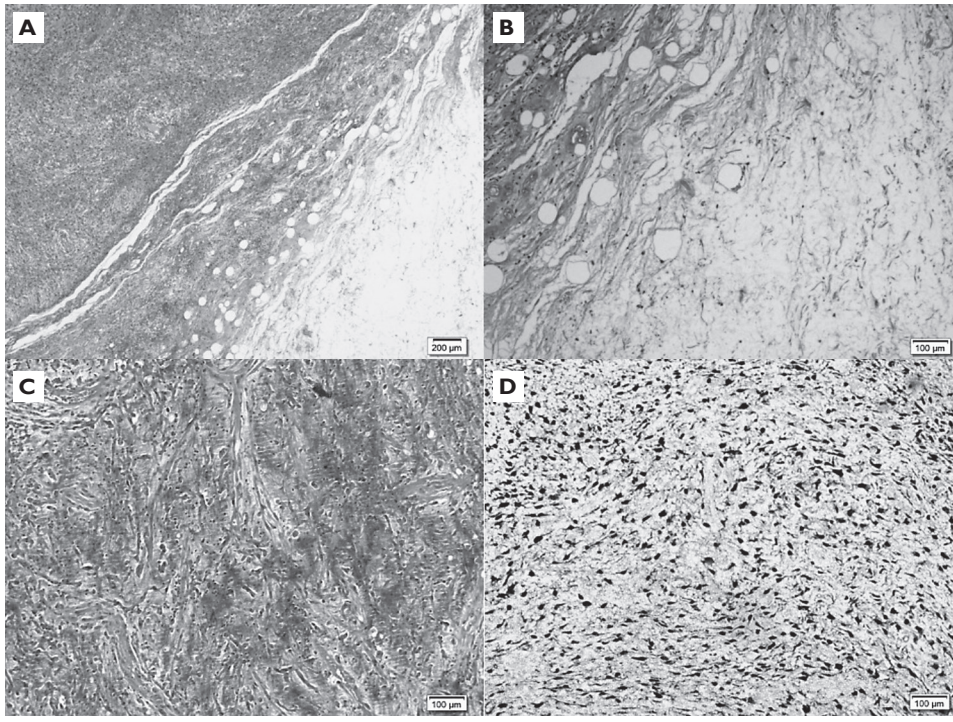

Figure 5 – Histologic and immunohistochemistry analysis of dedifferentiated liposarcoma specimens. (A) The transition zone of well-differentiated to the dedifferentiated tumour (hematoxylin-eosin stain). (B) Atypical lipomatous tumour area (hematoxylin-eosin stain). (C) Areas of liposarcoma on the myxoid floor with more spindle appearance (hematoxylin-eosin stain; 10×). (D) CDK-4 diffuse positive staining supports the diagnosis of dedifferentiated liposarcoma (immunohistochemistry).

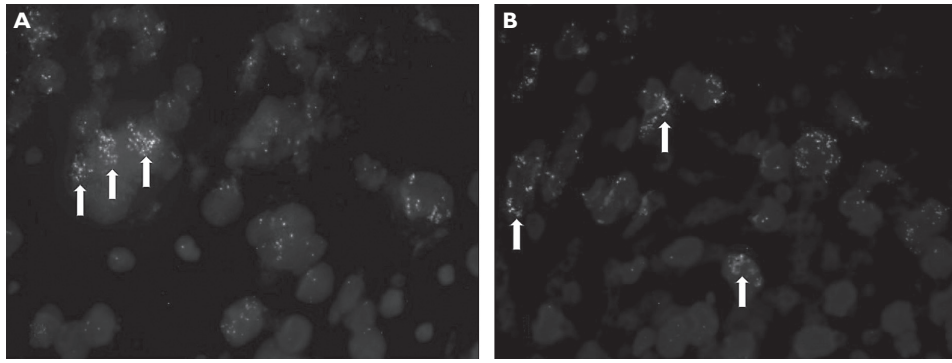

Figure 6 – Fluorescence in situ hybridization (FISH) of the CDK-4 (A) and MDM-2 (B) genes. FISH analysis confirmed CDK4 and MDM2 gene amplification in the nuclei of atypical cells (clustering of green signals indicated by arrows).

Histopathology revealed that the tumour was a DDLS (dedifferentiated liposarcoma) with rhabdomyoblastic differentiation, measuring 5×5×6 cm. CDK4(+); MDM2(+); ALK(–) immunohistochemical analysis supported this diagnosis (Figures 4 and 5). Fluorescence in situ hybridization (FISH) analysis showed the presence of CDK4 and MDM2 gene amplification supported the diagnosis of DDLS (Figure 6).

Discussion

Liposarcomas are classified into four types based on their histological appearance: myxoid (the most common; 40%), round cell, well-differentiated (subdivided into lipoma-like, sclerosing, inflammatory, and dedifferentiated), and pleomorphic (Logan et al., 2010). DDLS accounts for 18% of liposarcomas and was first described by Evans in 1979 as a well-differentiated liposarcoma adjacent to a cellular nonlipogenic sarcoma. Dedifferentiated, round cell and pleomorphic liposarcomas are high-grade, aggressive tumours with metastatic potential, whereas well-differentiated and myxoid liposarcomas are low-grade tumours with a more indolent clinical course (Dalal et al., 2006). DDLS can be identified on imaging as a heterogeneous non-lipogenic mass within an area of abnormal-appearing fat.

The diagnosis of DDLS is dependent on histology and immunohistochemistry. CDK4 and MDM2 were significant markers for diagnosing well-differentiated liposarcoma in one investigation (Pănus et al., 2015). FISH is especially beneficial when the presence of a well-differentiated liposarcoma component is unknown (Nishio et al., 2021). MDM2 and CDK4 amplification is helpful for differential diagnosis (Nishio et al., 2015).

Localized paratesticular liposarcoma can be treated with radical orchidectomy with a negative surgical margin. After orchidectomy, nearly one-third of the patients had local persistent lesions (Chiodini et al., 2015). Although some researchers

advocate adjuvant radiation therapy because of increased local control, its routine usage remains controversial (Li et al., 2018). Anthracycline-based therapy is the conventional first-line treatment for advanced DDLS. Trabectedin and eribulin are the two second-line therapeutic alternatives (Nishio et al., 2021). Several other medications were tested for advanced illnesses and with promising results. The prognosis and survival rate vary. Khandekar et al. (2013) reported a recurrence-free survival rate of 76% at 3 years and 67% at 5 years. Another study showed a 5-year survival rate of 75% and a recurrence rate of 50–70%. Large tumour size (>5 cm), pathologic degree of nuclear differentiation, and depth of invasion are the risk factors for recurrence (Schoonjans et al., 2016).

Conclusion

Paratesticular DDLS is a rare tumour with a painless scrotal mass. Radical orchidectomy with wide excision and high ligation is the standard treatment for localized disease. Although the prognosis is favourable, long-term follow-up is needed because the probability of recurrence is high. We must continue to examine the molecular mechanisms underlying liposarcomagenesis and work toward the development of novel therapeutic strategies for liposarcoma patients.

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