

EVALUATION OF THE SALIVARY PARAMETERS IN FACIAL MASK WEARERS DURING COVID-19 PANDEMIC

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ABSTRACT

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Introduction The COVID-19 pandemic situation forced governments to impose various measures to reduce the spread of the virus. The most used method is to wear facial masks, which can be found under several commercial forms. Wearing facial masks has caused a lot of controversy and rumors. Among them, many patients and practitioners have complained of a dry mouth after wearing different types of facial masks for a variable period of time. The aim of this study was to analyze the quantitative (un-/stimulated salivary flow rate) and qualitative (pH and buffer capacity) changes in saliva in mask wearers.

Methodology Forty subjects were selected for this study. All of them wore alternatively no mask, a surgical mask for 2 hours, and FFP2 mask for 2 hours (groups 1, 2, and 3). Saliva samples were collected from all the subjects in the groups and analyzed to determine the values of un-/stimulated salivary flow rate, the pH, and buffer capacity using GC Saliva-Check Buffer (GC Corporation, Japan). Descriptive and analytical statistics were performed using ANOVA and Bonferroni post-hoc test.

Results For unstimulated saliva samples, between groups 1 and 3, statistically significant differences were recorded, with a significance level of $0,02 < p = 0,05$. For stimulated saliva, salivary pH or buffer samples, no significant differences were found between groups.

Conclusion Wearing FFP2 masks for two hours showed a reduction in salivary flow rate compared to subjects who did not wear facial masks. Wearing surgical masks did not produce changes in salivary flow rates, pH or buffer capacity.

KEYWORDS

Mask; COVID-19; Saliva; pH; Buffer Capacity.

1. INTRODUCTION

The first cases of the novel coronavirus 2019 (COVID-19) appeared in Wuhan, China, in December 2019. The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) forced the World Health Organization (WHO) on March 11, 2020 to announce a global pandemic situation [1], disrupting and creating difficulties for governments and national health systems in managing it [2]. Throughout this period, the scientific communities around the world have been looking for some

methods to reduce the spread of the disease. The lack of effective treatments against the virus, as well as its rapid and uncontrollable evolution, forced the authorities to impose a series of non-pharmacological measures to limit the spread of the virus, such as maintaining hand hygiene, keeping social distance and wearing facial masks [1,2]. The existence of solid evidence that the SARS-CoV-2 virus would be transmitted by air has strengthened the belief that the use of face masks would be an effective way to reduce the spread of the virus [2,3,4].

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Since the beginning of the pandemic, protective masks have been recommended as protective measures due to the fact that the transmission of the virus is mainly respiratory. Countries have issued instructions and guidelines on the use of protective masks. In Romania, they must comply with the Personal Protective Equipment Regulation and with the relevant European standard EN 149. If they are manufactured in accordance with the current standards wearing a facial mask was considered one of the most effective ways to stop the spread of the virus.

Facial masks are devices that completely or partially cover the face with the role of protection against pollutants or pathogenic microorganisms. Surgical masks also known as medical mouth-nose protection (MNP) are devices that partially cover the face and act as protection against pathogenic microorganisms reducing the risk of spreading drops during expiration. Surgical masks are not watertight and cannot provide complete protection to the wearer [5].

FFP2 face masks (Filtering Face Piece) also known as N95 masks, provide 95% protection against pathogenic microorganisms. FFP2 masks may or may not be equipped with a valve. Masks without valves ensure effective protection of the external environment while masks with valves allow the exhaled air to escape without being filtered [4,5].

A number of studies have shown harmful effects of wearing a face mask on the cardio-respiratory system or on the neurological system [1,6], but no studies have yet been conducted to evaluate the effects of wearing a face mask on saliva parameters. Saliva has a multitude of functions that maintain the body homeostasis, but among them we focused on functions in the oral cavity. Saliva is very important in protecting dentition by salivary mucin or proline-rich glycoproteins pellicle, has a remineralizing role due to the content of calcium, phosphate and fluoride ions, buffering capacity due to bicarbonate and phosphate and antibacterial role due to the presence of IgA, IgG, IgM or some enzymes [7]. Therefore, any quantitative or qualitative change in saliva could impair the protective capacity of saliva on the dentition.

Therefore, the aim of this study is to evaluate the quantitative and qualitative parameters of saliva in surgical mask and FFP2 wearers during the COVID-19 pandemic.

2. MATERIALS AND METHODS

2.1. Subjects

The present study was conducted between June 1st, 2021 - September 30th, 2021, on a number of 40 subjects aged between 20 and 30 years old (18 females and 22 males) all of them being students of the Faculty of Dental Medicine, "Grigore T. Popa" University of Iasi. The subjects were informed about the procedures, they agreed to voluntarily participate in the study and signed an ethical agreement (no. 84/

26.05.2021) approved by the Ethics Committee of "Grigore T. Popa" University of Jassy, Jassy Romania. The selection of subjects was made in accordance with the inclusion and exclusion criteria presented in Table 1.

Table 1. Selection of the subjects: inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> - Patients aged between 20 and 30 years - Patients without associated general pathologies - Adult female / male volunteer - Agreement signed and dated for participation in the study - Ability to understand the purpose and type of the study - Willingness to cooperate with the doctor and to comply with the requirements of the study 	<ul style="list-style-type: none"> - Patients with general pathologies that affect salivary activity - Patients with chronic alcoholism - Patients with viral infections - C / B virus, HIV / AIDS - Patients who have an uncooperative attitude

2.2. Study design

For each subject, salivary parameters: unstimulated salivary flow rate, stimulated salivary flow rate, pH and buffer capacity were determined using GC Saliva Check Buffer test kit, GC Corporation, Japan, (LOT no. 2003102) at the same time of day (between 11-12 AM), in three different days. On the first day of testing, salivary parameters were evaluated in all subjects without wearing any mask (group 1), on the second day of testing after wearing a surgical mask for 2 hours (group 2) and on the third day of testing after wearing a FFP 2 mask for 2 hours (group 3). The individuals were recommended to follow the same schedule, to consume the same food and the same amount of liquids and use the same dental hygiene products and methods on each day of three testing days. The subjects were asked to avoid eating two hours before the salivary tests. Unstimulated salivary flow rate was initially assessed. From the collected saliva, the evaluations of the buffer capacity and salivary pH were performed, and finally the stimulated salivary flow was evaluated, after the subject chewed a piece of paraffin.

2.3. Salivary tests

The quantitative determination of stimulated and unstimulated salivary flow rate, buffer capacity and salivary pH were performed using the methodology presented in table 2.

2.4. Facial masks

The study participants wore two different types of facial masks:

1. surgical masks (AVE, Romania) with three layers: Outer layer-non-woven synthetic material - 20 g/sq; Middle layer-fused synthetic material, white; Inner layer-non-woven synthetic material, white - 20 g/sqm
2. FFP 2 masks (3M Aura 9320+, MN, USA) with four layers.

Table 2. Description of the saliva testing methodology.

Parameters to evaluate	Methodology
Stimulated salivary flow	The assessment was performed using a sialometer in which the patient eliminated the accumulated saliva at every minute, for a total period of 5 minutes. Stimulation of salivary secretion was done with a paraffin gum. The gum was by chewed by the subjects for 5 minutes during the whole period of stimulated saliva collection, according to the manufacturer's instructions. The measurement was made by three investigators. The values are expressed in ml/min.
Unstimulated salivary flow	The assessment was made using a sialometer in which the patient eliminated the accumulated saliva at every minute, for a total period of 5 minutes. The measurement was made by three investigators. The values are expressed in ml/min.
Buffer capacity	The assessment was made using an indicator strip for buffer capacity on which a drop of unstimulated saliva was placed. The color modification was optically assessed by three investigators. green – 4 points green/blue – 3 points blue – 2 points red/blue – 1 point red – 0 points. The points were counted and the result determined the buffering capacity score: 0 – 5 points as very low buffering ability 6 – 9 points as low 10 – 12 points as normal/high
Salivary pH	The assessment was performed using a pH indicator strip on which a drop of unstimulated saliva was placed. The color modification was optically assessed by three investigators.

2.5. Statistical analysis

The data were stored in the Office Excel program and the statistical analysis was performed using IBM SPSS 26 program. To determine the differences between the study groups we used the repeated measures ANOVA statistical test and the Bonferroni post-hoc test.

3. RESULTS

For unstimulated saliva samples, the mean values and standard deviation (SD) of group 1 was 3.53 (SD $\pm 0,68$), for group 2 was 3.19 (SD $\pm 0,58$) and for group 3 was 3.06 (SD $\pm 0,52$). Analyzing the mean variation, we can see that the values obtained in group 1 decrease in group 2 and the values recorded in group 2 decrease in group 3 (Fig. 1). Between groups 1 and 3, statistically significant differences were recorded, with a significance level of $0,02 < p = 0,05$. Between groups 1 and 2, and groups 2 and 3, no significant differences were recorded.

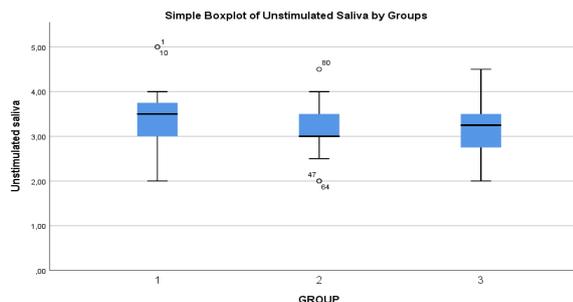


Figure 1. Boxplot presentation of the distribution of the values recorded in groups 1, 2 and 3 for unstimulated saliva samples.

For stimulated saliva samples, the mean values and standard deviation of group 1 was 10.95 (SD $\pm 2,58$), for group 2 it was 10.93 (SD $\pm 2,31$) and for group 3 it was 10.92 (SD $\pm 1,95$). Analyzing the mean variation, we can see that the values obtained in group 1 decrease in group 2 and the values recorded in group 2 decrease in group 3 (Fig. 2). Between groups 1, 2 and 3 no statistically significant differences were recorded for a significance level of $p = 0,05$.

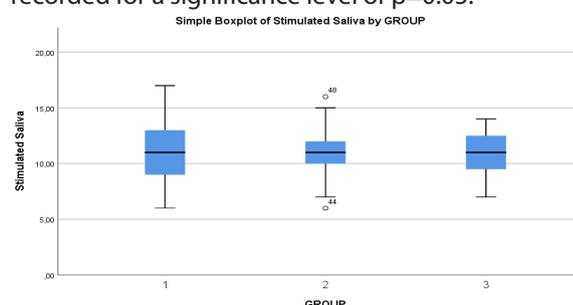


Figure 2. Boxplot presentation of the distribution of the values recorded in groups 1, 2 and 3 for unstimulated saliva samples.

For salivary pH, the mean values and standard deviation of group 1 was 6.72 (SD $\pm 0,19$), for group 2 it was 6.81 (SD $\pm 0,24$) and for group 3 it was 6.83 (SD $\pm 0,23$). Analyzing the mean variation, we can see that the values obtained in group 1 increase in group 2 and the values recorded in group 2 increase in group 3 (Fig. 3). Between groups 1, 2 and 3 no statistically significant differences were recorded for a significance level of $p = 0,05$.

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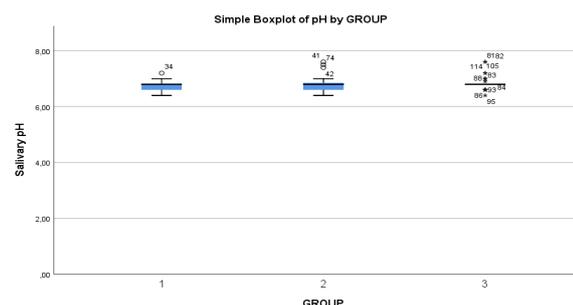


Figure 3. Boxplot presentation of the distribution of the values recorded in groups 1, 2 and 3 for salivary pH.

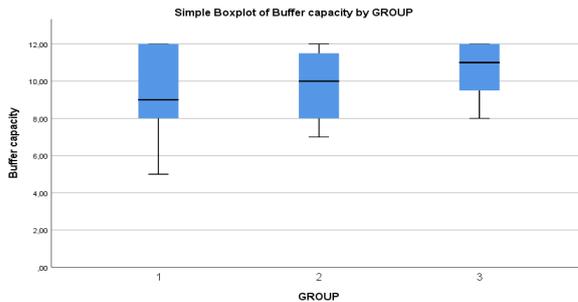


Figure 4. Boxplot presentation of the distribution of the values recorded in groups 1, 2 and 3 for buffer capacity.

4. DISCUSSION

In this study we evaluated the rate of unstimulated and stimulated salivary flow, buffer capacity and salivary pH in subjects wearing or not a surgical mask and a FFP2 mask. The results indicate a reduction in the resting salivary flow in FFP2 mask wearers compared to subjects that did not wore any type of mask, and these findings are supported by a recent review study conducted by Muley-Itke that described the phenomenon of "Mask mouth" as being a result of prolonged wearing of the facial mask. This condition can be a consequence of hyposalivation due to oral breathing that is a common behavior in facial mask wearers and is associated with an increased incidence of oral disorders, such as periodontal disorders, halitosis or an increased carious risk. Another justification may be poor hydration while wearing a mask that may be associated with general dehydration. Dental or periodontal diseases have an increased incidence in oral respiratory patients, as the mouth has a very high exposure to air flow, which decreases the rate of salivary flow [8,9].

A number of studies have shown a significant increase in the amount of acidogenic and aciduric bacteria such as *Streptococcus mutans* and *Lactobacilli* in patients with oral breathing or those with xerostomia. [8,10]. Other authors have described in patients with hyposalivation or oral respiration, an increase in bacterial plaque indices as well as a reduction in the buffer capacity of saliva and an increase in susceptibility to gingival inflammation or periodontal disease [8,10,11].

The decrease of the salivary flow rate will consequently decrease the antibacterial components increasing the potential for the development of bacterial or fungal infections, such as candidiasis. Decreasing the concentration of lysozyme, lactoferrin, lactoperoxidase or histatin will weaken the saliva's defense capacity [8,12,13,14].

Saliva has multiple roles: to ensure surface cleaning, substance clearance and an optimal pH by buffer capacity. In buffering effect, an important role is played by the bicarbonate-carbonic acid system, salivary phosphate and proteins [7,15].

Maldupa et al. described a different composition of unstimulated saliva when comparing to the stimulated one. Increased saliva production will decrease the concentration of potassium and phosphate and will increase the values of sodium, chloride and bicarbonate [7]. Most studies that evaluated the links between the saliva buffer capacity and the patient's

caries risk did not find significant correlations between them [7,16]. This conclusion is explained by the fact that bicarbonate and other buffering components do not come into direct contact with the surface of the teeth as they are covered by a layer of bacterial film or biofilm [7,17].

Another symptom of mask wearers is halitosis, also called "mask breath" caused by volatile sulfur compounds in the oral environment or by the remaining fermentable food residues on the dorsum of the tongue or on the dental surfaces and accentuated by oral dryness [8,18].

Wearing the facial mask not only produces imbalances in the oral environment, but also affects the perioral teguments, causing their dehydration and potentiating the bacterial action that will cause cracks or ulcerations of the tegument or angular cheilitis [8,19].

Some recent studies have described a number of recommendations to combat oral imbalances. Therefore, in addition to a rigorous oral hygiene that must include 2-3 toothbrushes, the use of mouthwash and dental floss to combat the phenomenon of "mask mouth", the surgical masks must be changed after 3 hours of wearing because the humidity expired degrades them. Oral breathing should be avoided as much as possible when the mask is applied. The mask must be applied correctly, as tightly as possible and used only once. To avoid perioral skin lesions, it is recommended to use creams with moisturizing and emollient effect [20-22].

5. CONCLUSION

Unstimulated salivary flow rate decreases in persons wearing FFP2 mask when comparing to the persons who wear no mask, but it remains unaffected by surgical mask wearing.

Stimulated salivary flow rate, salivary pH or buffer capacity are not influenced by any type of face masks.

AUTHOR CONTRIBUTIONS

IT: conception and design of the study, acquisition of data, statistical analysis and interpretation of data, drafting the article, final approval of the version to be submitted. SS: drafting the article, revising the article critically for important intellectual content. GI: acquisition of data, analysis and interpretation of data. AG: acquisition of data, analysis and interpretation of data. IN: acquisition of data, analysis and interpretation of data. ACTG: acquisition of data, analysis and interpretation of data. SA: conception and design of the study, revising the article critically for important intellectual content, final approval of the version to be submitted.

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Questions

1. What is the recommended time to wear a mask before it is necessary to change it?

- a. 1 hour;
- b. 5 hours;
- c. 3 hours;
- d. 30 minutes.

2. What is the meaning of FFP2?

- a. Frontal Filtering Piece;
- b. Filtering Face Piece;
- c. Face Filtering Product;
- d. Free Filtering Piece.

3. The decrease of the salivary flow rate will consequently:

- a. Decrease the antibacterial components;
- b. Decrease the growing potential of the bacteria;
- c. Increase the concentration of lysozyme;
- d. Increase the concentration of lactoferrin.

4. The most important role in buffering capacity is played by:

- a. Lactoperoxidase;
- b. Bicarbonate;
- c. Lactobacilli;
- d. Histatin.



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