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Non-Invasive Salivary Diagnostic Approach for Predicting Dental Caries

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ABSTRACT

Dental caries, often known as tooth decay, is a widespread public health concern that presents many difficulties, especially in developing nations like Pakistan. **Objectives:** To evaluate a non-invasive salivary diagnostic approach for predicting the risk of dental caries. **Methods:** In the comparative cross-sectional study, a total of 90 participants were recruited through purposive sampling technique belonging to the age group between 20 and 30 years, which was conducted in one of the private hospitals of Karachi, Pakistan. The participants were placed into two groups for the study according to their oral health state, as determined by the Decayed, Missing, and Filled Teeth index. Both informed consent and ethical approval were acquired. Samples of saliva were collected and examined utilizing standardized tools. Data were analyzed by statistical software version 23.0 by using the Chi-square test. **Results:** Participants with active caries (Decayed, Missing, and Filled Teeth >5) and those in optimum dental health (Decayed, Missing, and Filled Teeth=0) showed significant differences. Lower salivary pH (p=0.003), decreased flow rate (p=0.001), decreased buffering capacity (p=0.002), and increased viscosity in the high-risk group are important findings. These differences imply altered salivary dynamics, which raise the risk of dental cavities. **Conclusions:** It was concluded that a non-invasive and efficient method for determining the risk of dental cavities is salivary diagnostics, especially for communities with limited access to preventative dental treatment. Salivary evaluations incorporated into standard dental procedures may improve preventative measures.

INTRODUCTION

In developing countries like Pakistan, dental caries is very common, making it a significant worldwide public health concern [1]. Conventional caries diagnosis techniques, like visual inspection and radiographic imaging, frequently have drawbacks, such as subjectivity and the possibility of overlooking early lesions [2]. Non-invasive diagnostic techniques that can improve the early diagnosis and treatment of dental caries are therefore desperately needed. Recent developments in salivary diagnostics, which use saliva as a biomarker for dental health, have created new opportunities for non-invasive evaluation [3]. Salivary analysis has been used in medicine since ancient times when doctors used to taste patients' saliva to identify a variety of illnesses [4]. However, the first scientific investigations into salivary analysis did not take place until the late 1800s. Ivan Pavlov, a German scientist, found in 1880 that the autonomic nerve system controls salivary secretion. Numerous investigations into the different uses of salivary analysis in clinical practice have since been carried out. Saliva is a biological liquid and a unique medium

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in the oral cavity that directly contributes to the development of dental caries among all age groups [5]. Because of this, different caries-predicting models continue to consider variations in salivary flow rate and its content modulations. These models differ from one another in certain aspects, such as how different saliva-associated parameters affect the processes of demineralization and re-mineralization of hard dental tissues [6]. The maintenance of dental health is significantly influenced by saliva. Salivary glycoproteins and mucoid lubricate and protect the mucous membranes [7]. Saliva uses a variety of physical and biochemical processes to carry out its mechanical cleaning and protecting duties. The risk of oral illnesses, particularly dental caries, is increased by low salivary secretion rates. Both stimulated and relaxed circumstances can be used to gather saliva [8]. The most common dental condition affecting humans is dental caries. Dental caries is still a serious issue even if its frequency has greatly decreased. It is well-recognized that dental caries have a complex etiology and pathophysiology [9]. Saliva is a bio-fluid that has gained significant interest due to its non-invasive nature, which makes it a valuable diagnostic tool for analyzing various conditions and diseases. On the contrary major other diseases of the human body are often identified by examination of body fluids, such as urine, sputum, blood, and cerebrospinal fluid [10]. Saliva secretion rate and quality play a significant role in both the development of caries and re-mineralization. The health of the soft tissues and teeth both depend on saliva. Patients who have experienced dental caries or dental erosion frequently come with missing tooth structures. By evaluating significant salivary indicators, the most likely causes of the oral balance shift that favoured demineralization may be identified [11]. Salivary analysis is a new non-invasive and user-friendly method that has received a lot of attention lately. Salivary analysis, which makes use of the most recent findings about salivary biomarkers, has the potential to completely transform the healthcare industry by offering insightful data for tracking a person's health, identifying the course of a disease, and facilitating individualized therapy [12]. Healthcare practitioners may follow patient development and make informed treatment decisions with the support of healthcare diagnostics and monitoring, which are essential components of healthcare management. A variety of diagnostic instruments, such as blood tests, imaging tests, genetic testing, and monitoring technologies, such as wearables, remote monitoring systems, and electronic health records, are now available to healthcare professionals[13].

This study aims to evaluate a non-invasive salivary diagnostic approach for predicting the risk of dental caries.

METHODS

A comparative cross-sectional study was carried out in one of the private hospitals in Karachi, Pakistan. The sample size of the study was calculated through open EPI assuming a 95% confidence level and a 5% margin of error, 90 participants were selected through purposive sampling technique in the study belonging to the age group 20-30 years. Exclusion criteria included patients with xerostomia or poorly managed diabetes, smokers, pregnant women and chewers of betel leaf. Using a saliva testing kit, determine the salivary pH, stimulated salivary flow rate, and buffering capacity in stimulated saliva. While salivary diagnostics included assessing flow rate, pH levels and buffering capacity. These steps made it easier to determine how non-invasive salivary diagnostics affected dental caries in the targeted population. Ninety individuals were split into two groups for this comparative cross-sectional study according to the Decayed, Missing, and Filled Teeth (DMFT) index, which measures dental health. There were 45 participants in the control group (22 male and 23 female), all of whom had DMFT scores of 0, which indicates optimum dental health. The ethical approval (Ref.No 029 SSCMS-Ethics/2024) was taken from the institutional ethical committee board of Sir Syed College of Medical Sciences. Each participant provided informed consent and the dentist used the DMFT index to measure dental caries. Hence, a dental mirror for clear tooth visibility, a dental explorer for cavity detection, and an artificial light source for improved visibility are the three main tools used to evaluate the DMFT index. An enzyme-linked immunosorbent assay (ELISA) kit (Human Beta Defensin 3 ELISA kit Cat. No. E3240Hu) was used to assess the amount of Human beta-defensin-3 (HβD-3) in saliva from the Laboratory of Bioassay Technology [14]. A saliva testing kit was used to analyze the viscosity, pH, flow rate, and buffering capacity of saliva. Participants were instructed to sit up straight, tip their heads down, and keep their mouths open until saliva gathered on the floor of their mouths to collect unstimulated whole saliva. After that, participants let their saliva flow into a 15 ml Falcon tube. Until 4–5 milliliters of saliva were obtained, this procedure was repeated. Within 30 minutes, the tubes were carried to the lab in thermopile bags while resting on crushed ice in disposable glasses. To obtain clean saliva, samples were centrifuged in the lab for 15 minutes at 4500 rpm and 4°C. A micropipette was used to collect the supernatant, and 0.3 ml was kept in Eppendorf tubes at -20°C until it was further examined. The data were analyzed by using a statistical package (SPSS version 23.0). Hence Chi-square test was used to see the differences between the control group and group A.

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RESULTS

The demographic details of the study, participants were divided into two groups (Group A; DMFT >5) and the control group (DMFT=0). There were 90 participants in total. According to the gender distribution, Group A has 15 male and 30 female, whereas the control group had 22 male and 23 female. There are 50 female participants (55.6% of the sample as a whole) and 40 male individuals (44.4%).

Table 1: Demographic Details of selected Patients

Variables	Control Group (DMFT=0) n=45	Group A (DMFT >5) n=45	Total Participants (n=90)
Gender			
Male	22	15	37 (41%)
Female	23	30	53 (58%)
Age Group			
18-25 Years	14	15	33 (36.6%)
26-30 Years	13	14	26 (28.8%)
31-35 Years	11	12	19 (21.1%)
36-40 Years	7	8	12 (13.3%)

Further study compares the salivary parameter features of a control group (n=45) with a decayed, missing, and filled teeth (DMFT) score of 0 and Group A (n=45) with a DMFT score >5. There was a substantial difference in the groups' levels of hydration; 68% of the control group reached hydration in less than 30 seconds, whereas 95% of Group A did so (p=0.001). Significant differences were also observed in salivary viscosity; 84% of the control group had moderate viscosity, whereas Group A showed a greater range, with 37% having low viscosity, 42% having moderate viscosity, and 20% having high viscosity (p=0.002). The majority of the control group (77%) had a pH between 6.2 and 7.0, but Group A had a more acidic profile, with 42% having a pH below 5.6 (p=0.003). The control group's salivary flow rate was significantly higher, with 82% of them exhibiting a flow rate above 1.0 ml/min, while 80% of Group A had a flow rate below 1.0 ml/min (p=0.001). Lastly, the buffering capacity was low in Group A (75%) and primarily high in the control group (57%; p=0.002). These results underline the association between lower salivary function and higher DMFT scores, highlighting notable variations in salivary parameters (Table 2).

Table 2: Characteristics of Salivary Parameters in Patients

Salivary Parameters	Control Group (DMFT=0) n=45	Group A (DMFT >5) n=45	p-value
Hydration Status			
Less than 30 seconds	31 (68%)	43 (95%)	0.001
More than 30 seconds	14 (31%)	2 (4%)	
Viscosity of Saliva			
Low Viscosity	2 (4%)	17 (37%)	0.002
Moderate Viscosity	38 (84%)	19 (42%)	
High Viscosity	5 (11%)	9 (2%)	
pH of Saliva			
<5.6	1 (2%)	19 (42%)	0.003
5.6 to 6.2	6 (13%)	12 (26%)	
6.2 to 7.0	35 (77%)	6 (13%)	
7.1 to 8.0	3 (6%)	8 (17%)	
Flow Rate of Saliva			
Less than 1.0 ml/min	8 (17%)	36 (80%)	0.001
Greater than 1.0 ml/min	37 (82%)	9 (2%)	
Buffering Capacity			
Low Buffering Capacity (0 to 5)	5 (11%)	34 (75%)	0.002
Moderate Buffering Capacity (6 to 9)	14 (31%)	8 (17%)	
High Buffering Capacity (10 to 12)	26 (57%)	3 (6%)	

DISCUSSION

Dental caries is still one of the most prevalent oral health issues in the world, especially in poorer nations like Pakistan where the illness is made worse by a lack of awareness, poor oral hygiene habits, and restricted access to preventive therapy. This study investigated how non-invasive salivary diagnostics might be used to diagnose and treat dental caries in Karachi. Salivary characteristics such as hydration state, viscosity, pH, flow rate, and buffering capacity were found to differ significantly between people with high caries experience (DMFT>5) and those with optimum oral health (DMFT=0) [15]. One of the key factors affecting salivary pH and oral health is salivary pH. The acids in the oral cavity break down enamel when salivary pH falls below normal; the longer this situation persists, the greater the chance that caries will develop [16]. Dental caries is strongly prevented by a normal salivary flow rate, which includes both hydration status and stimulated saliva flow rate. Salivary characteristics, such as decreased flow rate, lower pH, and diminished buffering capacity, were significantly impaired in the high-risk group participants. These characteristics are known to foster the growth of cariogenic bacteria and the demineralization of tooth enamel These findings demonstrate saliva's diagnostic capacity in the treatment of dental cavities and emphasize its critical role in preserving oral health. According to the findings, just 2% of individuals in the Control Group had salivary pH values below 5.6, while 42% of people in Group A did. Previous research by Dawes and Wong conducted in 2019 has shown that enamel demineralization and the growth of cariogenic bacteria are facilitated by such an acidic environment [17]. This is consistent with the finding that a lower pH is directly linked to a higher risk of dental cavities. Saliva's ability to neutralize dietary acids is also hampered by poor buffering capacity, which was present in 75% of Group A participants. This is consistent with research by Barakzai *et al.*, in 2024 who highlighted how a decreased buffering capacity fosters an environment that

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accelerates the development of caries. These results support the buffering capacity's protective role and its vital role in maintaining oral health. The difference in salivary flow rates between the two groups was another noteworthy discovery. The average salivary flow rate for Group A participants was 0.4 mL/min, which was significantly less than the 0.8 mL/min recorded for Control Group participants [18]. The results of this study were further supported by Araujo *et al.*, (2020), who also found that lower salivary flow rates were linked to a higher prevalence of dental diseases. According to these results, preserving ideal salivary flow is essential for reducing the risk of dental caries [15]. Another measure that revealed notable variations between the two groups was salivary viscosity. Saliva in Group A participants was thicker and more viscous, which hinders teeth's ability to be mechanically cleaned and encourages the buildup of plaque. Although less research has been done on this topic, Halageri *et al.*, (2020) pointed out that increased viscosity is linked to decreased food debris clearance, which could raise the risk of dental caries [16]. Salivary dynamics were also discovered to be influenced by hydration status. Participants in Group A recovered from dehydration more slowly than those in the Control Group. To increase salivary secretion and enhance its quality, one must be properly hydrated. This result is in line with that of Thomas (2024), who emphasized the connection between caries prevalence and hydration levels [19]. Thus, encouraging proper hydration becomes a straightforward but powerful preventative measure. The salivary composition and function may also be impacted by age-related changes in hormone levels [20]. Hence the main limitation of the study was the smaller sample size because it would not accurately reflect the population, resulting in more sampling error, and decreased generalizability. Additionally, smaller samples limit subgroup analyses and can introduce bias, which can affect the findings' reliability. But anyhow the study can offer valuable information on the prevalence, correlations, and possible effects of diverse exposures across distinct groups within a community. A clinically significant decline in salivary functioning might be regarded as an etiologic factor that contributes to the development of dental caries because saliva has a general preventive effect.

CONCLUSIONS

It was concluded that this study emphasizes the vital role salivary parameters pH, buffering capacity, flow rate, and viscosity play in preserving oral health by highlighting notable variations between those with and without dental caries. These results highlight salivary measurements' diagnostic potential and provide a non-invasive method of evaluating caries risk, especially in high-prevalence populations like Pakistan. The use of salivary stimulants to improve salivary function, nutritional counselling, and hydration promotion are examples of practical uses. Although the study offers insightful information, its limitations such as its cross-sectional methodology and limited sample size warn against extrapolating the findings. Salivary diagnostics can be used in regular dental procedures to improve preventative and therapeutic strategies.

Authors Contribution

Conceptualization: WUN
Methodology: WUN, AI, AA
Formal analysis: AKH, SM
Writing review and editing: MK, AI, AA, FT

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

Conflicts of Interest

All the authors declare no conflict of interest.

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