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AVALIAÇÃO DA INFLUÊNCIA DO DISPOSITIVO DE COLETA DE SALIVA NA ANÁLISE DE IMUNOGLOBULINA A SECRETORA E ALFA-AMILASE

INFLUENCE OF THE SALIVA COLLECTION DEVICE ON THE ANALYSIS OF SECRETORY IMMUNOGLOBULIN-A AND ALPHA-AMYLASE

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Resumo

Introdução: A saliva humana tem sido amplamente utilizada para quantificar biomarcadores com finalidade científica e de diagnóstico, pois sua coleta é simples, não-invasiva e livre de estresse. O Salivette é o dispositivo de coleta de saliva mais empregado, devido à sua praticidade e aceitação pelo paciente, entretanto é desconhecida a influência de sua versão de poliéster nas análises das concentrações salivares de algumas substâncias. Objetivo: O objetivo deste trabalho foi avaliar a influência do Salivette poliéster na determinação das concentrações salivares de sIgA e alfa-amilase. Métodos: Foram coletadas amostras de saliva de 15 adultos jovens com bom estado de saúde, livres de infecções e mucosa bucal íntegra. Cada amostra de saliva foi dividida em duas porções: uma foi colocada sobre o poliéster do Salivette e a outra foi mantida no frasco original (controle), todas foram centrifugadas e armazenadas a -80ºC até a análise laboratorial. Resultados: As amostras salivares passadas pelo Salivette mostraram uma redução estatisticamente significante da concentração de sIgA, com uma redução de 17,3%±15,2, porém esta diferença não foi significante para as concentrações de alfa-amilase (p<0,05). Conclusão: A utilização do Salivette poliéster para coleta de saliva não influencia a concentração de alfa-amilase, porém altera de forma significante os níveis de sIgA.

Palavras-chave

Saliva. Imunoglobulina A. Alpha-amilase. Óxido nítrico.

Abstract

Introduction: Human saliva has been widely used to quantify biomarkers for research and diagnostic purposes, once its collection is simple, non-invasive and stress-free. Although Salivette is the most used saliva collection device, due to its practicality and patient acceptance, the influence of its polyester version on the analysis of the salivary concentrations of some substances is unknown. Objective: This study aimed to assess the influence of polyester Salivette on the salivary concentrations of secretory immunoglobulin-A (s-IgA) and alpha-amylase. Methods: Salivary samples from 15 young adults, in good general health, free from infections and with a healthy oral mucosa, were collected. Each salivary sample was divided in two portions: one was placed on the Salivette polyester and the other was kept in the original flask (control). The samples were centrifuged and stored at -80ºC, until laboratory analysis was performed. Results: The salivary samples which had contact with Salivette showed a statistically significant reduction of s-IgA (17.3%±15.2). The difference was not significant for alpha-amylase (p<0.05). Conclusion: The polyester Salivette collection device does not influence alpha-amylase concentrations, but significantly reduces s-IgA levels.

Key-words

Immunoglobulin A. Alpha-amylase. Nitric oxide.

1 INTRODUCTION

Human saliva contains 99.5% water, 0.3% proteins and 0.2% inorganic matter and trace substances, its protein component being composed of glycoproteins, enzymes, immunoglobulins and antimicrobial peptides (SCHIPPER et al., 2007). This fluid has a role in the formation of the alimentary bolus, mechanical protection of the oral mucosa, preliminary food digestion, maintenance of dental enamel and defense against microorganisms (AMERONGEN and VEERMAN, 2002; WALKER, 2004).

Salivary samples have been used to quantify biomarkers in clinical and laboratory research (SHIRTCLIFF et al., 2001; TAKAI et al., 2004; WALKER, 2004; STRAZDINS et al., 2005; MICHISHIGE et

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al., 2006; SCHIPPER et al., 2007; SHIRASAKI et al., 2007; KOKA et al., 2008), because its collection is simple, non-invasive and stress-free (TAKAI et al., 2004; SCHIPPER et al., 2007). Besides, storage is better accepted and contamination is reduced (LAC, 2001).

Among the salivary biomarkers, alpha-amylase and secretory immunoglobulin A (s-IgA) have bee widely used in clinical and laboratory research. Alpha-amylase is used as a parameter for the assessment of physical and psychological stress, due to its association with plasma norepinephrine levels (CHATTERTON et al., 1996; ROHLEDER et al., 2004; NATER et al., 2005; NATER at al., 2006; SHIRASAKI et al., 2007; DECARO, 2008). s-IgA is the predominant antibody in the saliva, being the first line of immunological defense of the oral mucosa, blocking microorganism adherence and penetration (VUDHICHAMNONG et al., 1982; ZEIER et al., 1996; WALKER, 2004).

Because of its practicality during collection, handling and processing, Salivette is one of the most used saliva collection devices (POLL et al., 2007; GRÖSCHL et al., 2008). It contains a cylinder of cotton or polyester absorbing material that is put in contact with the patient`s mouth. After centrifugation, the non-viscous saliva sample obtained is ready for laboratory analysis (POLL et al., 2007).

Previous studies have shown that cotton-based Salivette salivary samples had reduced s-IgA (SHIRTCLIFF et al., 2001; STRAZDINS et al., 2005; MICHISHIGE et al., 2006) and alpha-amylase (DECARO, 2008) levels. Polyester-based Salivette has shown good results for drugs (GRÖSCHL et al., 2008) and steroids (GRÖSCHL and RAUH, 2006; MYLONAS et al., 2006), its efficacy for s-IgA and alpha-amylase not having been assessed. This study aimed to assess the influence of polyester Salivette on the determination of s-IgA and alpha-amylase concentrations in the saliva.

2 METHOD

Salivary samples from 15 healthy young adults (6 males and 9 females, mean age 23.4 years) were collected. All the subjects had a healthy oral mucosa, did not have periodontal disease or infections, and were not on psychotropics, non-steroidal anti-inflammatory drugs, or hormones. Participants were asked not to eat, drink or smoke during the hour preceding collection.

The study was approved by the Committee of Ethics on Research of the Federal University of Juiz de Fora. All participants were volunteers and gave their informed consent.

Under direct supervision, the subjects washed their mouths for 20s with 25ml of distilled water, 10 minutes before collection. Each subject passively produced a 1-2ml saliva sample into a sterile container. From this sample, 500µl of saliva were obtained and dispensed on the top of the Cortisol Salivette® polyester cylinder (Sarstedt. Rommelsdorf,

Germany). After centrifugation for 5 minutes, at 1000g and 20°C, the latter formed the Salivette group. The containers with the remaining saliva volume were centrifuged for 5 minutes at 3500 RPM and 20°C, the supernatant being transferred to an Eppendorf tube, forming the control group. All samples were stored at -80ºC, until laboratory analysis was performed.

Alpha-amylase analysis: All samples were diluted to 1:100 for duplicate analysis with the kinetic method for alpha-amylase analysis kit (BioTécnica, Varginha, Minas Gerais, Brazil), according to the manufacturer`s instructions. Absorbance was assessed at 405nm wavelength, using the BTS-370 microtiter plate absorbance reader (BioSystems, Bucharest, Romania), with quantification through a standard curve.

s-IgA analysis: Salivary s-IgA was determined through antigen-specific enzyme-linked immunosorbent assay (ELISA), performed in duplicate with the Human IgA ELISA quantitation kit (Bethyl Laboratories, Montgomery, Texas), according to the manufacturer`s instructions. The capture antibody was diluted to 1:100, and anti-Human IgA-HRP diluted to 1:50.000 was used to detect the captured s-IgA. Absorbance was assessed at 450nm wavelength, using the Spectramax® 190 microtiter plate absorbance reader (Molecular Device, Sunnyvale, California). s-IgA was quantified through a standard curve that ranged from 7.8 to 500 ng/ml. The salivary samples were diluted to 1:100.

Statistical analysis: s-IgA and alpha amylase concentrations in the control and Salivette groups were compared with the Wilcoxon test, in order to determine the influence of the absorbing material on the salivary samples. Statistical significance was set at p<0.05.

3 RESULTS

Table 1 shows the means and standard deviation (SD) of the s-IgA and alpha-amylase concentrations in the control and Salivette groups. Salivary samples that had contact with the absorbing material (Salivette group) had a significant reduction of s-IgA concentration compared to controls (p=0.003). Although alpha-amylase concentrations in the Salivette group were slightly reduced compared to controls, this was not statistically significant (p=0.211).

Table 1: Effect of the absorbing material from polyester Salivette on saliva samples.

	Control group		Salivette group		Loss of the Salivette group compared to control (%)	
	Mean	SD	Mean	SD	Mean	SD
Secretory immunoglobulin A (µg/ml)	26.6	5.0	21.5*	2.9	17.3	15.2
Alpha-amylase (U/µl)	78.6	48.7	72.6	45.1	7.3	17.9

* - significant difference compared to control group (p<0.05).

4 DISCUSSION

Saliva collection with Salivette has been used because it is simple and patient-friendly (CHIAPPIN et al., 2007; GRÖSCHL et al., 2008). Result interpretation must be careful though, as the absorbing material can significantly affect the concentrations of some salivary biomarkers (SHIRTCLIFF et al., 2001; STRAZDINS et al., 2005; MICHISHIGE et al., 2006), as we demonstrated in this study. Specific validation of the collection technique must thus be performed prior to analyzing different biomarkers (SHIRTCLIFF et al., 2001; GRÖSCHL et al., 2008).

Studies have found that cotton Salivette significantly (30 to 72%) reduces s-IgA concentrations in salivary samples, because the molecules of the immunoglobulin are trapped by the cotton fibers (SHIRTCLIFF et al., 2001; STRAZDINS et al., 2005; MICHISHIGE et al., 2006). In our study, sample passage through polyester Salivette significantly affected s-IgA levels, although the reduction observed (17.3%±15.2) was smaller than that with cotton Salivette.

Differently from the s-IgA level, alpha-amylase concentrations in the salivary samples were not significantly reduced by polyester Salivette, with only a 7.3%±17.9 reduction, consistent with what has been observed with cotton Salivette (DECARO, 2008; PARK et al., 2008). In spite of similar results with both absorbing materials, DeCaro (2008) and Park et al. (2008) stated that saliva analysis with cotton Salivette should only be considered reliable if the cotton is fully saturated with saliva (around 1.5ml), as smaller volumes can lead to significant changes in alpha-amylase concentrations. We did not observe this limitation with polyester Salivette in our study, once the saliva volume dispensed on the polyester was 400µl.

5 CONCLUSION

Our study indicates that polyester Salivette used for sample collection may significantly reduce s-IgA concentration, although this reduction is smaller that that observed with cotton Salivette. The alpha-amylase levels, on the other hand, were not significantly changed, a result that strengthens the need to test the saliva collection method prior to conducting analysis of salivary biomarkers.

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