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Salivary Secretion and Composition in Malaria: A Case-control Study

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Summary: No previous studies have documented changes in salivary secretion in patients with malaria. This study aimed to compare salivary secretion and composition in malaria positive and malaria negative individuals. Ninety participants composed of 40 malaria parasite positive and 50 malaria parasite negative individuals (age and gender matched) were included. Malaria diagnosis was achieved by microscopic examination of Giemsa stained thick and thin film of blood smears. A self-administered questionnaire was used to assess presence or absence of oral symptoms in the malaria parasite positive individuals. Whole saliva samples were collected and analyzed for flow rate, pH, total protein and concentrations of electrolytes (K^+, Na^+, Ca^{2+}, Cl^-, $PO4^{2-}$ and HCO_3^{2-}). Data were analysed using Independent-Samples t-test and Spearman's correlation test. The salivary flow rate was significantly reduced in malaria parasite positive individuals (P = 0.001). Oral symptoms were present in 82.5% of the malaria parasite positive individuals. There was no significant difference in the salivary pH, total protein and electrolyte ion concentrations between the two groups. Also, Spearman's correlation test showed no significant relationship between the presence of oral symptom and the salivary parameters. Salivary flow rates are reduced in the individuals with malaria. However, presence of oral symptoms in these individuals may not be attributed to the reduced salivary flow rate. Further studies are needed to validate our findings and elucidate mechanisms involved.
Keywords*:* Saliva, Malaria, Salivary flow rate, Salivary electrolytes, Salivary pH, Salivary total protein.

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INTRODUCTION

Saliva is critical for preserving and maintaining the health of oral tissues and has been used as a source of non-invasive investigation of metabolism and the elimination of many drugs (De Almeida *et al.,* 2008). However, it receives little attention until its quantity diminishes or its quality becomes altered. At present, saliva represents an increasingly useful auxiliary means of diagnosis (Tabak, 2001; Malamud, 2010). Many researchers have made use of sialometry and sialochemistry to diagnose systemic illnesses, monitoring general health, and as an indicator of risk for diseases creating a close relation between oral and systemic health (Khalili and Biloklytska 2008; Lasisi and Fasanmade, 2012; Bertl *et al.,* 2013; Rathnayake *et al.,* 2013).

Several infectious diseases have been reported to produce marked and identifiable oral and salivary changes (Shahar *et al.,* 2008; Fung *et al,* 2012; Lazarevic *et al.,* 2013; Li *et al.,* 2014), and malaria being one of these diseases can affect the oral cavity, with salivary gland involvement. Since many oral and systemic conditions manifest themselves as changes in the flow and composition of saliva (King *et al.,* 1994; Dodds *et al.,* 2000), malaria may cause alteration in salivary gland function and saliva composition. More so, many metabolic and hematologic complications develop in patients with malaria infection (Bartoloni and Zammarchi, 2012) which can lead to changes in the oral cavity and salivary composition and secretion. Assessment of changes in salivary secretion and composition in malaria infected individuals can serve as indicators of their oral health condition and probably, biological markers of the disease in these individuals. Some oral symptoms of malaria include conditions such as taste impairment, altered sensation, oral dryness, metallic taste and oral ulcers (Owotade and Greenspan, 2008; Scully, 2008) which may be associated with changes in salivary secretion. However, comparative analysis of salivary secretion and composition in malaria positive and negative individuals has not been previously documented to the best of our knowledge. This study was therefore designed to compare salivary secretion and composition in malaria negative and malaria positive individuals.

MATERIALS AND METHODS

Study Population

The study included 90 human subjects (40 malaria

parasite positive and 50 malaria parasite negative individuals; age and sex matched). The participants were consecutive patients attending the General Out Patients Department, University College Hospital, Ibadan. The study received ethical clearance and approval by the University of Ibadan/University College Hospital Research Ethics Committee (UI/EC/13/0099). Participants were provided information regarding risks and benefit of the study and consent was taken. Individuals with severe malaria, other systemic diseases like diabetes mellitus and hypertension and those on medications were excluded. Biodata and oral symptoms (associated with malaria) of the participants were assessed using self-administered proforma.

Malaria Diagnosis

Malaria diagnosis was achieved by microscopic examination of Giemsa stained thick and thin film of blood smears by two independent microbiologists. Thick-film smears were prepared from blood (venipuncture) at the time of presentation, dried and stained with 10% Giemsa. The smears were inspected for parasites by microscopy under 200 x magnifications by the microbiologists. At least 100 malaria parasites and 200 white blood cells were counted. The density of parasites per microliter of blood was calculated with reference to 8,000 white blood cells per microliters according to the Guidelines for the laboratory diagnosis of malaria (Bailey *et al.*, 2013).

Saliva Collection

Saliva collection was undertaken between 8 a.m. and 10 a.m. and participants had not had meal for at least 2 hours. Unstimulated saliva was collected by spitting method. Participants were asked to spit (after rinsing the mouth with distilled water) into calibrated universal plastic bottles for a period of 10 minutes. Rate of resting saliva secretions were expressed in mls/mins and the pH of saliva samples were determined using a calibrated pH meter (PH-012 Portable pH Meter, China). Volumes of the secretions were recorded and stored at -20^{o}C until laboratory analysis. Saliva samples were defrosted at room temperature and then centrifuged at 3000 rpm for 10 minutes before being used for laboratory analysis in order to remove extrinsic contaminants such as oral epithelial cells, micro-organisms and food debris.

Analysis of Salivary Ions

Saliva collected was analyzed for the concentrations of K^+, Na^+, Ca^{2+}, Cl^-, PO_4^{2-} and HCO_3^{2-}. For the determination of salivary ions, saliva was diluted at 1/100 and K^+, Na^+ and Ca^{2+} concentrations were determined using flame emission spectrophotometry. Concentrations of Cl^- and HCO_3^- were determined by Schales method using mercuric nitrate (Schales and schales, 1941) while concentrations of PO_4^- was determined using Cyrus Fiske and Subbarow's method (Fiske and Subbarow, 1925).

Analysis of salivary total protein

Saliva samples were defrosted at room temperature and then centrifuged at 3000 rpm for 10 minutes before use. Total protein concentration expressed as mg/dl was determined using established colorimetric methods with the use of Helios spectrophotometer by reading samples at 720nm (Spectrumlab 23A, Techmel and Techmel, Texas, USA). Bovine serum albumin was used for calibration purposes.

Statistical Analysis

The main outcome variables were mean values of salivary flow rate, pH, total proteins, sodium, potassium, calcium, chloride, bicarbonate and phosphate in malaria positive and malaria negative individuals. Data are presented as median with interquartile range. Data were compared using Independent-Samples Mann-Withney U test. Spearman's correlation test was used to assess the relationship between oral symptoms in malaria parasite positive individuals and the salivary parameters. The level of statistical significance was set at $p < 0.05$.

RESULTS

There were 90 participants comprising 64 females and 26 males with a mean age of 29 ± 7.5 years (range: 18 to 45 years). Malaria parasite positive and malaria parasite negative participants were age and gender matched (Table 1).

Among the malaria parasite positive individuals, the reported oral symptoms are shown in table 2. There were no significant correlations between the presence of oral symptoms and the salivary parameters (Table 3).

The salivary flow rate was significantly reduced in malaria parasite positive) individuals compared to malaria parasite negative individuals (P = 0.001). The salivary pH and total protein levels were not significantly different comparing malaria parasite positive individuals and malaria parasite negative

Table 1: Demographic characteristics of the participants

	Malaria Parasite Positive Individuals	Malaria Parasite Negative Individuals
Males	13	15
Females	27	35
M:F	1:2.1	1:2.3
Age (yrs)	29 ± 7.73	29.76 ± 7.51

M:F= Male to female ratio

Table 2**.** Oral symptoms in malaria parasite positive individuals (N=40)

Oral symptom	Frequency	Percentage
Mouth bitterness	10	25
Dry mouth	2	5
Altered taste	8	20
Mouth bitterness + dry mouth	7	17.5
Mouth bitterness + altered taste	4	10
Mouth bitterness, dry mouth + altered taste	2	5
No symptom	7	17.5

Table 3**.** Salivary flow rate (SFR), pH and Total protein levels between the groups

	Malaria Parasite Positive N = 40	Malaria Parasite Negative N = 50	P value
Flow rate (mls/min)	1.65 (1.03)	2.70 (1.83)	0.001
pH	7.13 (6.91)	7.20 (7.04)	0.79
Total protein (mg/dl)	0.5 (0.3)	0.5 (0.3)	0.90

Table 4. Salivary electrolyte concentrations in malaria parasite positive and negative participants (Data are presented as median (interquartile range)

Salivary Electrolytes	Malaria parasite positive N = 40	Malaria parasite negative N = 50	P value
Na^+ (mmol/L)	15.45 (11.00)	16.00 (13.75)	0.53
K^+ (mmol/L)	25.10 (20.83)	24.60 (20.48)	0.89
Cl^- (mmol/L)	39.00 (29.78)	39.50 (31.75)	0.79
HCO_3^- (mmol/L)	4.00 (3.00)	4.10 (3.03)	0.93
Ca^{2+} (mmol/L)	3.55 (2.03)	4.4 (2.45)	0.39
PO_4^{2-} (mmol/L)	17.80 (15.15)	18.60 (13.68)	0.86

Table 5: Correlation between presence or absence of oral symptoms and salivary parameters

Salivary parameter	Correlation coefficient	P value
Flow rate	0.13	0.43
Total protein	0.03	0.86
pH	0.04	0.81
Sodium	0.19	0.23
Potassium	-0.15	0.35
Chloride	0.14	0.40
Bicarbonate	0.06	0.74
Calcium	-0.13	0.44
Phosphate	-0.14	0.38

individuals (Table 4). Although, there were changes in the salivary electrolytes (K^+, Na^+, Ca^{2+}, Cl^-, PO_4^{2-} and HCO_3^{2-}) concentrations in malaria parasite positive individuals compared to their concentrations in malaria parasite negative individuals, there was no significant difference in the electrolyte ion concentrations between the two groups (Table 5).

DISCUSSION

Saliva plays an essential role in the maintenance of oral health. It is a unique fluid that can be used to monitor both oral and systemic diseases. Its secretion and composition are the major factors responsible for its physiological functions (Tabak, 2001; De Almeida *et al.*, 2008). Alterations in these factors have been implicated in various oral and systemic diseases (Khalili and Biloklytska 2010; Lasisi and Fasanmade, 2012; Bertl *et al.*, 2013; Rathnayake *et al.*, 2013).

In this study, the decrease in salivary flow rate observed could be due pyrexia which usually accompanies malaria infection. Pyrexia is common clinical feature of malaria which may result in hyperthermia leading to dehydration. The hypothalamic control of temperature involves water loss as a compensatory mechanism to lower the raised body temperature (Vybiral *et al.*, 2000). This may explain the reduction in salivary flow rate of malaria positive individuals because dehydration has been associated with reduced salivary flow rate (Falcao *et al.*, 2013). In addition, malaria is commonly associated with nausea, vomiting, diarrhoea and abdominal cramps especially in children (Bartoloni and Zammarchi, 2012) which may also contribute to dehydration resulting in reduced salivary flow rate.

Although some studies have shown the presence of protein biomarkers in malaria positive individuals (Poinsignon *et al.*, 2008; Huang *et al.*, 2012), this did

not manifest as changes in the total protein concentration levels observed in this study. Lack of difference in the salivary total protein in malaria positive individuals compared to malaria negative individuals may be due to its generally low salivary level that is independent of blood protein level. However, there has been no notable report about decrease or increase of salivary total protein in malaria positive individuals. Also in this study, the salivary pH levels were within the normal range and also indicated that malaria infection did not affect the salivary pH level. This may imply that malaria infection does not affect the buffering capacity of saliva hence malaria positive individuals may not be more susceptible to dental caries.

In this study, there was no change in the electrolyte ion concentrations between the two groups. This could be due to the fact that the secretory mechanisms for the various electrolytes in saliva of malaria positive individuals are not affected. Generally, it is expected that the concentrations of the electrolytes would be affected due to the reduced flow rate observed in malaria positive individuals but the lack of significance difference might be due to whole saliva sample used in this study. Gland specific saliva samples are more appropriate for the assessment of secretory mechanisms of saliva (Lofgren *et al.*, 2012) which is one of the limitations of this study.

Similar to the report of previous studies (Scully, 2008; Owotade and Greenspan, 2008), malaria parasite positive individuals reported oral symptoms like dry mouth, altered or metallic taste, as well as bitter taste. However, these symptoms did not show correlation with the salivary factors assessed. Generally, it has been observed that feeling of dry mouth or altered taste may not indicate altered biochemical composition of saliva and more importantly that of the electrolytes. Dry mouth has been described as subjective sensations of dryness of the oral mucosa which may not be associated with salivary glands hypofunction in some individuals. Hence, lack of significant change in the salivary biochemical composition of malaria parasite positive individuals as well as the lack of association between these factors and oral symptoms reported support the subjective nature of the conditions. Also presence of these symptoms in the malaria parasite positive individuals may be attributed to other components of saliva that were not assessed in this study.

Malaria diagnosis in this study was made using the microscopic examination of giemsa stained thick film for malaria parasites which is the gold standard. The detection limit by thick film microscopy is claimed to be in the 100 to 5 parasites per microliter (pl) range on a microscopic slide (Wongsrichanalai *et al.*, 2007). Quality of the data obtained through microscopy can significantly vary, because microscopic accuracy largely relies on the experience and training of the assessor. Thus, in low parasitemia cases, microscopy is ineffective and unreliable in malaria detection (Harris *et al.*, 2010) which is another limitation of this study. Moreover, studies have shown that microscopy underestimates true parasite counts as parasites are likely to be washed off or lysed during Giemsa staining (Bejon *et al.*, 2006).

CONCLUSION

Findings from this study suggest that salivary flow rate is reduced in malaria parasite positive individuals. However, no significant change in salivary pH, total protein and electrolytes was observed in malaria positive individuals. Also there was no association between oral symptoms reported by malaria parasite positive individual and the salivary factors. Further studies including longitudinal follow up and experimental studies are needed in this area.

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