Alpha amylase activity in saliva of humans infected with
Plasmodium falciparum malaria

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ABSTRACT

The secretion of ptyalin (salivary α-amylase, SA-A) into saliva is stimulated by the sympathetic nervous system controlled by cerebral function. P. falciparum malarial infection alters cerebral activities, yet information on the effect of P. falciparum malarial infection on the production and activity of α-amylase in saliva of infected patients has remained scarce especially in Abraka, Delta State, Nigeria. In this study, the activities of salivary α-amylase (SA-A) in both male and female (20-34 years) subjects infected with varying severity (1+ to 3+) of P. falciparum were assayed. Thirty malarial infected patients were selected from the Out Patients’ Department, General Hospital, Abraka. Twenty consenting individuals in apparent good health and without malarial infection were randomly selected from the hospital’s community and included as controls. About 3ml of fresh saliva was collected from each volunteer and α-amylase was assayed by the p-nitrophenylheptaoside method as earlier described. Results show that P. falciparum malarial infection significantly (p<0.05) reduced SA-A activity value (117.62±30.25 IU/L) when compared with the value (251.83±26.52 IU/L) obtained from the uninfected (control) subjects. Observations show no gender or age bias but severity strongly influenced data. Experimental evidence (SA-A activity values) indicates alteration in cerebral function even when cerebral malaria has not been clinically recognized. Data also validate the usefulness of SA-A activity value as surrogate biomarker of cerebral function, especially regarding the activities of the autonomic and sympathetic nervous system.

Keywords: Plasmodium falciparum, malaria, saliva, α-Amylase, Abraka.

INTRODUCTION

Malaria is a mosquito-borne disease of the blood caused by a parasite: Plasmodium, which is transmitted to human by the bite of infected female Anopheles mosquito [1]. Although, this epidemic is highly prevalent in the undeveloped and developing countries it constitutes a major burden for health care providers globally [2]. Between 200 and 500 million new cases of malaria occur annually with an estimated 1.7 to 3 million deaths mostly among children in the Sub-Saharan African countries and sub-tropical regions including parts of America and Asia [2, 3, 4]. Malaria development occurs through two phases; an exoerythrocytic and erythrocytic phase [5]. The exoerythrocytic phase involves infection of the hepatic (liver) system whereas the erythrocytic phase involves infection of the erythrocytes [6]. In most severe cases of the malaria infection, fatality rates can exceed 20% even with intensive care and treatment [4], although, in most endemic areas, treatment is often less satisfactory and the overall fatality rate for all cases of malaria can be as one in ten [4].
The causative organism (Plasmodium) of malaria has been studied and observations have shown that only four species (P. falciparum, P. vivax, P. ovale and P. malariae) can effectively cause malaria in humans [7].

The most dangerous of these species of Plasmodia is the P. falciparum [7]. The dangerous potency of P. falciparum is centered on its ability to digest the blood cells it inhabits [7], and this causes the cells the cells to stick to the wall of the blood vessels including the capillaries of the brain; causing obstruction of blood flow which leads to a chemical pathological condition known as cerebral malaria [8, 9]. Therefore, P. falciparum malarial infection alters cerebral functions including the sympathetic and parasympathetic nervous system [10]. These nervous systems stimulate the production of saliva, the main package and carrier of ptyalin or salivary α-amylase [11].

Salivary alpha amylase (S-AA) is an enzyme that catalyzes the breakdown of starch into simple sugars [12]. This enzyme is present in the human saliva where it begins the chemical process of digestion [12]. It is a glycoside hydrolase that acts on α-1,4-glycosidic bonds [13]. Alpha amylases are calcium metalloenzymes, completely unable to function in the absence of calcium [14]. Alpha amylase is the major fast-acting digestive enzyme of carbohydrates in human with an optimum pH of 6.7 – 7.0 [14].

Several new studies [15, 16, 17] have shown salivary α-amylase to be a surrogate marker of autonomic and sympathetic nervous system. [18] demonstrated a positive correlation between salivary α-amylase and sympathetic tone. Albeit, the effect of P. falciparum malarial infection on the activity of salivary α-amylase and associated alterations in cerebral functions especially regarding the regulation of the sympathetic and parasympathetic nervous systems are scarcely documented in our environment. This article therefore, reports the effect of P. falciparum malarial severity, gender and age on salivary α-amylase activity.

**MATERIALS AND METHODS**

**Sample Collection:** Saliva samples were collected from 50 consenting individuals between the ages of 20 – 34 years. Thirty (30) of the subjects were infected with varying severity (1+1 to 3+) of P. falciparum malaria while the remaining 20 subjects were without P. falciparum malarial infection. Infected patients yet to be treated were selected from the Out patients’ Department, General Hospital, Abraka, Delta State, Nigeria. The gender and age-matched individuals in apparent good health and without the P. falciparum malarial infection were randomly selected from the hospital’s community. About 3 ml of fresh saliva was collected from each participant into plain, sterile container without preservative but was immediately ice cooled and analyzed within 4 hours of collection.

**Sample analysis:** α –Amylase activity values in the saliva samples were assayed by the p–nitrophenylheptaoside method as previously described [19]. The presence or absence of P. falciparum was confirmed by the Giemsa stain of thin and thick blood smears. The reagents used for the assay were supplied in commercial kits by Randox Laboratories, Ardmore, England.

**Statistics:** Student’s ‘t’ test was used employed to compare the means of the parameters and the differences between means were considered significant at p<0.05.

**RESULTS**

Results of the salivary alpha amylase (SA-A) activity values of patients with and without P. falciparum malarial infection are shown (Tables 1-4).

| Table 1: Salivary alpha amylase activity values of malaria infected and uninfected subjects |
|-------------------------------------------------|------------------|
| No of patients (n) | With P. falciparum infection | Without P. falciparum infection |
| SA-A (µ/l) | 117.62±30.25* | 251.83±26.52 |

*Significantly different when compared with subjects without malarial infection.

SA-A=Salivary α-amylase.

Subjects with P. falciparum malarial infection showed a significant lower SA-A mean activity value than their control; subjects without malaria infection at p≤0.05.
Table 2: Impact of malaria severity on SA-A activity value

<table>
<thead>
<tr>
<th>Salivary α-amylase activity (µ/l)</th>
<th>P. falciparum infection</th>
<th>No. P. falciparum infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++ (n=10)</td>
<td>72.30±9.82S</td>
<td>244.10±33.02</td>
</tr>
<tr>
<td>++ (n=8)</td>
<td>111.33±24.35</td>
<td></td>
</tr>
<tr>
<td>+ (n=12)</td>
<td>126.40±29.17</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for “n” subjects

*Significantly different when compared with subjects with malaria infection (P. falciparum)

SA-A=Salivary α-amylase.

In subjects with malaria infection, SA-A reduces with increase in seventy from 1+ to 3+, although differences were not statistically significant (p<0.05), but comparison of all the three malarial severity groups with the uninfected group show significant differences (p<0.05) in all cases.

Table 3: Gender difference in salivary amylase induced by malarial infection.

<table>
<thead>
<tr>
<th>Salivary α-amylase activity (µ/l)</th>
<th>Male (n)</th>
<th>Female (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum infection</td>
<td>108.84±39.49*</td>
<td>113.28±22.62*</td>
</tr>
<tr>
<td>No P. falciparum infection</td>
<td>248.40±30.37</td>
<td>239.80±34.94</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for “n” subjects

*Significantly different when compared with corresponding gender matched control subjects.

The male subjects with malarial infection, showed an insignificant (p>0.05) lower SA-A mean value compared with the female patients, but for the uninfected, female.

Subjects showed insignificant (p>0.05) lower mean SA-A value.

The SA-A mean values for both male and female patients infected with P. falciparum were significantly (p<0.05) reduced when compared with the gender-matched values obtained from the uninfected individuals.

Table 4: Group Significance of age in salivary amylase activity induced by P. falciparum malarial infection

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Salivary α-amylase activity (u/L)</th>
<th>P. falciparum infection</th>
<th>No P. falciparum infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-24 (n=13)</td>
<td>112.67±15.17</td>
<td>244.00±11.78</td>
<td></td>
</tr>
<tr>
<td>25-29 (n=10)</td>
<td>117.93±29.44</td>
<td>247.00±16.87</td>
<td></td>
</tr>
<tr>
<td>30-34 (n=17)</td>
<td>122.00±34.45</td>
<td>264.50±37.96</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for “n” subjects.

*Significantly different when compared with the same age group of the uninfected (control) subjects.

Among the P. falciparum infected subjects, SA-A showed a progressive but non-statistically significant (p>0.05) increase in mean values as age advanced.

For the uninfected subjects, a similar progressive, non-significant (p>0.05) increase was observed as age group increased.

Comparison of age group SA-A values among the P. falciparum infected subjects with the same age group in the subjects without P. falciparum infection showed a statistically significant (p<0.05) decrease in corresponding SA-A mean values.

DISCUSSION

The saliva obtained from the P. falciparum malarial infected patients had significantly reduced activity of α-amylase when compared with the value of the saliva collected from uninfected subjects. This reduction was observed to be strongly influenced by the malarial severity. The higher the severity, the lower the saliva α-amylase and this trend showed no gender or age bias. Male and female, young and old were affected alike.
The production of saliva: the main package and carrier of ptyalin (salivary alpha amylase) is stimulated by the sympathetic nervous system and the parasympathetic nervous system [11]. Sympathetic stimulation of saliva facilitate respiration whereas parasympathetic stimulation facilitates digestion through the release of acetylcholine (ACH) into the salivary acinar cells [11]. The release of ACH elicits a cascade complex of calcium concentration increment which causes the vesicles within the salivary acinar cells to fuse with the apical cell membrane to form secretion. The release of ACH also stimulates the release of kallikrein from the salivary gland and this converts kininogen to lysyl-bradykinin which functions to produce the saliva containing salivary alpha amylase [11].

The reduced SA-A mean value induced by *P. falciparum* infection is therefore not surprising, since malarial infection has been shown to cause alterations in cerebral functions including the sympathetic and parasympathetic nervous system [4, 10, 20]. This altered cerebral function results in appetite loss and decreased carbohydrate digestion observed in *P. falciparum* malaria infected subjects. As ACH production is reduced the cascade complex in the formation and secretion of salivary alpha amylase in saliva also reduces; and this is the hallmark of the cerebral disturbance.

Our study has proved experimental evidence (SA-A values) that demonstrates *P. falciparum* induced disturbances in cerebral functions especially concerning its role in regulating the activities of the sympathetic and parasympathetic nervous systems. This further validates the usefulness of salivary α-amylase (SA-A) as surrogate biomarker of the activities of autonomic and sympathetic nervous systems. Again, *P. falciparum* malarial infection could disturb cerebral functions even when cerebral malarial has not been diagnosed or clinically recognized.

REFERENCES