PLASMA NITRITE LEVEL, METHEMOGLOBIN LEVEL AND ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITY IN APPARENTLY HEALTHY INDIVIDUALS

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ABSTRACT

Nitrite is a well-known etiology of hemoglobin oxidation in biological system. It is present in a variety of daily diet apart from metabolism of nitric oxide and nitrate in the body. Once ferrous iron of hemoglobin is oxidized to ferric form, it loses the capacity of carrying oxygen molecules. On another hand, superoxide ions play a role in inactivation of nitric oxide in vivo. Superoxide dismutase (SOD) which neutralizes superoxides was poorly understood regarding with the metabolism of endogenous nitrite and methemoglobin formation. In this study, we aimed to determine the plasma nitrite level, methemoglobin level and erythrocyte superoxide dismutase activity in apparently healthy adults. We used simple spectrophotometric methods to determine the plasma nitrite level, methemoglobin level and erythrocyte superoxide dismutase activity in apparently healthy adults in Magway Township, Myanmar.Plasma nitrite level was 542 ± 139nmol/L in healthy adults. Methemoglobin constitutes to 1.88 ± 0.78 % of total hemoglobin while erythrocyte SOD activity was found to be 1.88 ± 0.78 U/g Hg in study population. In addition, we proved that there is a significantlypositive correlation between endogenous nitrite level and methemoglobin concentration (r = 0.6021). However, erythrocyte superoxide dismutase activity was evaluated to have no significant correlation with plasma nitrite level (r = -0.0137) nor methemoglobin level (r = 0.1644).

Keywords: nitrite, methemoglobin, superoxide dismutase, normal ranges

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INTRODUCTION

Methemoglobin is a form of hemoglobin in which functional ferrous iron is oxidized to ferric state. Once methemoglobin is formed, it loses the capacity of carrying oxygen [1]. Methemoglobin can result from a variety of etiologies including genetic conditions and acquired exposure to oxidizing agents. Among them, nitrite is a well-known compound that can oxidize ferrous iron of the heme [2]. In spite of being exposed to minute quantity of nitrite, methemoglobin level is normally kept at low values by cellular NADH cytochrome b_5 methemoglobin reductase system [3].

Nitrite is present in cereals, vegetables, baked goods and cured meat [4]. In addition, it can be generated from nitric oxide and nitrate in vivo [5,6]. Superoxides interact with nitric oxide to form nitrite inside the body [7]. Superoxides, on the other hand, are neutralized by an antioxidant enzyme, superoxide dismutase (SOD), and forming hydrogen peroxides [8].

The correlation between superoxide dismutase activity and nitrite in the blood is still poorly understood. The purpose of this study is to determine normal ranges of plasma nitrite level, methemoglobin level and erythrocyte SOD activity in healthy adults. Furthermore, we examine the possible correlations between plasma nitrite level, methemoglobin level and erythrocyte SOD activity in apparently healthy individuals.

MATERIALS AND METHODS

After providing all the necessary information about the study, written informed consents were taken from apparently healthy adults living in six different quarters of Magway Township, Myanmar. Subjects with chronic diseases, taking antioxidant supplements and oxidizing drugs and who have known hemolytic disorders were excluded from the study. Six milliliters of venous blood were taken and collected in EDTA tubes. The sample tubes were kept in ice-water slurry during the transport to the Common Research Laboratory of University of Medicine-Magway.

Methemoglobin determination was undertaken within 5 hours after sample collection by using Sato's method [9]. Together, total hemoglobin level was measured by using colorimetric kits available from Human Gesellschaft fur Biochemic and Diagonostic ambH, Germany. The instruction of the manufacturers was followed. Methemoglobin level was expressed in terms of percentage over total hemoglobin value. Erythrocyte SOD activity was analyzed by the method of Sun [10]. The activity was expressed in unit per gram of hemoglobin molecules (U/g Hb). One unit of SOD is the amount of the enzyme to reduce N-1-Naphthyethylenediamine dihydrochloride by 50%. Plasma nitrite level was determined by Titheradge's method [11]. The level was expressed in nanomole per liter (nmol/L).

The data were expressed as mean with standard deviation. Pearson correlation was used to determine the correlations between the parameters. p-value less than 0.05 was regarded to be statistically significant.

RESULTS

The biochemical parameters of the study group was shown in table (1). The mean age of the subjects was 33.3 ± 9.56 years. Plasma nitrite level was 542 ± 139 nmol/L, methemoglobin level was 1.88 ± 0.78 % of total Hb and erythrocyte SOD activity was 785 ± 310 U/g Hb. Figure (1) indicates the correlation between plasma nitrite and methemoglobin levels. The correlation coefficient (r value) was 0.6021

and it was statistically significant ($p < 0.00001$). In figure (2), the Pearson's correlation between methemoglobin level and erythrocyte SOD activity was -0.1644 and it was not statistically significant ($p = 0.07277$). As shown in figure (3), the correlation coefficient (r) between plasma nitrite and erythrocyte SOD activity was found to be -0.0137, and it was not statistically significant as well ($p = 0.8819$).

TABLE I: AGE AND BIOCHEMICAL PARAMETERS OF STUDY GROUP

	Mean ± SD
Age (years)	33.3 ± 9.56
Plasma nitrite (nmol/L)	542 ± 139
Methemoglobin (% of total Hb)	1.88 ± 0.78
Erythrocyte SOD activity (U/g Hb)	1.88 ± 0.78

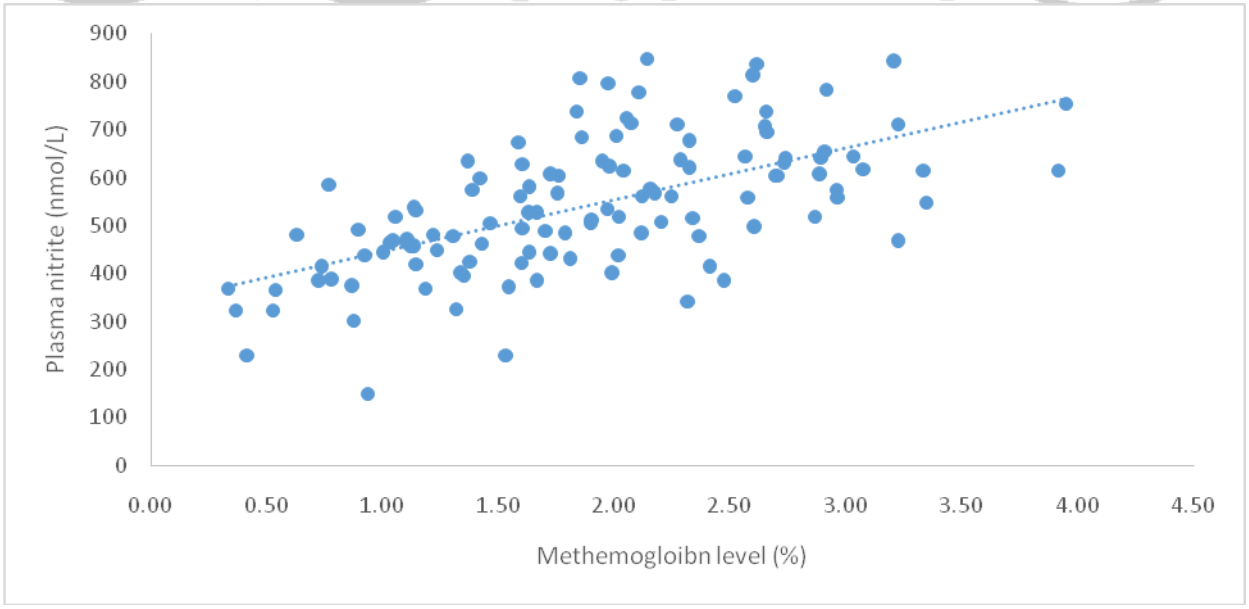

Fig. 1: Correlation between plasma nitrite and methemoglobin level

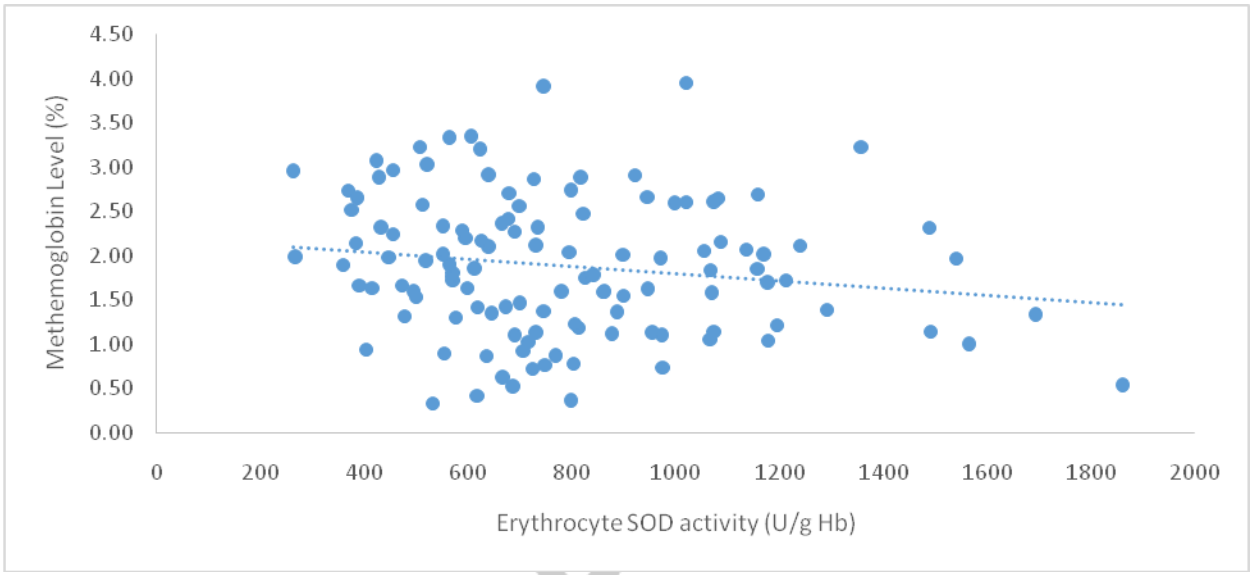

Fig. **2: Correlation between methemoglobin level and erythrocyte SOD activity**

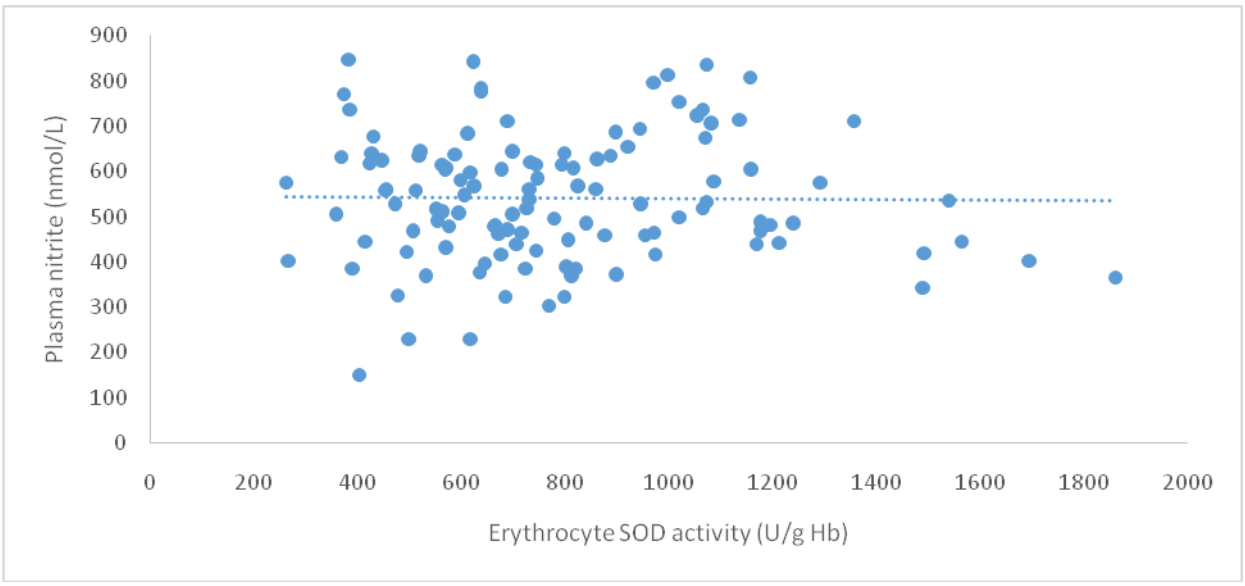

Fig. 3: Correlation between plasma nitrite and erythrocyte SOD activity

DISCUSSION

In this study, we have found out the normal values of plasma nitrite level as 542 ± 139 nmol/L in apparently healthy individuals in Myanmar.Kelm and colleagues stated the normal range for plasma nitrite was 127-748 nmol/L [12]. It has been reported that plasma nitrite level can increase in inflammatory conditions such as rheumatoid arthritis and in sepsis and after physical exercise [13-15]. The level may reduce in case of endothelial dysfunctions and is directly proportional to cardiovascular risks [16]. In this study, we could define the normal plasma nitrite level in apparently healthy individuals and this data could be utilized as a reference as compared with other conditions which can alter plasma nitrite level.

The present study indicated the normal methemoglobin level was 1.88 ± 0.78 % of total hemoglobin. Previous literature demonstrated the normal methemoglobin concentration was less than three percent of total hemoglobin [17, 18]. Agin and coworkers regarded methemoglobin level more than two percent as subclinical methemoglobinemia and found that six percent of the hospital professionals in Tehran, Iran had subclinical methemoglobinemia [19]. In our study, 43.33% (52 out of 120 healthy individuals) would have subclinical methemoglobinemia if we used such definition. However, the methods used were not the same as we performed Sato's method using potassium cyanide and potassium ferricyanide [9].

The mean of erythrocyte SOD activity in this study was 785 ± 310 U/g Hb for apparently healthy Myanmar adults. Dhana and coworkers indicated the erythrocyte SOD activity in healthy individuals as 1263.2 ± 7.22 U/gHb while another study conducted by Comelekoglu and Mazmanci stated the normal value as 884.51 ± 447.19 U/g Hb [20, 21]. It can be suggested that body weight, races, dietary factors and environmental factors might influence antioxidant enzyme activities from one nation to another.

There was a strong positive correlation between plasma nitrite level and methemoglobin concentration in our study. It might reflects nitrite-induced methemoglobin formation in the erythrocyte. Nitrite reacts with oxyhemoglobin to form nitrate and methemoglobin. One of the electrons from heme iron converts the bound oxygen molecule to superoxide radical. The other electron reacts with hydrogen peroxide to form water [22]. On another hand, nitrite reacts with deoxyhemoglobin to generate nitric oxide, methemoglobin and hydroxyl radical [23]. Hemoglobin acts as nitrite reductase with the peak nitric oxide production rates near relaxed to tense transition. It also deviates from the second order kinetics [24].

The weak negative correlation between methemoglobin level and erythrocyte SOD activity was found in present study but it was not statistically significant. In the study of Quebac and Chang, SOD can prevent methemoglobin formation in chemically cross-linked hemoglobin in Tris-hydrochloric acid buffer

[25]. Lynch et al stated that cells with reduced SOD activity can form methemoglobin readily on exposure to superoxide radicals [26]. In one of the animal studies, nitrite poisoning led to a significant fall in SOD activity in rats [27]. Although methemoglobin formation due to superoxide radicals can be prevented by SOD, further research is still needed whether SOD can prevent hemoglobin oxidation due to other kinds of oxidizing agents. In present study, as methemoglobin level had weak negative correlation with erythrocyte SOD activity, we postulatederythrocyte SOD may be one of the preventive factors in hemoglobin oxidation. Another possible link between erythrocyte SOD and nitrite-induced methemoglobin formation is the inactivation of SOD by the products of nitrite reaction. Hydroxyl radicals which is release from nitrite reaction on deoxyhemoglobin attack copper-binding histidine residues at the active site of SOD. This leads to copper loss and inactivation of SOD [28].

CONCLUSION

Plasma nitrite may influence the level of methemoglobin in apparently healthy individuals. In addition, erythrocyte SOD may a play role in the prevention against nitrite-induced methemoglobin formation in vivo. However, there was no association between plasma nitrite level and erythrocyte SOD activity in healthy adults.

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