Superoxide dismutase activity levels in parturients on intermittent preventive treatment with sulphadoxine pyrimethamine and their controls at current confinements

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Abstract

Malaria prevention in pregnancy especially in endemic areas has been greatly emphasized. This is especially so during the antenatal periods. Malaria prevention is important for reducing the problems of malaria in pregnancy. Early trimester miscarriages, preterm labours, anaemia in pregnancy, small for date babies and increased admissions into special care baby units are some of the problems encountered that are traceable to malaria infection. It is also noteworthy that some favourable antioxidants have been noted to help pregnant women enjoy and cope with their pregnancies. In that same way, it has also been noted that optimal levels of superoxide dismutase is equally necessary to help pregnant women enjoy uneventful pregnancies as well as stay free from some problems associated with malaria in pregnancy.

Objectives: We have comparatively and objectively assessed the plasma Levels of superoxide dismutase in Parturients on Intermittent Preventive Treatment with Sulphadoxine Pyrimethamine and their Controls at Current Confinements.

Method: This comparative study was conducted at the Obstetrics and Gynaecology Department of the Federal Medical Centre (FMC) Owerri, South East Nigeria. Owerri is widely known for its heavy malaria endemicity. Prior to participant recruitment, appropriate ethical clearance was applied for and obtained from the ethics committee of FMC Owerri. This enabled the commencement of the longitudinal recruitment of participants after due counseling and adequate informed consent involving both study groups. This bench-based research and cross-sectional descriptive study involved two hundred and ninety-six (296) consented participants. The participants were made to understand clearly the study protocol as well as satisfy the inclusion criteria for either the study (case) or control groups. Upon recruitment,
participants were then followed up all throughout their entire antenatal course culminating in the delivery. The follow up enabled the collection of blood samples for the estimation of Superoxide Dimutase (SOD) plasma levels at delivery. The SOD levels were estimated using the Misra and Fridovich method, 1972. The ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion (O2) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion introduced increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD.

Data analysis: Data computation and analysis were done using the computer Software Package for Social Science (SPSS) version 20.0 (SPSS, Inc, 2007, Chicago). Important descriptive statistics (mean, standard deviation, range, percentages etc) were determined for continuous variables. P-value less than (<0.05) at 95% confidence interval was considered statistically significant.

Result: The mean serum level of superoxide dismutase (SOD) in the study group was 12.61 U/ml ± 3.105 while the minimum and maximum serum superoxide dismutase were 7 and 20 U/ml respectively. For their controls, the mean serum level of superoxide dismutase was 11.69 U/ml ± 2.969. However, the minimum and maximum serum superoxide dismutase were 7 and 20 U/ml respectively. The difference was found to be statistically significant (p= 0.011) with odds ratio 1.12 (CI of 95% 1.024-1.193).

Keywords: Anaemia in pregnancy; Antenatal Course; Malaria endemicity; Sulphadoxine Pyrimethamine; Superoxide Dimutase

1. Introduction

The plasmodium parasites in mosquitoes that spread malaria belong to the plasmodium genius with over a 100 types of Plasmodium parasites capable of infecting a variety of species (Peter Lam, 2018). Plasmodium has about five identified disease-causing species namely: plasmodium falciparum, p. vivax, p. ovale, p. malariae and p. knowlesi. In Nigeria 98% of all important cases of malaria are caused by the most lethal specie, the plasmodium falciparum. This is the specie that is responsible for resistance and the severe forms of the disease that may lead to coma and death. (Getachew & Tsige, 2016; FMOH, Nigeria, 2005).

The World Health Organization (WHO), in 2017 defined Malaria as a life-threatening disease, caused by plasmodium protozoan parasites that are transmitted to people through the bites of an infected stubborn female Anopheles mosquitoes. It is a preventable and curable disease which in 2017 caused an estimated 219 million cases of malaria infection in 87 countries of the world with an estimated malaria death of about 435 000 in the same year (WHO, 2017).

Funding for malaria control and elimination in 2017 reached a total of about 3.1 billion US dollars. Of this total sum, the entire endemic countries were only able to contribute about 28% of the sum (WHO, 2017).

Every hour, it is estimated that about 720 Nigerian women become pregnant while an estimated 25 million pregnancies are believed to occur annually in malarious areas of the sub-Saharan Africa (Girma, et al. 2020; Issa, 2012; Falade et al., 2007). It is worthy of note that all these pregnant women are exposed to the dangers of malaria in pregnancy (asymptomatic/symptomatic/undetected/detected/untreated/poorly treated). On the other divide, are the treated patients who hardly get elaborate and desired relief for a good time owing to ready continuous re-infections.

Increased incidences of abortions (miscarriages), premature labour, intra-uterine death of the foetus with delivery of macerated foetuses are seen to be higher in women who had untreated malaria in pregnancy. While on the mother, we may notice lowered resistance to infections with increased incidence of puerperal sepsis. The incidences of intractable heart failures are equally increased. Chronic ill-health in pregnancy may results from untreated or inadequately treated anaemias. These anaemias are most likely from untreated malaria infections (Ojo and Briggs, 2006).

Nigeria is said to suffer the greatest malaria burden in the world, with approximately 51 million cases and 207,000 deaths reported annually representing an approximate of 30 % of Africa’s total malaria burden, while 173 million of her total populace, an approximate of 97% are at risk of infection (Salwa et al., 2016).
Virtually all pregnant women in malaria endemic areas predisposed to the dangers of malaria in pregnancy. It was reported in the past that malaria accounted for about 11% of maternal deaths (Isah, 2012; FIGO, 2010). It should be noted that in the past, considerable attention had been invested while trying to prevent malaria in pregnancy. The case of the present day and the expectations of the future certainly may not be entirely different. The numerous problems of malaria infection to the pregnant mother, her unborn fetus and the neonate clearly justifies all these efforts of malaria prevention. Owing to this, strategies for the prevention of malaria in pregnancy has evolved through several protocols from the past to the present day.

From the past antimalarial chemoprophylaxis has been generally recommended to prevent the adverse effects and consequences of malaria in pregnancy. To achieve this in the past, African countries adopted antimalarial prophylaxis using weekly pyrimethamine or chloroquine and this had wide acceptance in the past (Falade et al., 2007; WHO Report, 1986). It is rather unfortunate that the efficacy of these congeners as malaria chemoprophylactic agents has long been destroyed by the emergence of multi drug resistant strains of Plasmodium falciparum and poor compliance. In a study at Ibadan, Intermittent Preventive Treatment with Sulphadoxine Pyrimethamine (IPT-SP) was found to be effective in preventing maternal and placental malaria as well as improving pregnancy outcomes among parturient women in Ibadan, Nigeria (Falade et al., 2007). Currently, the use of IPT-SP for the prevention of malaria in pregnancy as recommended by the WHO is still in vogue as well as practiced in malaria endemic areas of the world. The practice needs continuous evaluations and emphasis to keep succeeding in preventing malaria in pregnancy.

Yet unfortunately for IPT-SP, its effectiveness is also being threatened by increasing levels of resistance to SP across Africa (Campbell, Baraah, Narauin & Rogers, 2006; Alloueche et al., 2004; Plowes et al., 2004; Ringwald, 2004; White, 2004; EANMAT, 2003).

A high energy demand and an increased oxygen requirement usually accompany the pregnant state even as an increased use of oxygen can lead to generation of oxidative stress. Patil and co noted that pregnant women were more prone to oxidative damage than their non pregnant counterparts. They concluded this by measuring the thiobarbituric acid reactive substance (TBARS), enzymatic antioxidants like Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and catalase in both the pregnant and non-pregnant women (Patil, Kodliwadmath, M. V. & Kodliwadmath, S. M, 2007).

Yet, the onset of many infections like malaria may trigger antigenic activation of the body's immune system with consequent release of reactive oxygen species (ROS). Besides this, the malaria plasmodium can also cause some cells to produce ROS and hence bring about haemoglobin degradation (Tiyong et al., 2009; Kulkarni, Suryakar, Sardeshmukk & Rathi, 2003; Das and Nanda, 1999).

1.1. Malaria, superoxide dismutase (sod) and antioxidation

Pregnancy is associated with a high energy demand of virtually all bodily functions and an increase in oxygen requirement which can increase the level of oxidative stress which itself is an increase in the levels of free radicals in the body. The Hydroxyl, Superoxide and Nitric oxide are free radicals derived from molecular oxygen and nitrogen. They are highly reactive metabolites named Reactive Oxygen Species, ROS (Bassi, Sharma, Maths, Kaur, M. & Kaur, D, 2017).

Claudio and co investigated Oxidative Stress (OS) and antioxidant capacity in pregnant women. Their results showed an increased amount of MDA, a prooxidant, in women during the third trimester of gestation. In parallel with this change, there was an increase in the amounts of the antioxidants SOD and CAT during the same period. These changes may be related to the pregnancy course itself and the placental circulation playing an important role in oxidative stress during this period (Claudio et al., 2011).

Other studies also reviewed literatures of enzymatic antioxidants. In one of such review, Superoxide dismutase (SOD) activity in erythrocytes and plasma Thiol levels were found to be lower while Ceruloplasmin levels were found to be higher in pregnant women than in their non pregnant counterparts, suggesting a diminished antioxidant defense (Wisdom, Wilson, Mckillop & Walker, 1991; Ilouno, Shu & Igboke, 1996; Adiga and Adiga, 2009).

Available articles on non enzymatic antioxidant levels in pregnancy reported diminished plasma ascorbic acid concentrations in normal pregnancy (Hubel, Kagan, Kisin, McLaughlin & Roberts, 1997; Adiga and Adiga, 2009) as well as a reduction in non enzymatic antioxidants (GSH, vitamins A, E, C) during normal pregnancy is also reported (Patil et al. 2006; Adiga and Adiga, 2009).
Shrivastava and co studied the Free Radicals and Antioxidant Enzyme Status in Normal Pregnant Women. They discovered a significant increase during normal pregnancy of the erythrocyte antioxidant enzyme, superoxide dismutase (SOD), p<0.01 and glutathione peroxidase (GPx), p<0.001 (Shrivastava et al. 2015).

SOD is the important antioxidant enzyme with an antitoxic effect against the super oxide anion. It's (SOD) over expression is likely an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide while GPX is an oxidative stress inducible enzyme that plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes (Chandra et al., 2000; Shrivastava et al. 2015).

In the Wdowiak and co study above, SOD activity in erythrocytes in the examined group was the lowest in the control group= 1375.33±60.31 U/gHb. In the erythrocytes of healthy pregnant women SOD was slightly increased, while a considerable increase in the activity of this enzyme was observed in erythrocytes of pregnant women burdened with diabetes. An increase in SOD activity in erythrocytes of pregnant women with diabetes was statistically significant, compared to the group of healthy pregnant women and the control group, p<0.001 (Wdowiak et al. 2015). This statistical difference may also be true for malaria infection.

Tiyong and co also evaluated oxidative stress and antioxidant status of pregnant women suffering from malaria in Cameroon. They discovered that Patients with malaria parasitemia had significantly higher levels of Superoxide Dimutase (SOD) than the healthy controls (Tiyong et al., 2009).

Wdowiak and co in their study concluded that, in pregnancies complicated by diabetes, the SOD activity in blood was higher than those for women with physiological pregnancy and the control group. However, the SOD activity was also noticed to be significantly reduced in the placental tissues of diabetic women (Wdowiak et al. 2015).

Hengbo and colleagues investigated MDA, SOD and interleukin (IL)-6 levels in the lung tissue of a rat model of acute pulmonary edema induced by acute hypoxia, and its pathophysiological significance and found out that the levels of MDA and SOD in the lung tissue of rats in the acute hypoxic groups B-D were measured as representative values in edema at different times, and compared with those of group A normal rat group. The results demonstrated that the MDA levels increased whereas SOD activity decreased in the rats in group B (acute hypoxic group), as compared with those in group A. This was not significant as P>0.05. However, the MDA levels and SOD activity in the lung tissue of rats with pulmonary edema were significantly altered as the duration of edema increased (Hengbo et al., 2016).

From the forgoing there is need to assay the levels of this antioxidant, SOD in pregnant women on Intermittent Preventive Treatment for malaria so as to compare that with their control. Hence the need for this study in Nigeria, a heavy malaria endemic country in Sub Saharan Africa.

Also, there is minimal scholarly works on Superoxide Dimutase levels and their interactions with Intermittent Preventive Treatment with Sulphadoxine Pyrimethamine in Parturients who received Intermittent Preventive Treatment with Sulphadoxine Pyrimethamine in pregnancy. Hence, this study was conducted to ascertain a comparative assessment of the Plasma Superoxide Dimutase Levels in Parturients who Received Intermittent Preventive Treatment with Sulphadoxine Pyrimethamine (IPT-SP), and their Controls during their Current Confinements. It is believed that the results of this study will help guide policy makers and stakeholders in obstetrics and gynecological practice in adopting strategies that will ensure better pregnancy outcomes for our women. The research question is: Does pregnancy affect the levels of Superoxide Dimutase Activity in malaria endemic areas of the world?

2. Material and methods

2.1. Study location

The study was done at Federal Medical Centre (FMC) Owerri. The facility is located at the Hospital Road (Amakohia Rd) Owerri, the capital city of Imo State South-East Nigeria. Owerri is a malaria endemic area and is densely populated. Malaria accounts for most of the out patient’s visits in the hospitals in Owerri. Owerri city though urban, is surrounded by sub-urban, semi-rural communities with lots of bushes, forests, slumps, unkempt gutters with dirty waters, open wells and swamps etc. All these factors help to encourage mosquito breeding and consequent malaria transmission.

2.2. Patient recruitment

Ethical certificate was applied for and obtained from the ethics committee FMC, Owerri. This is a prospective Cohort study in which the 296 participants were finally recruited following adequate counseling and informed consent.
Actually, a total of 456 parturients were initially assessed for eligibility. 56 of them were eliminated while about 400 completed the filling of the questionnaire. In the course of the study duration, some selected candidates defaulted at some stage of the study leaving us with 296 participants who were studied in detail and adequately followed up. A semi-structured interview based questionnaire was used as a qualitative study instrument.

2.3. Study population and recruitment

The population were those of pregnant women who used the Federal Medical Center, Owerri for their antenatal care within the period of study. The recruited antenatal women who sought for antenatal care services and satisfied the inclusion criteria were recruited longitudinally, enabling a Purposive Sampling technique that was used for participant selection. Initially, recruitment was based on those consented pregnant women without any symptoms nor signs of malaria (clinical diagnosis) and neither laboratory diagnosis of malaria (parasitological diagnosis). The recruited pregnant women must have witnessed quickening (first fetal movements en-utero) usually from about the 16th week of pregnancy depending on their parities.

2.4. Inclusion criteria

To qualify for recruitment into the study, consenting pregnant woman with the following characteristics were considered for inclusion. They included women without symptoms nor signs of malaria infection, those pregnant women that are resident in Owerri or its suburbs to encourage follow up and the willingness to deliver at FMC Owerri was made compulsory for their inclusion. Women who are ready to take IPT-SP as directly observed treatment (DOT) and have not had any antimalarial agent in the past 2 weeks.

2.5. The control group

On the other hand, the control group essentially consisted of pregnant women who only presented for delivery (unbooked) or those who registered very late for antenatal care (last month of pregnancy / late booking). Pregnant women with known allergies to sulphadoxine-pyrimethamine (SP) were also considered for control group inclusion. In considering the control participants, a good history was very essential to exclude the ingestion of SP before presentation. All these women must have also met other criteria to merit inclusion into the control group.

2.6. Exclusion criteria

Following adequate assessment, the following intending participants were excluded from the study: Those women with malaria infection as evidenced by the symptoms, signs and laboratory evidence of the infection. Equally all women with Haemoglobin AS were also excluded from the study. Also excluded were women with coexisting medical conditions like diabetes, hypertension, nephrosis, sickle cell disease (SCD), human immune deficiency virus (HIV) infection, congestive cardiac failure (CCF) or other conditions/co-morbidities suspected to be able to affect the results of the study. Also excluded were women who had taken antimalarial drugs within 2 weeks prior to recruitment and those with known allergies to any component of the drug combination, SP. Women who live far from study base at Owerri were also excluded since that might discourage effective follow-up and delivery in the hospital.

2.7. Statistical analysis

The results from this study were presented as mean ± std. deviation. Computer Software Package for Social Science (SPSS) version 20.0 was used to analyze the obtained results after computation (SPSS, Inc, 2007, Chicago). The mean, standard deviation, range, percentages (Descriptive statistics) were determined for continuous variables while independent Sample t-test was used to compare the means of continuous variables for the two groups studied. The results obtained were presented as tables, bar charts, histograms and box plots as seen in the next section while P-value less than (<0.05) at 95% confidence interval was considered statistically significant.

2.8. Determination of superoxide dimutase (sod) activity

The level of vitamin SOD was estimated by the Misra and Fredovich method (1972).

2.8.1. Principle

The ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion (O2) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion introduced increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of
adrenaline proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD.

2.8.2. Procedure

To 80 µl of sample/blank in a clean test tube was added 1000 µl of carbonate buffer (pH 10.2). The resulting solution was mixed thoroughly, and allowed to equilibrate by incubating at 37 °C for 5 minutes. Thereafter, 600 µl of freshly prepared epinephrine was added and the reaction mixture was read at 30 seconds interval for 150 seconds at 480 nm. The blank was treated the same way except that 80 µl of distilled water was used instead of plasma. The changes in absorbances of both test and blank were determined. The % inhibition of auto-oxidation of epinephrine by SOD was calculated and the plasma SOD activity was expressed as U/ml. One unit of SOD activity was equivalent to the amount of SOD that can cause 50% inhibition of epinephrine.

Calculation:

\[
\text{% inhibition} = \left( \frac{\Delta OD_{\text{blank}} - \Delta OD_{\text{test}}}{\Delta OD_{\text{blank}}} \right) \times 100
\]

Enzyme Unit (U/ml) = (% inhibition/50) X dilution factor (Misra and Fridovich, 1972).

3. Results

During participant recruitment, 456 pregnant women were assessed for eligibility out of which 56 were not eligible. Questionnaire was administered to the remaining 400 eligible participants with 90 of the defaulting at some point. 310 were enrolled (recruited) after questionnaire administration. 14 of these pregnant women were later excluded from the study. This left us with a total of 296 of the women that were analyzed. These 296 pregnant women comprised of 148 parturients that received the IPT-SP and served as the study / case group and 148 parturients in the control group that did not receive the IPT-SP.

Table 1 The Effects of Superoxide Dismutase in both the study and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>min</th>
<th>max</th>
<th>Mean</th>
<th>Std.dev</th>
<th>coef</th>
<th>S.e.</th>
<th>p-value</th>
<th>OR</th>
<th>95% C.I.for OR</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD Case</td>
<td>148</td>
<td>7</td>
<td>20</td>
<td>12.61</td>
<td>3.105</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD Control</td>
<td>148</td>
<td>7</td>
<td>20</td>
<td>11.69</td>
<td>2.969</td>
<td>0.010</td>
<td>0.039</td>
<td>0.011</td>
<td>1.12</td>
<td>1.024</td>
<td>1.193</td>
<td></td>
</tr>
</tbody>
</table>

The mean Superoxide Dismutase (SOD) for the women in the treatment group was 12.61 ± 3.11. The minimum and maximum SOD was 7 and 20 respectively. For the control group, the mean SOD was 11.69 ± 2.97 while the minimum and maximum SOD was 7 and 20 respectively.
3.1. Influence of Superoxide Dismutase in both the study and control groups

On box plot 1, the mean SOD was higher in the study group (12.61 U/ml) than what was found in the control group (11.9) U/ml. The boxes were heavier upwards indicating rightwards skewness (clearly observable of the Histogram 1 above). The difference in the mean values were found to be statistically significant with \( p = 0.011 \), 95% CI =1.024 – 1.193.

4. Discussion

The results of this study showed that the mean serum level of SOD in the study (case) group was 12.61 U/ml while in the control, it was 11.69 U/ml. The difference was found to be statistically significant (\( p = 0.011 \)) with odds ratio 1.12 (CI of 95% 1.024-1.193). This was also illustrated in the corresponding histogram in the result section above. The result obtained may mean that SP did alter the enzymatic antioxidant defense profile and possibly conferred the high SOD recorded in the study group relative to their controls.

In the work by Adisa and co, the effect of orally administered SP on the erythrocytic SOD activity of rabbits was studied. Data obtained demonstrated a significant increase (\( p = <0.05 \)) in the activity of erythrocytic SOD with time and compared with the results of this result. SOD activity increased by 4.9, 63.4 and 120.8% at 6, 12 and 24 h, respectively after drug...
administration. Similarly, CAT activity increased by 44.5, 82.6 and 116.3% at 6, 12 and 24h respectively, compared to the control values. This was able to demonstrate that treatment with SP alters the enzymatic antioxidant defense profile and induces oxidative stress in the body (Adisa et al., 2011).

In another report, the level of SOD was higher in the malaria infected and anaemic women compared to their non-infected and non-anaemic counterparts, respectively though the difference not statistically significant. The levels of SOD, NO and CAT increased with decreasing leukocyte accumulation in the intervillous space. Baby birth weight increased significantly with SOD and CAT levels, but decreased with levels of GSH (Megnekou, et al., 2015).

The Atiba et al study above also demonstrated that Superoxide dismutase activity was significantly lower in the third trimester of pre-eclamptic pregnancy when compared to the second trimester (110.40±59.47 Vs 118.01±64.41 U/ ml) (p<0.039). Similarly, superoxide dismutase activity was significantly lower in the third trimester of normal pregnancy (110.40±59.47U/ml) than in the second trimester (153.01±71.85U/ml) and p<0.0001 (Atiba et al., 2014).

5. Conclusion

From the study also we can deduce that treatment with SP alters the body’s enzymatic antioxidant defense profile. The plasma superoxide dismutase level was higher among the study group than the control and the differences were statistically significant as well. While the role of antioxidants in pregnancy course helps to underscore the importance of malaria prevention in pregnancy, the practice of intermittent preventive treatment for malaria in pregnancy should continue to receive due emphasis even as the component drugs of Intermittent Preventive Treatment with Sulphadoxine Pyrimethamine needs to be protected from drug resistance.

Compliance with ethical standards

Acknowledgments

Several people whose names were not enlisted as authors above helped us in the carrying out of this research work. The effort of our research assistants (house officers, nurses and midwives at FMC Owerri) and other laboratory attendants are worthy of mentioning. May the good LORD bless you all and meet you at the point of your needs, Amen

Disclosure of conflict of interest

The authors unanimously hereby declare that there is no conflict of interest till date and do not envisage any in the future

Statement of ethical approval

Ethical certificate was applied for and obtained from the ethics committee Federal Medical Center.

Statement of informed consent

All the participants were duly counselled on the extent of their participation and the need and importance to be compliant with the drug, Sulphadoxine Pyrimethamine (for the study/cases) was equally discussed. The need to also deliver the babies at the study center, FMC Owerri was discussed too and this agreement was gotten from each of the participants before final enrollment. The above enabled them to make their informed consent before they were finally recruited into the study. This was done for both the study and control groups.

References


