JASEM ISSN 1119-8362

Full-text Available Online at www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *September*, 2009
Vol. 13(3) 13 - 18

Comparative Study of Glutathione S-Transferase Activity of Three Human Erythrocyte Genotypes Infected With *Plasmodium falciparum.*

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ABSTRACT: Investigation to ascertain the levels of glutathione S-transferase (GST) activity of three human erythrocyte genotypes (HbAA, HbAS and HbSS) obtained from apparently healthy and clinically confirmed malarious subjects/volunteers was carried out. The incubation of human erythrocytes with 1-chloro-2,4-dinitrobenzene (CDNB) resulted in almost quantitative conjugation of glutathione (GSH) to form S-(2,4-dinitrophenyl) glutathione. The reaction formed the basis for the spectrophotometric determination of GST activity. The levels of GST activity of the red cell genotypes was in the order of HbAA<HbAS<HbSS. There was no significant difference (p>0.05) between GST activity of HbAA and HbAS erythrocytes. The results also showed red blood cells infected with *Plasmodium falciparum* exhibited significantly lower levels of GST activity compared to erythrocytes obtained from apparently healthy non-malarious individuals. Furthermore, gender did not significantly (p>0.05) affect erythrocyte GST activity of non-malarious subjects/volunteers. The study suggests that GST activity evaluation might be a reliable biochemical marker and possess promising rational for diagnostic potential in malaria. @ JASEM

Four species of intracellular protozoa of the genus *Plasmodium* cause malaria. *Plasmodium falciparum* is the most dangerous and remains the world's most common devastating human parasitic infection (AMR, 2003; Snow *et al.,* 2005). This parasitic infection is affecting more than 500 million people and causing from 1.7 million to 2.5 million deaths each year of which children less than 5 years of age, pregnant women and non-immune individuals are preferentially affected (WHO, 1995). Human beings usually are affected by sporozoites by bite of infected female mosquitoes (genius Anopheles); although malaria can be transmitted by transfusion of infected blood (Ali and Kadaru, 2005) and by sharing needles (Tracy and Webster, 2001). The parasites have a complicated life cycle that requires a vertebrate host for the asexual cycle and female *Anopheles* mosquitoes for completion of the sexual cycle. Marcus *et al.*, (1978), in their findings on the purification and characterization of glutathione-S transferase from human erythrocyte reported that the enzyme molecular weight of the enzyme is 47,500MW and is composed of two subunits. The enzyme was active with a variety of compound bearing an electrophilic centre. Although the physiological role of this enzyme is not fully known, it has been suggested that the location of the enzyme in erythrocytes is ideal for the removal of circulating xenobiotics (Marcus *et al.*, 1978; Anosike *et al.,* 1991). Studies suggest that glutathione-S transferase occur in the erythrocytes primarily for the protection of erythrocytes against electrophilic compounds, rather than serving a general protective function in the body (Harvey and Beutler, 1982; Anosike *et al.,* 1991). Many studies of both humans and laboratory animals have documented strong associations between impaired or deficient GST activity and various diseases connected to oxidative stress injury. Such diseases include lung cancer, acute leukemia, and many others (Strange *et al.,* 1998; Ye and Song, 2005). Since malarial pathology progresses with increasing oxidative stress, the present study seeks to ascertain the influence of gender of human population on GST activity in malaria pathology. In addition, the study will investigate the influence of *P. falciparum* on metabolism of human three red blood cell genotypes (HbAA, HbAS and HbSS) with respect to levels of glutathione-S transferase activity. The findings may serve to provide reliable biochemical parameter for diagnosis in relation to the pathophysiology of malaria disease.

MATERIALS AND METHODS

Selection of Volunteers/ Experimental Design:

Non-Malarious Subjects/Volunteers (Control Group): Seventy (70) male (61-73kg) subjects/volunteers of confirmed HbAA (25), HbAS (25) and HbSS (20) genotypes between the ages of 20-28years enrolled for this study. The female category was made up of forty-two (42) subjects/volunteers (55-69kg); HbAA (20), HbAS (20) and HbSS (12). Their age bracket was between 18-27years.

Malarious Subjects/Volunteers (Test Group): Sixty-one (61) male (59-79kg) subjects/volunteers – HbAA (24), HbAS (25) and HbSS (12) and forty-three (43) female (55-76kg) subjects/volunteers, HbAA (15), HbAS (15) and HbSS (13) enrolled for this study. The male and female subjects were between the age brackets of 21-34 and 20-25 years respectively.

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Ethics: The institutional review board of the Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria, granted approval for this study and all subjects/ volunteers involved signed an informed consent form. This conducted study was in accordance with the ethical principles that have their origins in the Declaration of Helsinki. Individuals drawn were from Imo State University, Owerri, Nigeria and environs. The research protocols were in collaboration with registered and specialized clinics and medical laboratories.

Collection of Blood Samples/Preparation of Erythrocyte Haemolysate: Five milliliters (5.0ml) of venous blood obtained from the volunteers by venipuncture was stored in EDTA anticoagulant tubes. Blood of HbSS genotype and malarious blood samples were from patients attending clinics at the Federal Medical Center (FMC), Imo State University Teaching Hospital (IMSUTH), Orlu, St.John Clinic / Medical Diagnostic Laboratories, Avigram Medical Diagnostic Laboratories, and Qualitech Medical Diagnostic Laboratories. These centers are located in Owerri, Imo State, Nigeria. The erythrocytes were washed by methods as described by Tsakiris *et al.,*(2005). Within 2hours of collection of blood samples, portions of 1.0ml of the samples were introduced into centrifuge test tubes containing 3.0ml of buffer solution pH=7.4: 250mM tris (hydroxyl methyl) amino ethane–HCl(Tris-HCl)/140mMNaCl/I.0mMMgCl_2/10mMglucose).

The erythrocytes were separated from plasma by centrifugation at 1200g for 10minutes, washed three times by three similar centrifugations with the buffer solution. The erythrocytes re-suspended in 1.0ml of this buffer were stored at 4^0C. The washed erythrocytes were lysed by freezing/thawing as described by Galbraith and Watts, (1980) and Kamber *et al.,* (1984). The erythrocyte haemolysate was used for the determination of GST activity.

Determination of Erythrocytes Haemolysate Haemoglobin Concentration: A modified method (Baure, 1980), based on cyanomethaemoglobin reaction was used for the determination of haemolysate haemoglobin concentration. The expressed values were in grams per deciliter (g/dl). A 0.05ml portion of human red blood cell haemolysate was added to 4.95ml of Drabkin reagent. The mixture was left to stand for 10minutes and absorbance read at λ_{max}=540nm against a blank (Drabkin reagent only). The absorbance was used to evaluate for haemolysate haemoglobin concentration by comparing the values with the standards.

Determination of Erythrocyte Haemolysate Glutathione S-transferase Activity: GST activity was assayed spectrophotometrically by monitoring the conjugation of 1-chloro-2,4dinitro benzene (CDNB) with glutathione (GSH) at λ_{max}=340nm at 37^oC. (Habig *et al*., 1974).

$$CDNB + GSH \rightarrow CDNB - S - glutathione.$$

The enzyme assay was according to methods of Habig *et al.,* (1974) with minor modifications (Anosike *et al*.,1991).The 1.0ml in 2% ethanol enzyme assay mixture contained 0.5mMCDNB (0.02ml),1.0mMGSH(0.05ml),0.68ml of distilled water and 100mMPhosphate buffer (K_2HPO_4/KH_2PO_4; pH=6.5) (0.2ml).

The CDNB was pre mixed with the Phosphate buffer before use. The Phosphate buffer-CDNB mixture was pre-incubated for 10 minutes at 37^oC and the reaction started by adding GSH, followed immediately by an aliquot (0.05ml) of the haemolysate. The rate of increase in absorbance at λmax = 340nm was measured for 10minutes at 37^oC against a blank solution containing the reaction mixture, in which; the haemolysate was substituted with distilled water.

Statistical Analyses: The experiments were designed in a completely randomized method and data collected were analyzed by the analysis of variance procedure while treatment means were separated by the Least Significance Difference (LSD) incorporated in the Statistical Analysis System (SAS) package of 9.1 version.(2006).

Calculation of Enzyme Activity: The expression below was used to evaluate erythrocyte GST activity in international unit per gram haemoglobin (iu/gHb).

$$E_A = \frac{100}{[Hb]} \times \frac{0.D/min}{\sum} \times \frac{V_C}{V_H}$$

Where,

E_A = Enzyme activity in iu/gHb

[Hb] = Haemolysate haemoglobin concentration (g/dl)

0. D/min=Change per minute in absorbance at 340nm.

$\sum$ =Millimolar extinction coefficient = 9.6, in reaction in which 1mole of glutathione (GSH) is oxidized or reduced.

V_C = Cuvette volume (total assay volume) = 1.0ml.

V_H = Volume of haemolysate in the reaction system (0.05ml).

RESULTS AND DISCUSSION

The mean (±S.D) activity of glutathione S-transferase (GST) of three erythrocyte genotypes (HbAA, HbAS and HbSS) of blood samples obtained from non-malarious and malarious human subjects/volunteers is presented in Table 1 below.

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Table 1: Glutathione S-transferase Activity of Human Erythrocyte Haemolysate:

Genotype/Gender	GST Activity (iu/gHb) (X+/-S.D)	
	Non-malarious	Malarious
1).HbAA		
Male (n=25$^{NM;}$ 24^{M})	3.34±0.10^{a}	2.65±0.46^{b}
Female (n=20^{NM};15^{M})	3.35±0.10^{a}	3.05±0.49^{a}
2).HbAS		
Male (n=25$^{NM;}$ 25^{M})	4.29±0.11^{a}	2.79±0.20^{b}
Female (n=20^{NM}; 15^{M})	4.30±0.10^{a}	3.34±0.38^{a}
3).HbSS		
Male (n=20$^{NM;}$;12^{M})	12.22±1.50^{a}	12.26±0.81^{b}
Female (n=15$^{NM;}$ 13^{M})	11.57±0.95^{b}	13.19±1.04^{a}.

M and NM= number of malarious and non-malaroius blood samples respectively. Means in the column with the same letter are not significantly different at $p< 0.05$ according to LSD.

The mean (±S.D) activity of glutathione S-transferase (GST) in the three genotypes was in the order: HbAA<HbAS<HbSS irrespective of malarial status of the human subjects/volunteers. However, there was no significant difference ($p>0.05$) between mean (±S.D) erythrocyte GST activity of HbAA and HbAS erythrocyte genotypes of human subjects/volunteers (i.e. male and female individuals) irrespective of malaria status. On the contrary, the three erythrocyte genotypes of malarious female individuals exhibited significantly ($p<0.05$) higher erythrocyte GST activity than their male counterpart. A cursory look at Table 1 above showed that mean (±S.D) erythrocyte GST activity was significantly ($p<0.05$) decreased in *P. falciparum* infected blood when compared to non-malarious blood samples with the exception of those obtained from male subjects/volunteers of HbSS genotype. The recorded lowest value of mean (±S.D) erythrocyte GST activity was 2.65±0.46 iu/gHb for blood samples obtained from malarious male subjects/volunteers of HbAA genotype. Paradoxically, the highest mean (±S.D) erythrocyte GST activity of blood samples obtained from malarious female subjects/volunteers value was 13.19±1.04 iu/gHb. Furthermore, there was no significant difference ($p>0.05$) in erythrocyte GST activity between non-malarious and malarious male subjects/volunteers of HbSS genotype. Glutathione S-transferase (GST) activity, though not routinely assayed in clinical laboratories, could serve as a useful marker enzyme in diagnostic pathology. Over-expression of GST in the erythrocytes of patients with chronic renal failure (Galli *et al.,* 1999) and uremia (Galli *et al.,* 1999: Carmagnol *et al.,* 1981) have received immense attention and documentations. Patients with hepatocellular damage present elevated plasma GST activity (Mulder *et al.,* 1999: Beckett and Hayes, 1993). In addition, low GST activity and consequently impaired placental detoxification may represent a risk factor for recurrent early pregnancy loss (Zusterzeel *et al.,* 2000) and as an indicator of oxidative stress at birth (Neefjes *et al.,* 1999). The level of expression of GST could provide useful diagnostic parameter in carcinoma of the breast (Forrester *et al.,* 1990) and bladder (Engel *et al*., 2002). These events are of immense relevance from clinical pathology and toxicological standpoints.

Previous investigations by Sarini *et al.,* (1993), reported that increased parasitaemia accompanied decrease in the activities of enzymes of the glutathione system, namely glutathione peroxidase (GPx), glutathione reductase (GRx) and glutathione S-transferase (GST) in the red blood cells (RBC) lysates. In the present study, GST activity was significantly ($p<0.05$) decreased in *P. falciparum* infected erythrocytes, compared to red cells obtained from apparently healthy non-malarious individuals (Table 1). This observation was in concordance with the reports of Sohail *et al.,* (2007). In their views, decreasing GST activity might play important role in host defense mechanisms against malarial infection by up-regulating oxidative defense mechanisms. They further suggested that GST activity evaluation might be a reliable biochemical marker and possess promising rational for diagnostic and therapeutic potential in malaria. In the same vein, previous reports have shown that antioxidants such as glutathione (GSH), catalase and α-tocopherol were lower in patients with malaria (Becker *et al.,* 2004; Kavishe *et al.,* 2006) and visceral Leishmaniasis (Neupane *et al.,* 2008) than the control groups. Therefore, inoculation of the malarial parasites usually accompanies production of reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl radicals and nitric oxide as a host defense mechanism (Becker *et al.,* 2004). The accumulated ROS because of decreased antioxidant scavenging activities engenders vast membrane lipid peroxidation with concomitant membrane damage and oxidation of haemoglobin to methaemoglobin (Nuepane *et al*., 2008). Since there was no significant difference ($p>0.05$) in erythrocyte GST activity between non-malarious and malarious male subjects/volunteers of HbSS genotype (Table 1), it is probable the host erythrocytes did not deploy oxidative up-regulatory mechanism as a means for parasitic control and elimination. The available high oxidative potentials of these erythrocytes as earlier reported by Anosike *et al.,* (1991) must have fulfilled the antifecundity property of this erythrocyte genotype.

Another plausible reason for decreased GST activity of erythrocytes obtained from malarious human subjects; hinged on the pathophysiology of malaria disease have been described (Dubios *et al.,* 1995, Liebau *et al.,* 2002). Consequent upon ingestion and digestion of large quantities of erythrocyte haemoglobin by the parasite, it subsequently has to

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handle and deal with high amount of potentially parasitotoxic ferroprotoporhyrin IX (FPIX) - haemin. As mentioned earlier, *P. falciparum* GST (pfGST) are able to bind efficiently to FPIX (Harwaldt *et al.,* 2002). The uncompetitive interaction inhibits GST activity preferentially by binding to preformed GST-GSH complex (Hiller *et al.,* 2006). The significantly higher erythrocyte GST activity of malarious female erythrocytes (HbAA, HbAS and HbSS) compared to malarious male red cells (Table 1) seem to suggest the influence of sex hormones on *in vivo* enzyme activity. Reports indicate that mammalian GSTs are involved in intracellular transport of varieties of endogenous metabolites including sex hormones by their ability to bind to these compounds (Abramovitz *et al.,* 2006; Listowsky *et al.,* 1998). This interaction often impairs the catalytic activity of the enzyme (Salinas and Wong 1999: Sheeham *et al.,* 2001: Deponte and Becker, 2005). For instance, it has been demonstrated that testosterone and progesterone have varied capacities to bind to mammalian GSTs with moderate ($10^{-6} < K_d < 10^{-4}$ M) or high ($K_d < 10^{-6}$ M) affinity respectively (Listowsky *et al.,* 1998). Previous studies have also showed specific binding of testosterone to parasitic *Schistosoma haematobium* GST protein (Sh28GST) exhibited high affinity ($K_d = 2.57 \times 10^{-7}$ M) (Remoue *et al.,* 2002). This mechanism was closely associated with the reduction of *Schistosoma* fecundity in male individuals. Therefore, in human schistosomiasis, this GST-dependent effect of sexual steroids could have an influence on the age- and sex-dependent level of infection. Indeed, observations showed gender-dependent pattern of prevalence and intensity of infection after puberty for several parasitic species (Bundy, 1988). Since variability in erythrocyte GST activity of malarious human subjects/volunteers was gender dependent, the findings of the present study suggest sex hormones might have contributed to the significant difference ($p<0.05$) in GST activity between the two sexes infected with *P. falciparum*. Therefore, gender-dependent level of malarial infection showed similar patterns as described in human infection of *Schistosoma haematobium*. On the contrary, with respect to HbAA and HbAS erythrocytes of non-malarious individuals, there was no significant difference ($p<0.05$) between levels of male and female GST activity. In agreement with this observation, Zhang *et al.,* (2006) and Anosike *et al.,* (1991) stated that gender did not significantly influence GST activity of apparently healthy Chinese and Nigerian subjects respectively.

Comparative studies of human erythrocyte GST showed a variable activity in the order HbAA<HbAS<HbSS (Table 1). This observation conformed to previous reports as described by Anosike *et al.,* (1991). They noted that increased activation of the enzyme activity in HbSS and HbAS erythrocytes was because of the presence of high levels of oxidants in these cells (Shalev *et al.,* 1995). Therefore, there was a positive activation of this redox enzyme engendered by higher levels of oxidants in HbSS and HbAS compared to HbAA erythrocytes (Anosike *et al.,* 1991). The intermediate values of the enzyme activity of HbAS erythrocytes were obvious reflections of the hybrid nature of the heterogeneous the red blood cells (Anosike *et al.,* 1991).

* Corresponding author: Chikezie, P.C.

REFERENCE

Abramovitz, M; Homma, H; Ishigaki, S; Tansey, F; Crammer, W and Listowsky, I. (2006). Characterization and localization of glutathione S-transferases in rat brain and binding of hormones, neurotransmitters, and drugs. J. Neurochem. Vol.50. Issue 1, 50-57.

Ali, M.S.M and Kadaru, A.G.M. (2005): *In vitro* processing of donor blood with sulphadoxine/pyrimethamine for eradication of transfusion induced malaria. An. J. Trop. Med. Hyg. 73(6). 1119 – 1123.

Anosike, E.O; Uwakwe,A.A; Monanu, M.O and Ekeke, G.I.(1991).Studies on human erythrocyte glutathione-S transferase from HbAA, HbAS and HbSS subjects Biochem. Biomed. Acta. 50:1051-1055.

African Malaria Report (2003). Africa malaria report 2003. Geneva. WHO/UNICEF: http://www.rbm.who.int/amd2003/amr_toc.htm.

Baure, J.D.(1980). Laboratory investigation of hemoglobin. In: Gradwohl's Clinical Laboratory Methods and Diagnosis, (Editors) Sonnenwirth AC and Jarett L. St. Louis, MO: Mosby.809-902.

Becker, K; Tilley, L; Vennerstrom, J.L; Roberts, D; Rogerson, S and Ginsburg, H. (2004).Oxidative stress in malaria parasite infected erythrocytes: host – parasite interactions. Int J Parasitol. 34: 163 –189.

Beckett, G.J. and Hayes, J.D. (1993). Glutathione S-transferases: biomedical applications. Adv. Clin. Chem., 30, 281–380.

Bundy, D. A. P. (1988). Gender-dependent patterns of infection and disease. Parasitol. Today. 4**:**186–189.

Carmagnol, F; Sinet, P.M; Rapin, J and Jerome, H.(1981).Glutathione *S*- transferase of human red blood cells; assay, values in normal subjects and in two pathological circumstances hyperbilirubinemia and impaired renal function. Clin. Chim. Acta, 117: 209-217.

Deponte, M and Becker, K.(2005). Glutathione S transferase from malarial parasites-structural and functional aspects. Methods Enzymol. 401: 240 – 252.

Dubios, V.L; Platel, D.F; Pauly, G and Tribouley - Duret, J.(1995). *Plasmodium berghei*: Implication of intracellular glutathione and its related enzyme in chloroquine resistance *in vivo*. Exp. Parasitol. 81: 117-124.

Engel, L.S; Taioli, E; Pfeiffer, R; Garcia-Closas, M; Marcus, P.M. *et al.,*(2002). Pooled analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer: A HUGE review. Am. J.Epidemiol. 156: 95-109.

Forrester, L.M; Hayes, J.D; Millis, R; Barnes, D; Harris, A.L; Schlager, J.J; Powis, G; Wolf, C.R. (1990). Expression of glutathione *S*-transferases and cytochrome P450 in normal and tumor breast tissue. Carcinogenesis. 11**:**2163–2170.

Galbraith,D.A and Watts,D.C.(1980).Changes in some cytoplasmic enzymes from red cells fractionated into age groups by centrifugation in Ficoll^{TM}/Triosil^{TM} gradients. Comparison of normal human andpatients with Duchenne muscular dystrophy. Biochem.J.191:63-70.

Galli, F; Rovidati,S; Benedettis, S; Buoncristiani, U; Covarelli, C; Floridi, A and Canestrari, F. (1999). Over expression of erythrocyte glutathione S-transferase in uremia and dialysis. Clin. Chem. 45: 1781-1788.

Habig, W. H; Pabst, M. J and Jacoby, W. B. (1974). Glutathione *S*-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem.,249:7130-7139.

Harwaldt, P; Rahlfs, S and Becker, K. (2002). Glutathione S- transferase of the malaria parasite *Plasmodium falciparum* characterization of a potential drug target. Biol. Chem. 383: 821-830.

Harvey, J.W and Beutler,E.(1982).Binding of heme by glutathione-S-transferase:The first step in mercapturic acid formation. J.Biol.chem. 245:7130-7139.

Hiller, N; Fritz-Wolf,K; Deponte,M; Wende,W; Zimmerman,H and Becker,K.(2006). *Plasmodum falciparum* glutathione S-transferase-structural and mechanistic studies on ligand binding and enzyme inhibition. Protein Science. 15:281-289.

Kamber, K; Poyiagi, A; Delikonstantinos, G.(1984).Modifications in the activities of membrane-bound enzymes during *in vivo* ageing of human and rabbit erythrocytes. Comp. Biochem. Physiol. *B*.77B: 95-99.

Kavishe, R.A; Koenderink, J.B; McCall, M.B; Peters, W.H; Mulder, B; Hermsen, C.C; Sauerwein, R.W; Russel, F.G and Van Der Ven, J.A. (2006). Severe Plasmodium falciparum malaria in Cameroon: Associated with the glutathione S-transferase M1 null genotype. Am. J. Trop. Med. Hyg. 75(5): 827-829.

Liebau, E; Bergmann, B; Campbell, A. M; Teesdale – Spittle, P; Brophy, P.M; Luersen, K and Walter, R.D. (2002).The glutathione S-transferase from *Plasmodium falciparum*. Mol. Biochem. Parasitol. 124: 85-90.

Listowsky, I; Rowe, J.D; Patskousky,T.V; Tchaikovskaya, T,S; Shintani, N;Novikova,E and Nieves, E. (1998). Human testicular glutathione-S-transferase: Insight into tissue-specific expression of the diverse subunit classes. Chem. Biol. Interact. 111-112:103-112.

Marcus, C.H; Habig, W.H and Jacoby, N.B.(1978).Glutathione-S-transferase from human erythrocyte. Arch. Biochem. Biophys. 38:287-293.

Mulder, T.P.J; Court, D.A and Peters, W.H.M.(1999). Variability of glutathione S-transferase α in human liver and plasma. Clin. Chem. 45: 355-359.

Neefjes, V.M.E; Evelo, C.T.A; Bears, L.G.M and Blanco, C.E. (1999).Erythrocyte glutathione S-transferase as a marker of oxidative stress at birth. Arch. Dis. Child. Fetal Neonatal Ed. 81:F130 -F133.

Neupane, D.P; Majhi, S; Chandra, L; Rijal,S and Baral, N. (2008).Erythrocyte glutathione status in human visceral Leishmaniasis. Indian. J. Clin. Biochem. Vol. 23(1): 95-97.

* Corresponding author: Chikezie, P.C.

Remoue, F; Mani, J; Paungniere, M; Schacht, A; Capron, A and Riveau, G. (2002). Functional specific binding of testosterone to Schistosoma haematobium 28-Kilodalton glutathione S-transferase. Infect. Immun. 70(2): 601-605.

Salinas, A.E. and Wong, M.G. (1999).Glutathione *S*-transferases—A review. Curr. Med. Chem. 6**:** 279–309.

Sarin, K; Kumar, A; Prakash, A and Sharma, A . (1993). Oxidative stress and antioxidant defense mechanism in *Plasmodium vivax* malaria before and after chloroquine treatment. Indian J.malariology. Vol. 30, n^{o}3, pp. 127-133.

Shalev, O; Repka, T; Goldfarb, A; Grinberg, L; Abrahamov, A and Olievieri, N.F. (1995) Deferiprone (L1) Chelates pathologic iron deposites from membranes of intact thalasaemic and sickle red blood cells both *in vitro* and *in vivo*. Blood. 86: 2008-2013.

Sheehan, D; Meade, G; Foley, V.M and Dowd, C.A. (2001). Structure, function and evolution of glutathione transferases: Implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J. 360**:** 1–16.

Snow, R.W; Guerra, C.A; Noor, A.M; Myint, H.Y and Hay, S.I.(2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature. 434:214-217.

Sohail, M; Kaul, A; Raziuddin, M and Adak, T. (2007). Decreased glutathione S-transferase activity: Diagnostic and protective role in vivax malaria. Clin. Biochem. Vol.40. issue 5-6: 377-382.

Statistical Analytical System (SAS).(2006): Package 9.1 Version.

Strange R.C; Spiteri, M.A; Ramachandran, S and Fryer, A.A.(2001). Glutathione-S transferase family of enzymes. Mut. Res. 482:21-26.

Tracy, J.W and Webster, L.T.(2001).Drugs used in the chemotherapy of protozoan infections. In: (Adam, J.G, Limbird, L.E and Gilman, A.G (eds). Goodman and Gilman's Pharmacological Basis of Therapeutics. 10th Edition, McGraw-Hill,U.S.A.

Tsakiris, S; Giannoulia-Karantana, A; Simintzi,I and Schulpis, K.H. (2005).The effect of aspartame metabolites on human erythrocyte membrane acetylcholinesterase activity. Pharmaco. Res. 53:1-5.

World Health Organization Study Group, (1995). WHO vector control for malarial and other mosquito borne disease. World Health Organ. Tech. Rep. Ser. 857. World Health Organization, Geneva.

Ye, Z and Song, H.(2005). Glutathione s-transferase polymorphisms (GSTM1, GSTP1 and GSTT1) and the risk of acute leukaemia: a systematic review and meta-analysis. Eur. J. Cancer. 41: 980–989.

Zhang, J; Krugliak, M and Ginburg, H.(1999).The fate of ferriprotoporphyrin IX in malaria infected erythrocytes in conjunction with the mode of action of antimalarial drugs. Mol. Biochem. Parasitol. 99: 129 - 141.

Zusterzeel, P.L.M; Nelen, W.L.D; Roelofs, M.J; Peters, W.H.M; Blom, H.J and Steegers, E.A.P. (2000). Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. Molecular Human Reproduction. Vol. 6 No.5:474-478.

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