Relationship between Glutathione Levels and Activities of Detoxification Enzymes in *Anopheles gambiae* from Northwest Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author AAI designed the study, conduct the experiments, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author YD managed and supervised the study. Both authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The aim of this study is to investigate the relationship between levels of the three forms of glutathione and the activities of detoxification enzymes in *Anopheles gambiae* from northwest Nigeria.
Study Design: *Anopheles gambiae* larvae were sampled from breeding sites with marked differences in physico-chemical characteristics, grouped into three different study zones A, B & C on the bases of human related activities (intensive agriculture, domestic, and petrochemical activities, respectively) taking place within and/or around the breeding sites. The sampled larvae were reared until they emerged into pupae and adult.
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Methodology: Activities of the 3 major detoxification enzymes (P450, GST and α & β-esterases) were assayed on the sampled larvae and the emerged pupae and adult followed by the determination of the levels of total, oxidized and reduced glutathione across the three life stages.

Results: Following various statistical analyses, the activities of the detoxification enzymes were higher in zones A and C (which also recorded higher levels of the physico-chemical factors) compared to zone B. Also, the relative distribution of glutathione showed that the levels of total and reduced GSH across the three study zones appeared to be similar while the levels of oxidized glutathione (GSSG) appeared to be higher in study zone A and C compared to zone B. A deduced statistical model established the different forms of the glutathione variables as having a significant effect on the activities of the detoxification enzymes across the three life stages of *An gambiae* from northwest Nigeria.

Conclusion: These observations could allow for an evaluation of the possibility of targeting control of the activities of detoxification enzymes in mosquitoes through manipulation of the availability of GSH in the management of insecticides resistance in mosquitoes.

1. INTRODUCTION

According to the world health organization (WHO), about half of the world population (3.3 billion) is at risk of malaria. More than 216 million cases were reported in 2010 alone, with over 660, 000 deaths recorded [1]. Over 300 million people are infected yearly, mostly in areas of poverty and low economic growth [2]. According to the world health organization, about 109 countries were endemic for malaria in 2008, out of which 45 are found within the who African region [1]. Sub-Saharan Africa accounts for almost 90 percent of all malaria cases and deaths attributed to malaria alone stands at about 20 percent [1]. While many drugs have been and are still being developed to combat malaria, vector control by recourse to environmental management, educational programmes and the use of chemical and biological agents still remain the most effective alternative to reducing the risk of malaria parasite transmission [3]. Out of these alternatives, the use of chemicals in the form of insecticides and insecticide treated bed nets is probably the best available option for vector control in sub-Saharan Africa and other less developed parts of the world. With the evolution of resistance to insecticides, this too is facing serious challenges.

The physiological and biochemical role of glutathione in various organisms is multidimensional. In xenobiotic metabolism, the cysteinyl residue of glutathione provides a nucleophillic thiol responsible for the detoxification of electrophilic metabolites and metabolically generated oxidizing agents. The overall hydrophilicity and the net negative charge of glutathione are very important in its function of conjugation because they greatly increase the solubility of the lipophillic moieties with which it is conjugated. In addition, the high molecular weight of GSH increases the preference for the secretion of its adducts in the billiary system. Finally, its unique structure, including the N-terminal glutamyl residue linked through γ-glutamyl bondage to L-cysteine ensures high specificity for GSH-enzymes [4]. Glutathione is an important redox buffer because of its ability to remove excessive free radicals which is made possible by its cycling between the reduced and oxidized forms catalyzed by the enzymes glutathione reductase and glutathione peroxidase. In this process, reactive oxygen species (ROS) such as hydrogen peroxides produced during cellular respiration and metabolism of toxic substances are removed to protect cells from oxidative damage [5]. Glutathione protects cells from oxidative injury induced by free radicals and xenobiotic overload through either direct thiol conjugation or acting as substrate in GSH-dependent enzyme catalyzed reactions. thus GSH provides a first line of defense against reactive species by scavenging free radicals. Glutathione-dependent enzymes such as GST provide the next line of defense by using GSH to conjugate and ultimately detoxify various ROS and xenobiotic metabolites. Evidence has shown that many of the GSH-dependent enzymes including GST, glutathione reductase, glutathione peroxidase are coordinately induced through the antioxidant response elements (AREs) in response to oxidative stress induced by xenobiotic overload [6]. Several other studies have previously established the role of AREs and other cis and trans-acting transcription factors in the regulation of synthesis and activities of glutathione [7,8]. This suggests that GSH and GST-dependent

enzymes could be under coordinate control and therefore levels of various forms of GSH could be an important factor in the activities and regulation of synthesis of some of the GSH-dependent enzymes.

Many of the available information have tended to focus on the relationship between glutathione and GSH-dependent enzymes, evidence from studies in higher organisms have however shown that an indirect relationship could also be established between availability of GSH and other detoxification enzyme systems. For instance, a concomitant increase in the level of total glutathione and the activity of P450 enzymes was demonstrated in response to increased oxidative stress induced by a dose-wise treatment of benzo[a]pyrene in human hepg2 cells [5]. Findings from this and similar studies suggests that agents which induce oxidative stress in response to xenobiotic overload could induce a coordinated up-regulation of glutathione in conjunction with various xenobiotic detoxification enzymes other than GST.

The aim of this study is to investigate the relationship or correlation between levels of the three forms of glutathione and the activities of the major xenobiotic detoxification enzymes (GST, P-450 and Alpha & Beta-esterases) in *Anopheles gambiae* from northwest Nigeria. The role of these enzymes in the metabolism of environmental xenobiotics insecticides inclusive cannot be overemphasized. Managing metabolic insecticides resistance in mosquitoes (which involves activities of these enzymes) is one of the major global strategy in the fight against malaria. Since upregulation of these enzymes is one major mechanism mosquitoes have developed to resist insecticides, and the fact that glutathione is required by these enzymes (either directly or indirectly) as an integral part of the detoxification process, it is necessary to study the interrelationship between levels of glutathione and the activities of these enzymes in mosquitoes. This study, which, to our knowledge is the first of its kind conducted on *Anopheles gambiae* could provide a novel strategy in molecular and biochemical management of insecticides resistance in mosquitoes.

2. METHODOLOGY

2.1 Study Sites and Zones

The study was conducted across three different breeding sites designated as study zones A, B &

C. These zones were differentiated by the type of human related activities taking place around the mosquito breeding sites i.e. A; intensive agricultural areas; B, residential areas; and C, areas where petrochemical products are sold, processed, used and/or discharged. The breeding sites located in intensive agricultural zones and petrochemical areas consist of small puddles of stagnant water bodies. The water was found to be muddy, dirty, oily and obviously contaminated. The breeding sites in the domestic areas were larger with higher water volume and relatively clean. A total of three sites in study zone A, four in zone B and three in zone C were visited and sampled across the Nigerian states of Kano and Jigawa. Kano is situated in the northwest and has a four-season climate with a typical temperature range of 11-44°C and yearly rainfall of 1000 mm. Jigawa is also situated in the northwest, and is characterized by a Sahel savannah climate with a typical temperature range of 10-42°C and a yearly rainfall of less than 800 mm [9,10].

2.2 Preparation of Mosquito Homogenate

Mosquito samples, about 20 each of the larvae, pupae and adult, were homogenized in ice-cold phosphate buffer (0.1 m; ph7.2) in 1.5 ml microfuge tubes with pellet pestle motor (Kontes Anachem, Mettler Toledo, Luton, Bedfordshire, UK). The homogenization was carried out on ice and the homogenates were centrifuged for 1 min in a refrigerated centrifuge (Eppendorf Centrifuge 5417R, Motor Park Way, New York, United States) and the supernatants used for protein and detoxification enzymes assays. All the mosquito larvae used were of 4th instar, roughly of the same size and all the adult mosquitoes were one day old [11,12].

2.3 Protein Determination Assay

Protein concentration of each homogenate was determined by using Bradford reagent in a 96 well plates following manufacturer's instructions.

2.4 Determination of Reduced (GSH), Oxidized (GSSG) and Total Glutathione (tGSH)

Reduced, oxidized and total glutathione levels in the mosquito samples were determined using glutathione assay kit (NWLSSTM NWK GSH01, Northwest Life Science Specialties, LLC Vancouver, WA 98683). The assay was

conducted on the three life stages of *Anopheles gambiae* (larvae, pupae and adult) following manufacturer's instructions and procedures. The procedure employed for the assay is based on the enzyme recycling method described by Teitze [13]. The general thiol reagent, 5',5'-dithiobis[2-nitrobenzoic acid] (DTNB, Ellman's Reagent) reacts with GSH to form 5-thionitrobenzoic acid (TNB) which can be measured photometrically at 412 nm. In order to measure the levels of total glutathione (tGSH), any oxidized glutathione (GSSG) present in the sample was converted to GSH by glutathione reductase. Levels of oxidized glutathione was determined by first removing or quenching all reduced GSH present with 4-vinyl pyridine before addition of the Ellman's reagent and glutathione reductase. The difference between total and oxidized glutathione was used to obtain the levels of reduced GSH. All the glutathione levels were corrected for the milligrams of protein present in the samples and expressed as nmol/mg protein.

2.5 Data Analysis

Firstly, significance in mean distribution of the various forms of glutathione across the three study zones was investigated using mixed effect linear model with study zones as fixed factor and sites as random variables followed by Bonferoni post-hoc test for multiple comparisons. Then, linear regression analysis was carried out to investigate correlations or associations between detoxification enzymes activities and the levels of the three forms of glutathione. Furthermore, to assess the effect of the glutathione forms on the activities of the detoxification enzymes, preliminary multiple regression analysis indicated strong colinearity between model covariates. As a result of colinearity, the standard error estimates of the linear regression model get inflated and so the P-values indicating the contribution of different covariates to the model became unreliable. The colinearity problem was addressed by performing a regression in principal components, extracted from the model covariates. Factor analysis was employed to extracts principal components from the detoxification enzyme activities as well as the three forms of glutathione studied across the three life stages of *An. gambiae.* Finally, redundancy analysis was carried out to examine the association between a combination of the extracted glutathione components and those of the detoxification enzyme activities.

3. RESULTS AND DISCUSSION

3.1 Mean Distribution of the Three Forms of Glutathione across the Three Life Stages of *Anopheles gambiae*

The result of the one way ANOVA, which was used to investigate the differential mean distribution of the three forms of glutathione across three study zones showed that there were no significant differences (P= .562 and .139) in mean distribution of total glutathione (tGSH) at the larval and pupal stages of *An. gambiae* respectively (Fig. 1). The mean distribution at the adult stage was moderately significant (P= .033). While the significant differences in distribution was low in the two aquatic immature stages (larvae and pupae) and moderate in the adult stage, the P-values nonetheless showed a gradual progression in significance across the three life stages i.e. from larvae to adult.

The *An. gambiae* mosquitoes were sampled from three different breeding sites categorized into three study zones in Nigeria. zone A; intensive agriculture; B; domestic/residential areas and C; petrochemical/hydrocarbon laden breeding ecologies.

Bonferoni pairwise comparism test was used to examine zone-wise differences in mean total glutathione (tGSH) distribution across the three study zones. The result showed that at the larval stage, no significant difference (P=1.000) was detected when the three zones were compared against one another. The degree of significance improved a little at the pupal stage between A and B and B and C (P= .394 and .181) respectively while that between A and B remain the same (P=1.000). Finally, further increase in the degree of significance was recorded at the adult stage between A & C and B & C (P= .045 and .097) respectively. The pairwise significance between A and B remain the same at this stage (P=1.000) as in the two lower life stages (larvae and pupae). This means that the result of Bonferoni pairwise comparism followed the same pattern as that of the mixed linear model; significance increases across the life stages from the lowest to the highest (i.e. from larvae to adult). Similar observations were also recorded for the reduced glutathione (GSH).

However, observations recorded for oxidized glutathione (GSSG) were markedly different from those of total and reduced glutathione forms.

Firstly, the result of the one way ANOVA, which was used to analyze the differential mean distribution of GSSG across the three study zones, showed that there were statistically significant differences (p= .010, .000 and .000) in distribution of GSSG at the larval, pupal and adult life stages respectively, across the three study zones (Fig. 2). As in the other two forms of glutathione described above, the degree of significance also appeared to increase across the three life stages.
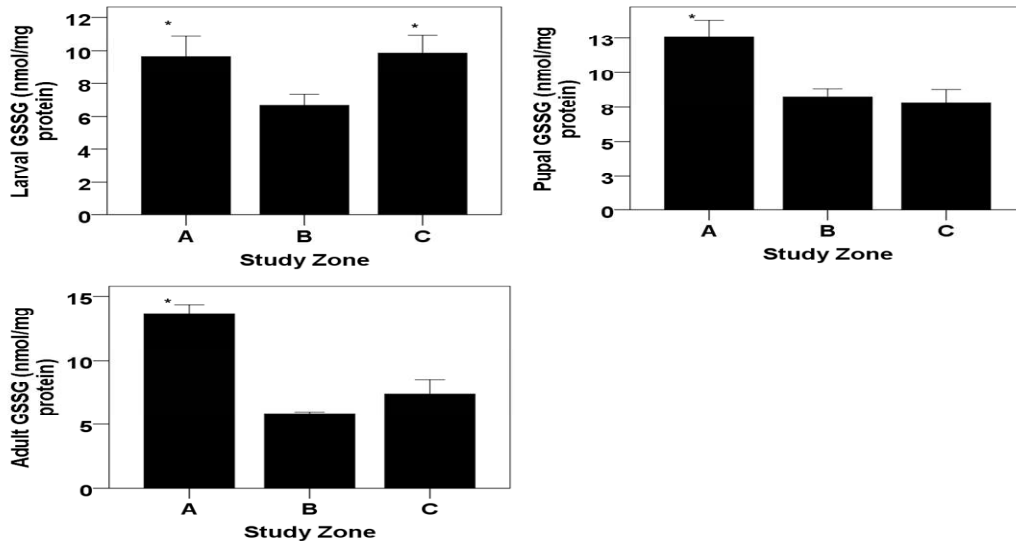

Fig. 1. Mean distribution of total glutathione (tGSH) across the three life stages; larvae, pupae and adults, of *An. gambiae*
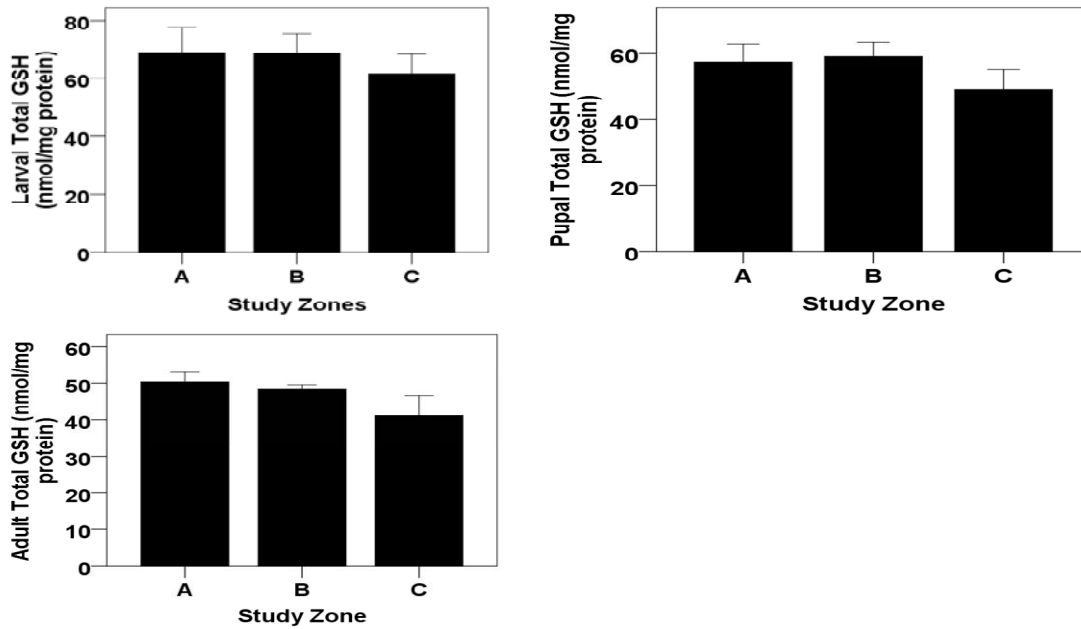

Fig. 2. Mean distribution of oxidized glutathione (GSSG) across the three life stages; larvae, pupae and adults, of *An. gambiae*

The *An. gambiae* mosquitoes were sampled from three different breeding ecologies categorized into three study zones in Nigeria. zone A; intensive agriculture; B; domestic/residential areas and C; petrochemical or hydrocarbon laden breeding ecologies. * indicates significant values (P<.05) relatives to those of the other zones.

The result of Bonferoni pairwise comparism showed that zone A & B and B & C pairwise comparism were highly significant (P= .030 and .021) respectively, while that between A and C was not statistically significant (P= 1.000) at the larval stage of *Anopheles gambiae*. However, at the pupal stage, comparism between A & B and A & C was statistically significant (P= 0.001) while that between B & C was not (P= 1.000). Finally, as observed with the two other forms of glutathione, the degree of significance increased at the adult life stage. A & B and A & C zone-wise comparisms recorded P-value of .000 while B against C recorded P-value of .069.

3.2 Associations or Correlations between the Three Forms of Glutathione and Activities of Detoxification Enzymes

Furthermore, the result of the linear regression model, which examined the correlation or association between levels of total glutathione and activities of P450, GST and α/β-esterase, showed that there was a moderate significant association with P450, which increases across the life stages, while there was little or no association between total glutathione and GST and α/β-esterase activities (R^2 =0.164, 0.100, 0.198 and 0.120 respectively). Total glutathione was moderately negatively associated (P= .319, .051 and .026) with P450 activities at the larval, pupal and adult life stages respectively. However, the association or correlation between total glutathione and GST and α/β-esterase were not highly significant. The P-values for GST at the larval, pupal and adult life stages were .987, .707 and .089 respectively while the P-values for α-esterase at the larval, pupal and adult life stages were .505, .794 and .014 respectively and P-values for β-esterase at the larval, pupal and adult stages were .886, .476 and .060 respectively. While the association was not highly significant for GST and α/β-esterases and moderately significant for P450, the significance nonetheless improved across the three life stages. Similar observations were also recorded for the reduced form of glutathione.

However, there was a statistically significant positive association or correlation (P= .000) between GSSG and larval P450 activities, although the significance (P= .449 and .663) decreased at the pupal and adult stages respectively. In contrast however, GST and α & β-esterases showed highly statistically positive associations (P= .000) with levels of GSSG across all the three life stages. The P-values for association between GSSG and GST activities at the larval, pupal, and adult stages of *An. gambiae* were .151, .000 and .000 respectively. For α-esterase activities, the *P*-values at the larval, pupal, and adult stages were .070, .000 and .000 respectively while associations between GSSG and larval, pupal and adult stage β-esterase activities recorded *P*-values of .205, .000 and .000 respectively. These results were in line with the previous observations made with the other two forms of glutathione: significance in distributions and associations appeared to increase across the three life stages i.e. from larvae to adult.

3.3 Mean Distribution of the Detoxification Enzymes in the Three Life Stages of *An gambiae* Across the Three Study Zones

Various statistical analyses have established highly significant difference in the mean distribution of the detoxification enzymes (P450, GST and α & β-esterases) across the three study zones. The mean distribution is similar irrespective of life stage. A deduced statistical model was used to established a significant relationship between the activities of these enzymes and the levels of the physico-chemical factors present in the *An. gambiae* breeding sites across the three study zones [14,15].

3.4 Effect of the Three Forms of Glutathione on Activities of the Detoxification Enzymes

Due to the results of the factor analysis conducted on the detoxification enzyme variables [14,15] which extracted the three enzymes (P450, GST and α & β-esterases) as principal components irrespective of the *An. gambiae* life stage, it was necessary to perform the same analysis on the three forms of glutathione (total, oxidized and reduced) studied, so as to also extract the glutathione principal components that explains all the variability in these forms of glutathione across the three life stages studied.

Redundancy analysis could then be used to determine the glutathione components that produce a combined effect on the extracted principal components of the detoxification enzymes activities.

The results of the factor analysis on the three forms of glutathione studied across the three life stages of *An. gambiae* showed that five principal components (PCs) were extracted and they explained more than 99% of the variability in the overall glutathione variables. PC 1 correlates strongly with total and reduced GSH at pupal stage, PC 2 was strongly associated with only oxidized (GSSG) glutathione at both pupal and adult stages, PC 3 correlates strongly with total and reduced GSH at larval stage, PC 4 was explained by total and reduced glutathione at the adult stage and finally PC 5 correlates strongly with only GSSG at the larval stage of *An. gambiae* (Fig. 3). Thus in summary, PCs 1, 3, and 4 correlated strongly with both total and reduced glutathione (tGSH & GSH) across the three life stages while PCs 2 and 5 was strongly associated with oxidized glutathione (GSSG) also across the three life stages. Thus in contrast to the results of factor analysis for the detoxification enzyme variables [15], the life stages of *An. gambiae* appeared to produce an impact on the variability of the three forms of glutathione studied. this results was consistent with the results of the linear regression analysis

carried out between the levels of these three forms of glutathione and the detoxification enzyme activities.

Finally, redundancy analysis (regression in principal factors or components) was carried between the extracted factors or components of the glutathione variables and those of the detoxification enzymes [15] to determine the combination of the forms of glutathione that produce a combined effect on each of the three detoxification enzyme factors. The result of the redundancy analysis (Table 1) between the glutathione factors and GST and α & β-esterase showed that a combination of total and reduced glutathione (tGSH & GSH) at only the pupal stage and oxidized glutathione at all the three stages (factors 1, 2, and 5) produced a combined effect on GST and α & β-esterase activities.

Lastly, the result of the redundancy analysis (Table 2) between the glutathione extracted PCs and P450 activities showed that four out of the five glutathione extracted principal components (PCs 1, 3, 4, and 5) produced a combined effect on P450 activities. Thus total and reduced glutathione at the three life stages (PCs 1, 3, and 4), and oxidized glutathione at only the larval stage were responsible for the combined effect of glutathione on the activities of P450 in *An. gambiae* from northwest Nigeria.
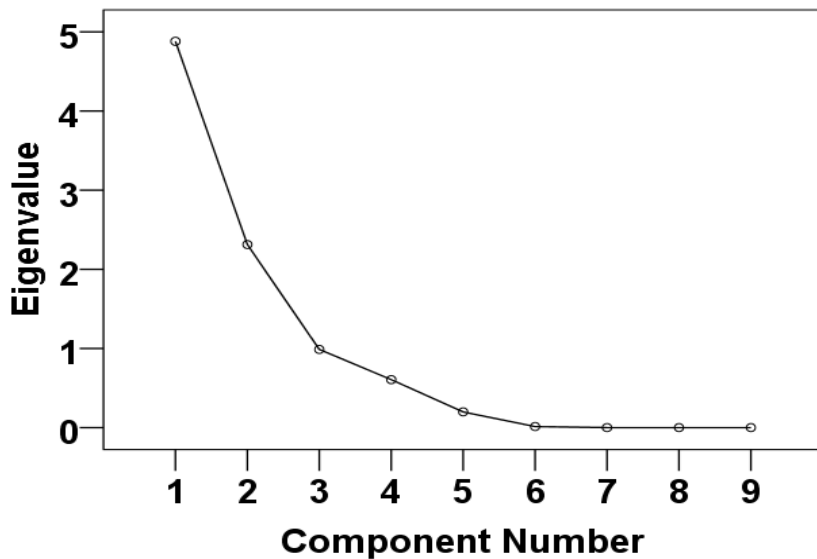

Fig. 3. A scree plot of the extracted factors or components from the factor analysis of the glutathione variables
Factors 1-5 explained 99% of the variability in the data

Table 1. Glutathione factors or components with combined effect on GST and α & β-esterases across the three life stages of *An. gambiae*

Parameter	Coefficient	Std. error	Wald Chi-Square	Df	Sig.
Intercept	-1.032E-015	0.0406	0.000	1	1.000
PC1	-0.114	0.0412	7.586	1	0.006
PC2	0.965	0.0412	547.384	1	<0.001
PC3	0.009	0.0412	0.051	1	0.822
PC4	0.012	0.0412	0.085	1	0.770
PC5	-0.074	0.0412	3.191	1	0.074

Table 2. Glutathione factors or components with combined effect on P450 activities across the three life stages of *Anopheles gambiae*

Parameter	Coefficient	Std. error	Wald Chi-Square	Df	Sig.
Intercept	8.967E-016	0.0505	0.000	1	1.000
PC1	-0.236	0.0513	21.218	1	<0.001
PC2	0.012	0.0513	0.055	1	0.815
PC3	-0.304	0.0513	35.029	1	<0.001
PC4	-0.296	0.0513	33.254	1	<0.001
PC5	0.828	0.0513	260.239	1	<0.001

3.5 Discussion

This study demonstrated the presence of the two forms of glutathione; oxidized and reduced, their role in xenobiotic detoxification process and their relationship with detoxification enzymes across the three life stages of *An. gambiae*. Glutathione plays a vital role in both enzymatic and non enzymatic routes for xenobiotic detoxification in most organisms. Similar process of regulatory mechanisms has been reported for both glutathione and many of the detoxification systems in organisms [6,16]. Furthermore, availability and levels of the different forms of glutathione, as well as synthesis and activities of detoxification enzymes have been found to responds to changes in the degree of oxidative stress induced by xenobiotic overload in different organisms [5,17]. The ubiquitous nature of mosquito breeding ecologies in most malaria endemic countries means that mosquito breeding sites can be created in varieties of environments where they could be exposed to arrays of different environmental xenobiotics resulting from various human related activities [18,19]. Due to these observations, monitoring the levels of various forms of glutathione and examining the relationship between these forms and activities and the major detoxification enzymes in *An. gambiae* is necessary to provide an alternative approach for management of insecticides resistance, hence this study.

The *An. gambiae* in this study were sampled from three different breeding ecologies defined by the type of human related activities taking place within and/or around the mosquito breeding sites. Levels of physical environmental factors (i.e. ph. temperature, conductivity, transparency, dissolved oxygen and biological oxygen demand) and chemical environmental parameters (i.e. total dissolved solids, sulphates, phosphates, nitrites, nitrates, carbon content and oil and gas) were determined from all the *An. gambiae* breeding sites visited across the three studied zones. The results [14,15] showed that the distribution of the levels of many of these factors (especially the chemical environmental parameters) varied significantly across the three studied zones. Furthermore, activities of the three major detoxification enzymes (GST, P450 and α & β-esterases) were determined in the three life stages of *An. gambiae* collected from all the breeding sites across the zones. The results [14,15] also showed that the mean activity distribution of the three enzymes varied significantly across the three study zones.

In this present study, levels per mg protein, of three forms of glutathione; total, oxidized and reduced, were determined in the three life stages of *An. gambiae* collected from breeding sites located across the three study zones. The relative zone-wise mean distribution of these forms of glutathione and the correlations or associations between their levels and the activities of the three detoxification enzymes (P450, GST and α & β-esterases) was investigated. The results showed that the three forms of glutathione were all detected in the

three life stages of *An. gambiae* in varying concentration across the three studied zones. Over 70% of the assayed glutathione was present in the reduced forms (GSH) across the three life stages. Also, the levels of total tGSH) and reduced (GSH) glutathione appeared to be higher in the larval stage of *An. gambiae* compared to the other two stages. There was not much variation between the pupal and adult stages. These observations are consistent with the findings of a previous study [20] which investigated the relationship between ageing and glutathione levels in mosquito.

Hence, since GSH is an integral part of the overall detoxification process; acting directly to conjugate xenobiotics and indirectly as substrate to GST, the levels of the resultant GSSG correlated significantly with the activities of the detoxification enzymes. The positive association of GSSG with GST was obviously due to the role of GSH as a substrate for GST in the metabolism of xenobiotics, while the association with P450 and α & β-esterases could be due to their role in the further metabolism of GSH-conjugated metabolites [5]. Finally, the increased accumulation of GSSG as indicated by the lower GSH/GSSG ratios in zone A and C, as compared to zone B could be due to the increased oxidative stress as a result of the higher xenobiotic overload. The increased oxidative stress was evidenced by the significantly higher levels of environmental chemical factors recorded in these zones compared to zone B [14]. Also, these two zones recorded higher activities of the detoxification enzymes [15], hence the significant positive associations with GSSG. Observations from previous studies [21,22] have established increase in oxidative stress induced by xenobiotic overload as a source of generation and accumulation of GSSG, leading to lower GSH/GSSG ratio in various organisms.

Distribution and changes in the levels of various forms of glutathione and their relationship with the activities of detoxification enzymes has not been largely investigated in insects. However, findings from previous studies [5,23,24] involving other organisms showed that changes in the levels of glutathione and activities of various detoxification enzymes do occur in response to different conditions of oxidative stress.

In this study, a low to moderate association was established between levels of total and reduced glutathione and activities of detoxification enzymes while a highly significant positive correlation was demonstrated between oxidized glutathione and activities of the three major detoxification enzymes (P450, GST and α & β-esterases) in *An. gambiae*. In many studies, contradictory observations were reported regarding the levels of various forms of glutathione and their association with detoxification enzymes under conditions of oxidative stress in both plants and animals. In most instances [25], the levels of total and reduced glutathione may increase, reduce or may not change significantly under conditions of oxidative stress. However, levels of oxidized glutathione and the ratio between oxidized and reduced forms of glutathione is usually used as the more accurate indicator of the redox state of a cell and in most organisms, this ratio defines the degree of oxidative stress induced either by xenobiotic overload or free radical formation, especially in instances where no apparent and significant induction in the synthesis of glutathione occurred [21,24]. Hence, finding from this present study appeared to be consistent with these observations. Despite the low significance in the levels of total and reduced glutathione across the three study zones, the highly statistically significant differences in the levels of GSSG across these zones could be used to explain the varying oxidative stress conditions as indicated by the significant changes in the activities of the detoxification enzymes across these zones.

Furthermore, another line of argument in support of the observed low changes in the levels of the total and reduced glutathione across the three study zones despite significant differences in the levels of environmental chemical factors is that glutathione is constitutively synthesized and abundantly available in all organisms. Under normal physiological conditions, its concentration in higher organisms such as mammals ranges between 2-10 mmol [6]. Synthesis of glutathione can however be induced under increased conditions of oxidative stress [6]. Thus, it is possible that the levels of reduced glutathione recorded in this study represent the normal threshold levels in *An. gambiae*. This means that despite the observed inductive effect of the chemical environmental factors on the activities of the detoxification enzymes studied [15], these levels of glutathione was sufficient for its role in the overall detoxification process. Hence, since levels, availability and activities of glutathione has been shown to respond to changes in oxidative stress induce by xenobiotic overload [8], the sources of oxidative stress in our

samples of *An. gambiae* (i.e. the chemical environmental factors) may not be significantly sufficient enough to cause the induction of glutathione synthesis above the normal threshold levels even though it was sufficient to induce increased activities of the detoxification enzymes.

4. CONCLUSION

This study was carried out to investigate the distribution of the various forms of glutathione and their relationship with activities of detoxification enzymes in *An. gambiae* under varying degree of oxidative stress induced by environmental xenobiotics. The results and observations recorded suggest that while significant induction in synthesis of glutathione may not have occurred, there was however an approximately 2-fold increase in the utilization of glutathione which corresponds with the degree or levels of xenobiotic concentrations across the three zones studied. The relationship between oxidized glutathione (GSSG) and the activities of the three major detoxification enzymes (i.e. P450, GST and α & β-esterases) in *An. gambiae* demonstrated in this study suggest a close coordinated relationship between activities of glutathione and these detoxification enzymes in xenobiotic metabolism. Since many of the environmental chemical factors investigated in this study have been established [14] to share similar structures and activity relationship with many of the chemical insecticides used in malaria vector control, their routes of metabolism and hence, the response they elicited in mosquito detoxification process could also be similar. Therefore, observations from this study and inferences from previous findings suggest a close coordination and relationship in the synthesis, activities and regulation of both glutathione and the detoxification enzymes. This could be harnessed in designing a novel strategy for the regulation of synthesis and activities of these enzymes, as a tool for the management of insecticides resistance in *An. gambiae*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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