Role of Glutathione S-Transferase (GST) in Mosquito Physiology and Anti-Plasmodial Activity

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Abstract- Malaria transmission depends on the competence of selective *Anopheles* mosquitoes that plays role of intermediate host. The innate immune system of most mosquitoes (non-vectors) is able to completely clear a *Plasmodium* infection, preventing parasite transmission to humans. Mosquito defense against malaria parasites involves variety of biological processes, among which ROS (Reactive Oxygen Species) are important determinants of parasite invasion to the mosquito. ROS are toxic by-product of cellular metabolism in all living beings. The living cells create potent antioxidant enzymes which detoxify ROS. The detoxifying enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase and glutathione reductase. The Glutathione-S-transferases (GSTs) are a diverse family of enzymes involved in a wide range of biological processes. Our previous study demonstrated that elevated GST activity played a role of resistant phenotype (to insecticide) in the mosquito, which is directly related to vectorial capacity and competence. The study of role of GST in mosquito physiology will give an insight in terms of effect of insecticide application on the vector and *Plasmodium* parasite development inside the vector, which has potential to explore more powerful and effective malaria control tool.

Index Terms- Glutathione S-transferase, mosquito physiology, insecticide resistance, anti-Plasmodial activity

I. INTRODUCTION

The global public health problem of malaria persists today. Malaria transmission depends on the competence of selective *Anopheles* mosquitoes that plays role of intermediate host. They pick up gametocyte of *Plasmodium* from infected human host and transmit the sporozoites into the healthy, which initiates a complex physiological mechanism inside the mosquito midgut (including mosquito immune system in the hemolymph) to sustain *plasmodium* development (susceptibility) (Kumar *et al.*, 2003). . Oocyst formation in mosquito gut may pose bottleneck in the parasite life cycle, as most parasites die either during midgut invasion or as they come in contact with components of the mosquito immune system present in the hemolymph (Vlachou & Kafatos, 2005; Blandin *et al.*, 2004). The innate immune system of most mosquitoes (non-vectors) is able to completely clear a *Plasmodium* infection, preventing parasite transmission to humans. Mosquito defense against malaria parasites involves variety of biological processes, among which ROS (Reactive Oxygen Species) are important determinants of parasite invasion to the mosquito.

ROS are toxic by-product of cellular metabolism in all living beings. The living cells create potent antioxidant enzymes which detoxify ROS. The detoxifying enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase and glutathione reductase. Foreign infections in the host cell cause oxidative stress and generates higher ROS production which disturbs the balance between ROS and antioxidant defenses. Similar mechanism occurs during *Plasmodium* infection in the mosquito. Previous studies have shown that higher levels of ROS are synthesized in mosquito hemolymph during *Plasmodium* development and limit *Plasmodium* development (Dejong *et al.*, 2007; Molina-Cruz *et al.*, 2008).

The Glutathione-S-transferases (GSTs) are a diverse family of enzymes involved in a wide range of biological processes. They play a central role in detoxification of both endogenous and xenobiotic compounds and are also involved in physiological processes such as intracellular transport, biosynthesis of hormones and protection against oxidative stress (Enayati *et al.*, 2005). It is also believed that these enzymes are a family of detoxification enzymes that have essential roles to play in the survival of insects exposed to endogenous and erogenous xenobiotics (Kostaropoulos *et al.*, 1996). The enzyme plays an important role in maintaining the redox status of the mosquito cell, particularly in relation to vectorial capacity and resistance (Ranson and Hemingway, 2005). Many studies have shown a decrease in reduced glutathione (GSH) concentrations during aging (Abraham *et al*, 1978; Hazelton and Lang, 1980; Tripathy & Kar, 2015 in press). Our previous study demonstrated that elevated GST activity in the mosquito played a role of resistant phenotype (to insecticide) and directly related to vectorial capacity and competence (Tripathy *et al.*, 2011). The above mentioned importance of glutathione-S-transferases in detoxification encouraged us to review its role in mosquito physiology. The review will give an insight in terms of effect of insecticide application on the vector and *Plasmodium* parasite development inside the vector, which has potential to explore more powerful and effective malaria control tool.

II. Role of GST in mosquito physiology

Today, insecticide resistance has been reported in all main mosquito vector species and geographical regions with high parasite-related mortality and morbidity (Roberts & Andre 1994; Ranson *et al.*, 2011). Main physiological mechanisms of insecticide resistance have evolved in mosquitoes: (i) the overproduction of detoxifying enzymes that sequester and/or degrade the insecticide before it reaches the nervous system (metabolic resistance) and (ii) mutations in the insecticide neural targets that render them less sensitive to the insecticide's active ingredient (target site resistance). It has been observed by Poupardin *et al.*, (2008) that the potential of xenobiotics present in polluted mosquito breeding sites affected their tolerance to insecticides through cross-induction of particular detoxification genes. Similarly the xenobiotics present in the mosquito blood meal might have affected their tolerance to insecticide. An enhanced ability of the insecticide resistant insects to tolerate oxidative stress has also been implied by the protective role of glutathione S-transferases (Vontas *et al.*, 2001). Mittapalli *et al.*, (2007) studied the GST expression in the Hessian fly and found that the product of the Delta GST genes aid in detoxifying exogenous allelochemicals from the host plant (wheat), while that of a Sigma GST could function in providing protection against toxic oxygen species generated endogenously during development. An enhanced ability of the insecticide resistant mosquitoes to tolerate oxidative stress was implied by protective role of Glutathione S- transferase (Tripathy *et al*, 2011).

Blood feeding or hematophagy is a behavior exhibited by female mosquitoes which is essentially required for reproduction and transmission of pathogens (Dana *et al*, 2005). For most mosquitoes living in optimal field or laboratory conditions, this cycle requires about 72 hours and involves a complex series of biological events including peritrophic matrix formation, blood digestion, oocyte development, vitellogenesis and excretion (Dana *et al*, 2005). Acquisistion of a blood meal stimulates midgut proteolytic activity such that approximately 80% of the protein content is digested within 24 hours (Billingsley and Hecker, 1991; Jahan *et al*, 1999; Lemos *et al*. 1996). Multiple aminopeptidases have been isolated from hematophagous insects and it has been suggested that they may play different roles in digestion (Billingsley, 1990; Hori *et al.*, 1983; Cheeseman & Gooding, 1985; Ferreira & Terra, 1986); thus the GST activity may be associated with the above physiological processes of the mosquito, as they (GSTs) are involved in metabolic cycle for the transport of certain amino acids across the membrane of the malpighian tubules (Enayati *et al*, 2005).

Mosquitoes such as Anophelines must ingest a blood meal to obtain the nutrients required for oogenesis. The blood meal itself brings metabolic changes and induces a state of oxidative stress (Felix *et al.*, 2010). The blood is digested by the midgut and nutrients are transported to the fat body where vitellogenin and other major proteins of the egg yolk are synthesized (Attardo *et al*, 2005). Blood fed females respond to oxidative stress by increasing systemic expression of ROS detoxification enzymes (Molina-Cruz *et al.*, 2008). In *An. gambieae* Dejong *et al.*, (2007) observed that H_2O_2 levels in hemolymph increased dramatically after a blood meal, due to increased metabolic activity during the process of blood digestion and oogenesis. Hence it may be postulated that higher GST activity during gonotrophic cycle might be associated with the enhanced levels of H_2O_2 as GST scavenges H_2O_2. According to Dejong *et al*, (2007), ROS detoxification by catalase increases the reproductive output by protecting the ovary and the early embryo from oxidative damage but the transient and local accumulation of ROS appears to be necessary for normal mosquito physiology. Kumar *et al.*, (2003) analyzed ROS levels in hemolymph of the refractory strain of *An. gambie* and suggested that clearance of H_2O_2 a rate limiting step in free radical detoxification. Their study also confirmed that H_2O_2 level increase in responses to blood feeding. According to Lumjuan *et al.*, (2007), out of the three mosquito specific GSTs, GSTX2-2 showed an affinity for hematin and this, indicates a role of these enzymes in protecting mosquitoes against heme toxicity during blood feeding.

III. Does insecticide resistance alter vectorial competence and impact transmission?

Insecticide resistance has an impact on the transmission of diseases directly by increasing the number of mosquitoes in the population. It has been recently suggested that insecticide resistance may also have an impact on the quality of these mosquitoes (McCarroll *et al.*, 2000; Rivero *et al.*, 2010). Mosquitoes indeed provide a very specific environment in which the parasites differentiate, proliferate and migrate to the appropriate host to ensure transmission to the next host. A modification in any of the factors that make up this complex physiological environment can drastically alter the vectorial competence of mosquitoes (Dong *et al*, 2006; Garver *et al*, 2009). The mosquito immune system appears to be one of the most important of these factors. Mosquitoes exhibit sequential immune responses to combat infection. These responses can be classified into two types: constitutive (which are always present and ready to act) and induced (which are expressed only after the host has been exposed to an infection (Hamilton *et al*, 2008). Endogenous innate immune molecules of mosquitoes have been shown to hinder the development of malarial (Luckhart *et al.*, 1998) and filarial (Shiao *et al.*, 2001) parasite; also arbovirus infection (Sanchez-Vargas *et al.*, 2009). Vontas *et al.*, found a differential expression of some of these immune effectors genes (2005, 2007) suggesting a potential link between insecticide resistance and the insect immune system. In this context it needs to explore qualitative effects insecticide resistance on the ability of mosquitoes to transmit malaria; are insecticide resistant mosquitoes as good vectors of *Plasmodium* as susceptible ones? Surprisingly little is known as to whether insecticide resistance interferes with the subsequent development of *Plasmodium* within the vector.

IV. Does parasite alter the behavior of mosquito vector?

Malaria parasites have been suggested to alter the behavior of mosquito vectors to increase transmission (Cator *et al*, 2012). Parasite manipulates the vector and increases vector feeding rate once they have become infectious (sporozoite stage) (Alano, 2007). According to the manipulation hypothesis, malaria parasites decrease mosquito blood-feeding and other risky

behaviors during the pre-infectious phase thereby decreasing the risk of host death during parasite development (oocyst stage) and parasite then increase vector feeding rate once they have become infectious (sporozoite stage) (Cator *et al*, 2012). This manipulation hypothesis has been supported by many laboratory and field based evidences (Cator *et al*, 2012). In laboratory studies, female mosquitoes infected with oocyst-stage malaria parasites were less persistent at blood-feeding and less likely to resume feeding if interrupted (Sinden, 1998). Other studies reported that sporozoite infected females probed more frequently than uninfected controls (Sinden, 1998; Ayala, 1999). It was reported by Sinden (1998), sporozoite-infected females also took less blood per meal, which in the field may lead these females to feed more than once within a gonotrophic cycle. Other studies found that infection status did not affect blood-meal size (Liu *et al.*, 2010) or probing duration (Prugnolle *et al.*, 2010). The multiple feeding behavior of vector can increase transmission of a vector-borne pathogen by four times through enhanced survival, fecundity and vectorial capacity (Scott & Takken, 2012). Due to multiple feeding behavior of mosquitoes the infection rates in mosquito vectors can range from < 1% to 5% for the incidence of malaria and dengue (Gilles and Warrell, 1993). Several studies suggest that malaria parasites manipulate mosquito behavior to facilitate transmission but the nature and extent of the phenomenon remains unclear. Changes in mosquito behavior following parasite invasion could be a pathological consequence of infection or a manifestation of the mosquito immune response to infection or an interaction between the two [39]. Based on above it is yet to reveal the factors that favour frequent and preferential feeding on humans and its epidemiological consequences are needed. Increased research attention on the frequency of mosquito vector-host contact will enhance prospects for developing more successful disease prevention tools, strategies and also add to the capacity of risk assessment. The behavioral changes differences between infected and uninfected females should be considered in epidemiological models and in development /implementation of control measures.

V. Does Plasmodium infection alter detoxification gene expression in mosquito vector?

The mosquito becomes infected with the malaria parasite by taking a blood meal. The blood meal itself brings metabolic changes and induces a state of oxidative stress [47], [48]. This further increased by the presence of *Plasmodium* parasites in the blood meal [5]. During mosquito response to infection, active nitrogen and oxygen radicals are produced to contain *Plasmodium* infection [47], [5]. These products may represent potential oxidative stress that can be ameliorated or eliminated by detoxification enzymes. For example several glutathione S-transferases (GSTs) have peroxidase activity and help to eliminate ROS [8]. GST expression can also be induced by reactive oxygen species (ROS) [49], [16]. The studies by Jaramillo-Gutierrez *et al*, revealed that OXR1 gene regulates the basal levels of catalase (CAT) and glutathione peroxidase (Gpx) expression and in *An. gambiae* this gene silencing decreases *Plasmodium* infection in the mosquito[50].

VI. Possible anti-Plasmodial role of GST in mosquito vector

Previous studies have shown that higher levels of reactive oxygen species (ROS) in mosquito hemolymph limit the *Plasmodium* development [4], [5]. The intake of blood meal by the mosquito brings metabolic changes and induces a state of oxidative stress and further it is increased by the presence of *Plasmodium* parasites in the blood meal [26]. Elevated level of reactive oxygen species plays a role in contributing to the parasite melanotic encapsulation [1], [51].

However, till date the anti-Plasmodial role of GST in the mosquito vector is not known. The recent study by Oliveira *et al*, revealed that long proved anti-plasmodial role NOS (Nitric Oxide Synthetase) induction is not sufficient to achieve an effective anti-plasmodial response [52]. In this context it draws our attention towards the protective role of Glutathione S-transferase which may have anti-plasmodial role in the mosquito vector.

As regards the role of GST during developmental stages of mosquitoes, it showed that there is a high level of production of induced GST in blood fed mosquitoes and during gonadial development stages [11]. Taking into account all the above facts, it is hypothesized that there might be a strong association between the response to *Plasmodium* infection and insecticide resistance, thus enhancing the significance of further studying their interactions through the GST pathway. So the following possibilities need to be explored: 1) The GST may themselves exert irreversible damage to the parasite, which secondarily triggers immune recognition and parasite melanization.2) Increased level of GST may accelerate immune activation and parasite melanization. 3) As GST is a resistant phenotype, it may favor the parasite development inside the mosquito vector. It is important to explore the role of differential expression of the ROS and GST on the vectorial capacity and competence of the mosquito vector. Further study is needed to explore the mosquito immune defense mechanism, genetic pathway of vector physiology, vectorial capacity and competence. The research will ultimately lead to novel measures for malaria control.

Conflict of interest: No

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