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Development of Resistance Mechanism in Mosquitoes: Cytochrome P450, the Ultimate Detoxifier

Arif Malik[1], Khalid Mahmud Khan[2], Muhammad Asif,[3]Hafiz Muhammad Arsalan[1], Sara Zahid[1], Abdul Manan[1], Hafiza Shamaila Murawat[1], Mahmood Husain Qazi[4], Mahmood Rasool[5]

[1]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, [2]Fatima Jinnah Medical College, Sir Gangaram Hospital Lahore, [3]Office of Research Innovation and Commercialization, Balochistan University of Information Technology Engineering and Management Sciences, Quetta, [4]Center for Research in Molecular Medicine (CRiMM), The University of Lahore, [5]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia.

Abstract

The development of resistance in different species of mosquitoes increases day by day. Malaria, yellow fever, dengue and many other diseases spread by the mosquitoes. According to the WHO report, it is estimated that about 247 million individuals are infected by malaria every year. Biochemical, physiological and behavioral resistance exists in mosquitoes. Glutathione-S-transferase (GST), carboxylesterase and Cytochrome P450 are enzymes families playing role in the metabolic resistance. These enzymes play key role in pyrethroid, carbamate and xenobiotic detoxification and metabolism. Several cytochrome P450 enzymes present in mosquitoes cause resistance. In Anopheles gambiae, cytochrome P450 found to be the main cause of resistance to insecticides. Over expression of P450 is reflected by the cause of development of resistance. GSTs are present in cytosol detoxify endogenous and xenobiotics. Inhibiting the expression of resistance causing enzymes will lead to overcome the resistance produced in mosquitoes.

Keywords: GST, Carboxylesterase, cytochrome P450, pyrethroid, lipid peroxidation, metabolic resistance.

Corresponding author's email: *asifjallali@yahoo.com*

INTRODUCTION

Currently 3,300 mosquito's species have been discovered which belong to 41 genera. All species belong to family culicidae. (Suman et al., 2012). They are categorized into 3 sub families, Culicinae, Anophelinae and Toxorhynchitinae. Recently 176 species has been reported in the United States. *Anopheles gambiae*, a new specie was found from the Florida Keys in 2001 (Darsie et al., 2002). Each mosquito specie consists of Latin scientific name. For example, *Anopheles quadrimaculatus* is the name which represents individual group. These names used in descriptive way which means that the name describe particular mosquito, i.e. Anopheles is Greek word, means hurtful and *Quadri manculatus* is Latin word which mean "four spots". Some species has both names (common and scientific). For example, *Ochlerotatus taeniorhynchus*, "black starch mosquito". In spanish, mosquitoes called as musketas. Mosquito is Spanish word mean as "little fly". The word mosquito was firstly used in North America in 1583. In Scandinavian countries, mosquitoes has several names i,e, "myg" and "myyga". Aristotle called mosquitoes as "empis" in his book "Historia Animalium" where he mentioned their life cycles and metamorphic abilities. Modern writers used "culex" and retained as name of mosquito genus. Mosquitoes are distributed in everywhere in the world and found in tropical and temperate regions. But not found in Antarctica and island. Mosquitoes are present in 1250m depth at sea level (Braverman 1994). The important genera are Anopheles, Culex, Psorophora, Sabethes and Aedes. *Aedes agypti* is small, dark mosquito have white marking or dots on the banded legs scutum, it cause dengue and yellow fever. It also causes chikungunya and in Australia it cause Ross river virus. *Aedes*

albopictus also called Asian tiger also cause or transmit dengue fever and encephalitis. *Anopheles stephensi* is used in laboratories to propagate malarial agent. Specie called *Plasmodium berghei*, infects the rodents.

In countries like Iran, India and neighboring area, three types of mosquitoes are differentiated on the basis of their eggs i.e. *Anopheles stephensi* and *stephensi mysorensis*. Both are explained to be zoophilic, preferring cattle in rural areas and humans in urban areas. It is examined that *Anopheles stephensi* could fly from 800m (half mile) to 5 km. Mosquitoes are usually found in buildings, cattle sheds or underground shelters i.e. walls. Compatible condition of this mosquito is wells, empty containers, and roofs gutters, fountains and rice fields. Rainy season is favorable for larval breeding. Adult male and females at second night become sexually mature. *Anopheles stephensi* life span can up to 26 days in laboratories. But in natural conditions, they die in 11 days. During one oviposition, female lays about 100 eggs. 247 million people worldwide and one million deaths are reported due to malaria transmitted by Anopheles, and major African vector known as *Anopheles funestus* each year (WHO, 2008, Gillies and De Meillon, 1968; Fontenille *et al*., 1990 and Hargreaves *et al*., 2000).

By the development of resistance to insecticides, effective malarial vector control is hampered. In vector populations, understanding of resistance mechanism enables the resistance management strategies development. Reduced target site sensitivity and mediated detoxification of enzymes are most common modes of insecticide resistance. Reduced affinity of target receptor to insecticide results the target site insensitivity mutation (Brogdon and McAllister, 1998). Sodium ions channel gene alteration, GABA receptor and acetylcholine esterase reduce the binding affinity of pyrethroid and DDT, Fipronil and Dieldrin, Organophosphates (Hemingway and Ranson, 2000). About 31 partial monooxygenase P450 sequences were isolated (Amenya *et al*., 2005) in which over expression of CYP6P9 showed highly expressed features in eggs and adult stages of laboratory strain pyrethroid resistance. In *Anopheles funestus* strain, quantitative trait locus (QTL) which confirmed increase in CYP6P9 transcription was identified. The quantitative trait locus markers included p450 gene clusters also contain CYP6P9. Duplicate genes named as CYP6P9b and CYP6P13 was identified respectively. Similarities of amino acids between CYP6P9b and CYP6P13 are the confirmation of these same genes (Wondji *et al*., 2009).

Differentiation between two closely related genes that used as primer for both these informations were designed in a region sharing 100 percent sequence identification between them was not given (Amenya *et al*., 2008 and Wondji *et al*., 2009). In *Anopheles funestus*, increased mRNA expression of both these genes was confirmed (Morgan *et al*., 2010). In recent studies while over expression of one of the duplicate gene (CYP6P9a) was identified (Cuamba *et al*., 2010). In microarray study, increase in gene transcription for these genes was showed (Christian *et al*., 2011). A gene p450 was only gene that was upregulated (CYP6M7) but quantitative real time PCR this gene was not validated (Christian *et al*., 2011). In older mosquitoes, characterization of insecticide resistance is important, mosquitoes are able to transmit malaria only after 10-14 days age. Estimation of pyrethroid resistance level is main purpose in the anopheles resistant strain at different stages (3, 5, 10,14,20,30 days). Transcription level estimation was also observed in CYP6P13 and CYP6P9 duplicated genes, whether there is direct relationship between pyrethroid resistance phenotype and gene transcription by age (Cuamba *et al*., 2010). In the world, *Anopheles gambiae* is the major malarial vector. Due to malaria, about 500 million people severely ill every year. Asian, Latin American, and European countries are also affected by malaria. In the south Asian countries and Middle East, the major human malarial vector is *Anopheles stephensi*. It is related to African malarial vector's subgenus as *Anopheles gambiae*. The biology of *Culex erraticus* is not much known. Important vector of yellow fever is Aedes. It is also a vector of *Brugia malayi* and *Wuchereria bancrofti* in the restricted areas. They also cause dengue, Arboviruses and Encephalitis

viruses. In Central and South America, yellow fever and few arboviruses caused by the *Haemogogus sabethes* mosquitoes (Reiter, 2010).

MORPHOLOGY

Mosquitoes have only one pair of wings, called as fore-wings. A pair of small, knob like halters represents the hind wings. They are differentiated from each other by some similar shape and size by **a)** Possession of a conspicuous forward- projecting proboscis. **b)** Presence of scales on thorax, legs, abdomen, wings etc. **c)** Fringe of scale. They are 3-6 mm in length. And they have head, thorax and abdomen. Eyes are present on the head. A pair of antennae is present between eyes. A pair of palps is present below to antennae. Thorax is dorsally and laterally with colour of scales. Veins of mosquitoes are covered by scales. Legs are long and slender. Abdomen is composed of 10 segments but visible segments are only 7 or 8 (Coetzee and Fontenille, 2004).

In population of *Anopheles funestus*, pyrethroid resistance has been reported in South Africa. Pyrethroid resistance has been reported in Southern parts of Mozambique since 2000 and implication of elevated P450 monooxygenase were takes place. Laboratory seleted resistance strain, FUMOZ-R is used to further justify pyrethroid resistance mechanism in *Anopheles funestus.*In conferring pyrethroid resistance, the role of P450 genes has confirmed. But fully characterization of specific P450 involvment has still not developed. There are some markers which are used to design diagnostic assays to monitor and detect the resistance. For the identification of these markers, characterization is necessary. It is notify that female mosquitoes bite. Heat, light, smell, lactic acid, and carbon dioxide are favourable things to attract them. Female mosquitoes come on the skin and enter the proboscis into the skin. Anti-coagulants are present in her blood. She sucks the blood into her abdomen. If sensory nerve of her abdomen were cut then she burst if abdomen full of blood. There are some factors which are considered: **i)** Susceptibility to plasmodium. **ii)** Choice for meal. **iii)** Life span. (Wondji et al., 2007).

Some species of anopheles exist in which parasite development not well within them and these are poor malarial species. A strain of *Anopheles gambiae* that cannot become infected by malarial parasites has been possible to select in the laboratory. The stage to which a specie prefer to feed on human or animals is important factor to understand the Anopheles and malaria transmission relationship. Humans or animals do not exclusively prefer by the most Anopheles mosquitoes. Malaria parasite before infectious to humans, it must undergo the process of development. 10 to 21 days are required for the Plasmodium development in Anopheles and this time period is depends on temperature and parasite specie. Only 20% Anopheles survive more than 14 days. (Wondji et al., 2007).

DISEASES ASSOCIATED TO MOSQUITOES

Several viral and parasitic diseases caused by different mosquitoes. Viral diseases i.e. dengue fever, yellow fever and chikungunya are caused by *Aedes aegypti*. Parasitic disease i.e. malaria caused by Anopheles. (Wondji et al., 2007).

ENCEPHALITIS An inflammation of brain caused by *Culex tarsalis*. It transmits the virus to mules, horses, birds and sometime people. In disease cycle, birds are the host for the virus reservoir. To determine the incidence of virus and potential for transmission, health officers survey the bird populations. (Wondji et al., 2007).

DENGUE Between humans, by the Aedes aegypti, a complex of virus transmission causes dengue. It is sporadically occurs in Southern Texas and endemic (naturally transmitted) in Mexico. It is categorized in three forms. Symptoms include high fever, severe joint pain, vomiting and rash. Dengue affected people have also chances of dengue hemorrhagic fever and/or dengue shock syndrome (DSS) which is sever forms of dengue. Both forms can be fatal (World Health Organization, 2008).

NON-CHEMICAL CONTROL MEASURES

If certain sources which are necessary for the development of mosquitoes are removed (i.e. stagnant water control, garbage disposal and old tires) from the residential areas then mosquitoes development become lesser.

The flowerpots, discarded container and ditches are favorable places for the mosquitoes breeding. Water is very necessary for the completion of mosquito's life cycle. The ponds and lakes where contain fish and no weeds, algae free places are not major breeding areas of mosquitoes. The places of water which contain fish and other predator insects keep mosquitoes from reaching dangerous level. But storms and floods disturb this system which is beneficial for rapid growth of mosquitoes. Human's activities are also favorable for the breeding of mosquitoes in clean water. Such as maintenance and road building which impede the drainage of rain runoff. In used and maintained swimming pools, mosquitoes will never breed. (Wondji et al., 2007).

RESISTANCE TO INSECTICIDE

Several ways to measure the control which based on insecticides such as, insecticides spray nets that are insecticide treated. Due to these ways Anopheles is killed. But after a long time mosquitoes and other insects develop the resistence against insecticides. The resistence of Anopheles has been reported against insecticides within few years of their introduction. (Wondji et al., 2007).

REPELLENTS

Repellents are present in many forms such as chemical sprays, lotions, and electronic devices**. Deet (N, N diethyl-m-toluamide)** is considered as well known mosquitoe repellent (Mittal et al., 2011). Deet is available commercially and long lasting effects and repellent. Best personal protection from bites of mosquitoes provided by Deet. It has different concentrations such as 2% to 100 %. Because Deet is chemical so try to avoid it. It applied on clothes instead of skin. It does not give permanent relief from mosquitoes so must apply daily. (Wondji et al., 2007).

CITRONELLA OIL

It protects from the bites of mosquitoes, fleas and ticks. It can use in form of oils or liquid. When it is used as tropical insect repellent, accordingly to EPA (Environmental Protection Agency) it has very little or no toxic effect. It can irritate eyes or skin or it can be toxic when it takes in large quantity. As compared to Deet, citronella oil is less toxic. Deet may have chance to go into blood stream through skin which disturb the central nervous system (Mittal et al., 2011).

COILS Coils are also used as protective agent against mosquitoes or bites of mosquitoes. Smoke of coils release due to which mosquitoes to go away. But due to this smoke, eyes and throat also irritated and also cause air pollution. Mats also prevent from mosquitoes. Small in size and have slight smell. Electricity is required**.** A new advancement is from mats to liquidator. Liquids present in machines when it switched on, liquid act as repellent due to which mosquitoes goes away. A special kind of candle is made for mosquitoes. Not dangerous for environment. Many types are available in sweet fragrance and used as room fresheners. Sprays are present in aerosol cans. (Mittal et al., 2011).

PESTICIDES RESISTANCE MECHANISM

Three types of mechanisms exist to reduce toxicants**.**

1 Biochemical resistance**.**
2 Physiological resistance.
3 Behavioral resistance.

BIOCHEMICAL RESISTANCE

Insecticides, before reaching to his target side, are detoxified by the action of one or more enzymes. i.e. mixed function oxidases. (Wei *et al*., 2001).

PHYSIOLOGICAL RESISTANCE

A mechanism of resistance in which basic physiological changes reduces the toxicity. Physiological functions of chemicals are altered by the insect rather than chemical break down into less toxic and insects accommodates it. i.e. activity of acetylcholinesterase altered by organophosphate (OP) and carbamate. Acetylcholinesterase less sensitive to inhibition, i.e. Anopheles mosquitos' resistance to organophosphate (OP) and carbamate have been noticed to have an altered acetylcholinesterase. (Wei *et al*., 2001).

BEHAVIORAL RESISTANCE

Insects change the behavior by which they avoid from insecticides. For example, *Anopheles gambiae* considered indoor strain, was susceptible to indoor walls DDT sprays applied. At same time out-door strain try to avoid exposure to insecticides due to their behavior.

METABOLIC RESISTANCE

Metabolic resistances contain enzymes over-expression that detoxify the insecticides or modified the amino acid sequences and as a result changes in the detoxifying proteins activity and levels. Metabolic resistance has three types of enzymes families, 1 Glutathion-s-transferase (GST), 2 carboxylesterase, 3 cytochrome P450 (Wei *et al*., 2001).

a.Glutathione S-transferases

The glutathione S-transferases (GSTs) are multi-functional intracellular enzymes which detoxify the endogenous and xenobiotic compounds through the glutathione conjugation, dehydrochlorination and through the Glutathione peroxide (GPx) activity (Pickett and Lu, 1989, Yang *et al*., 2001). They also act as non-enzymatic binding proteins in the intracellular transport (Listowsky *et al*., 1988) and also in the signaling processes (Adler *et al*., 1999 and Cho *et al*., 2001). In many insects, the higher or increased levels of GSTs have been reported which is related to insecticide resistance. Some GSTs in the house fly (*Musca domestica*) have been resistant to Organophosphates (OPs) (Wei *et al*., 2001) and Organochlorine (OCs) resistant in the fruit fly *Drosophila melanogaster* (Tang and Tu,1994) and also reported in pyrethroid resistance strain of plant hopper, *Nilaparvata lugens* (Vontas *et al*., 2001). Major mechanism of DDT resistance in mosquitoes is GST based metabolic resistance (Hemingway and Ranson, 2000).

GSTs are belongs to the enzymes family that are abundant to the transferases and present in most Eukaryotes and Prokaryotes (Sheehan *et al*., 2001). Three types of GST proteins found in Eukaryotes i.e. Microsomal (Jakobsson *et al*., 1999), Cytosolic and Mitochondrial (Kappa) (Pearson, 2005). Mammalian mitochondria and peroxisomes contain Kappa or mitochondrial GSTs (Pemble *et al*., 1996) but this family not found in Diptera specie (Ding *et al*., 2003). Three different Microsomal GSTs have been reported in *Anopheles gambiae*. Before the discovery of genome of *Anopheles gambiae* and GST containing genetic map, the amino acid sequence homology and immunological properties were the major and basic criteria for the estimation of GSTs to a particular class (Toung *et al*., 1990, Fournier *et al*., 1992, Ranson *et al*., 2002).

Now GSTs are classified as by the identification of 40% amino acid sequence and some other properties i.e. immunological properties, Phylogenetic properties and Tertiary structure and their heterodimer formation abilities (Ding *et al*., 2003; Hemingway *et al*., 2004 and Ranson and Hemingway, 2005). There are six different types of cytosolic proteins reported in Dipteran and other species which have similar characteristics to the GSTs, named as Delta, Epsilon, Omega, Sigma, Theta and Zeta (Ding *et al*., 2003 and Tu and Akgul, 2005). These types are also identified in *Anopheles gambiae* and *Aedes aegypti*.

b. Carboxylesterase

Carboxylesterase play a role in carbamate and pyrethroid resistance and in organophosphate. Cytochrome P450 play a role in pyrethroid metabolism organophosphate carbamate detoxification (Hemingway and Ranson., 2000). Xenobiotics are detoxified by glutathione-S-transferase containing organochloride insecticide DDT (Enayati *et al*., 2005). By the elevation activity of the detoxifying enzymes, metabolic resistance to insecticides can be conferred in *Anopheles gambiae*.In several insects including mosquitoes, organophosphorus and carbamate insecticide selection pressure has been reported by the carboxylesterases over expression as an evolutionary response (Vulule *et al*., 1999 and Zhu *et al*., 1999). Rapid esterification in the active site of serine residue is result of inhibition of B esterase by carbamate and organophosphorus, and for this slow hydrolysis of new ester bond usually followed. Esterases are inhibited by these insecticides due to their poor substrates with high affinity of these enzymes (Hemingway and Karunaratne, 1998). As the insecticides rapidly sequestered the large amount of carboxylesterase causing resistance even before reaching to the target-site acetylcholinesterase (Hemingway and Karunaratne, 1998). In insecticide resistant mosquitoes including *Anopheles gambiae*, carboxylesterases over expression was reported even in permethrin resistant mosquitoes, carboxylesterases enhanced

production was observed (Vulule *et al*., 1999). Increase in the amount of one or more enzymes cause higher enzyme activity, either due to amplification of gene or increased transcriptional rate instead of in individual enzymes qualitative changes may occur (Hamingway *et al*., 2004).

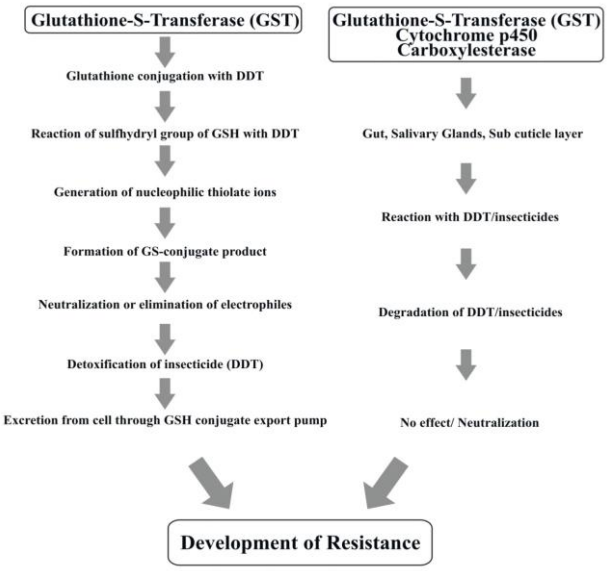
Detoxification of endogenous and xenobiotics is the major function of GST. Detoxification either by directly or by catalyzing the secondary metabolism of compounds that are oxidized by Cytochrome P450 (Wilce and Parker., 1994). Insecticides are metabolized by the GST enzymes by facilitate the reductive dehydrocholorination or the water soluble metabolites produced by the conjugation reaction with reduced Glutathion. They are also helpful in removal of toxic oxygen free redical species which produced through the pesticides action (Enayati *et al*., 2005). Levels of elevated GST associated with DDT resistance were found in *Anopheles gambiae* (Ranson *et al*., 2001). In *Anopheles gambiae* genetic mapping of the major loci DDT resistance implicate cis and trans acting factors in the GSTs over-expression (Ranson *et al*., 2000b). In a DDT resistant strain, GSTs were over-expressed in *Anopheles gambiae*, but DDT is metabolized by only one GSTE2-2 (Ortelli *et al*., 2003).

c. Cytochrome P450

Xenobiotics are metabolized by Cytochrome P450 enzymes and also play role in endogenous metabolism. Most frequent resistance was that which was mediated by Cytochrome P450. They play role in insecticide metabolism, in case of organophosphorus insecticides leads to molecular activation (Scott and Wen, 2001). In most cases insecticide resistance and activity of elevated P450 was shown as a link, CYP6 family contain Cytochrome P450 (Djouaka *et al*., 2008; Muller *et al*., 2007 and Mclaughlin *et al*., 2008). The specific cytochrome P450 that is associated with resistance is being difficult to be identified, insecticide resistant strain are source of P450 isolation (Sabourault *et al*., 2001).

RESISTANCE MECHANISM

Resistance mechanism of insecticides contain biochemical resistance divided into two classes i.e. Target site resistance. Detoxification enzymes based resistance. In which different enzymes (GSTs, Oxidases and Esterases) prevent the insecticides.



GENERALIZED MECHANISM OF DEVELOPMENT OF RESISTANCE IN MOSQUITOES

Besides these, some thermal stress response based mechanisms also proposed (Patil *et al*., 1996).

TARGET SITE MECHANISM

The insecticides are less effective or ineffective because amino acids alterations are responsible for the binding of insecticides at its site of action. In nerve synapses, acetylcholinesterase is the target of carbamate and organophosphorus. While sodium channels are the target of organochlorine and synthetic pyrethroids. In the insecticide binding site of axonal sodium channel, single amino acid change produce pyrethroid-DDT cross resistance (Miyazaki *et al*., 1996 and Williamson *et al*., 1996). A shift is produce in the sodium current activation curve by the cross resistance and low sensitivity to pyrethroids also cause (Vais *et al*., 1997). Same as cyclodiene (dieldrin) examples at molecular level, biochemical resistance mechanism in Anopheles gambiae target site DDT-pyrethroid resistance confers by the single amino acid mutation in IIS6 membrane-spanning region of sodium channel gene. Barbie box is the regulatory element present upstream of coding sequence. The induction of esterase resistance gene and insecticide detoxifying oxidase takes place due to Barbie box. Vector resistance enzymes have many putative control elements. A2-B2 esterase

amplicon within the amplification unit, esterase resistance genes present at 5'end to 5'end. Single mosquito has more than 100 amplicon copies. It is example of amplified esterase genes family. For the GABA receptor within the same codon of a gene, change in a single nucleotide confirmed the resistance (Ffrench Constant *et al*., 1993).

MECHANISM OF DETOXIFICATION

GSTs, Esterases and Oxidases are large multi-gene family members transcribed the enzymes which are xenobiotics detoxification responsible in living organisms. Activities of esterase detoxification enzymes or modified levels are most common resistance mechanisms in insects. Esterases have six proteins families belong to a/β hydrolase fold super family (Cygler *et al*., 1993 and Oakeshott *et al*., 1993). On the same chromosome, they are present in form of cluster of gene in *Diptera* (Campbell *et al*., 1997). Modification of the individual member of cluster of gene in instances of insecticide resistance i.e. the specificity of esterase convert in to hydrolase insecticide by single amino acid change (Newcomb *et al*., 1997) or by amplification of multiple gene copies in resistant insect (examples are B1 (Mouches *et al*., 1990) and A2-B2 (Vaughan *et al*., 1997) amplicon in *culex pipiens* and *culex quinquefasciatus*).

By the O-, S-, and N-alkyl hydroxylation, aromatic hydroxylation, ester oxidation, epoxidation and aliphatic hydroxylation and nitrogen and thioether oxidation, insecticide metabolism takes place by Cytochrome P450 oxidases. Four families (4, 6, 9 and 18) of P450 isolated from insects. Family 6 contain P450 oxidases which is responsible for resistance (Maitra *et al*., 1996). Cluster members are expressed in form of multiple alleles (Tomita *et al*., 1995). Constitutive over-expression instead of amplification cause oxidases enhanced levels in resistant insects (Tomita and Scott, 1995 and Carino *et al*., 1994).

In resistance oxidase overproduction mechanism is under investigation process and expressed as a result from cis and trans acting factors, may be related to induction phenomenon (Cohen *et al*., 1994; Brun *et al*., 1996 and Liu and Scott, 1997). Multiple GSTs are present in most organisms (Hayes and Pulford, 1995). In DDT insecticide resistance, GST is implicated and present as gene cluster and by recombination they further shuffled through genome (Zhou and Syvanen, 1997). Resistant GSTs gene members characterized in vector in the same insect. (Ranson *et al*., 1997).

MECHANISM OF ACTION OF GST

The GSTs which present in cytosole are homodimer or heterodimer protein that is formed by 2 poly-peptide chains. Out of these two chains, each chain has 25kDa size (Armstrong, 1991). Each chain consists of two terminals i.e. N-terminal and C-terminal. About 1-80 amino acid residues are present in N-terminal and have a same confirmation as present in thioredoxin and thioredoxin present in GST structure. N-terminal contains active site or G-site which is the binding site of Glutathione (GSH). On the other hand, C-terminal have variable number of alpha helices which contains substrate binding site. The G-site or active site residues are highly conserved within the GST classes but they differs in between the classes. The active or G-site residue is responsible for the activation of GSH thiol residue in catalysis appears to be a tyrosine (Sheehan *et al*., 2001), but serine residue is responsible in case of epsilon and delta type (Ranson and Hemingway, 2005 and Udomsinprasert *et al*., 2005).

Endogenous and xenobiotic compounds can be detoxified by either directly or by the breakdown of reactive products (Yu, 1996 and Sheehan *et al*., 2001). The active thiolate ions are formed by the reaction of active site residue to the sulphydryl group of GSH. Thiolate ion is nucleophilic in nature and able to form GS-conjugate by attacking to the electrophilic centre of lipophilic compound (Armstrong, 1991). As a result of GS-conjugation, substrates electrophilic sites become neutralize and then detoxification occurs with the elimination of highly reactive electrophiles (Habig *et al*., 1974 and Hayes and Wolf, 1988). By the help of Glutathione S-conjugate export pump, the conjugates are removed from the cell (Sheehan *et al*., 2001). GSTs also used in to dehydrochlorinate the insecticides i.e. DDT in which GSH work as co-factor. Passive binding to insecticides or Reactive Oxygen Species (ROS) elimination

and lipid peroxidation (LPO) products detoxification also parts or methods of detoxification. ROS and LPO are products of the oxidative stress (Clark and Shamaan, 1984).

TARGET SITE RESISTANCE

In the site of action, amino acid alteration cause target site resistance. Single or multiple substitutions in sodium channel produce the knock down resistance (kdr) (Martinez *et al*., 1998 and Ranson *et al*., 2000a). Insecticide decreased sensitivity cause due to alteration in acytylcholinesterase (Mutero *et al*., 1994). In all insects which were dieldrin resistant, single codon mutation in the Rdl (resistance to dieldrin) has been reported, and decrease rate desensitization and insecticide insensitivity confers (Ffrench Constant *et al*., 1998).

ORGANOPHOSPHATES (OPS)

O-dealkylation and O-dearylation are two different detoxification pathways. In O-dealkylation, conjugation of GSH and insecticide alkyl portion occurs while in O-dearylation, GSH react with leaving group. O-dealkylation and O-dearylation identified in *Musca domestica* (Ugaki *et al*., 1985) and in *Plutella xylostella* (Chiang and Sun, 1993) and their verification occurs by recombinant GST enzymes usage in both species (Huang *et al*., 1998).

ORGANOCHLORINES (OCS)

Dehydrochlorination and GSH conjugation are two reactions to detoxify the halogenated hydrocarbons. Major detoxification pathway in mosquitoes is DDT-dehydrochlorination (Brown, 1986 and Hemingway, 2000). The generations of GS ions in the active site in GSH dependent DDT dehydrochlorination cause the removal of hydrogen from DDT which causes removal of chlorine for the production of DDE which is non toxic. Increased levels of Glutathione-dependent dehydrochlorination confirm the DDT resistance in *Aedes aegypti* (Grant *et al*., 1991 and Lumjuan *et al*., 2005), *Anopheles dirus* (Prapanthadara *et al*., 1996, 2000b) and *Anopheles gambiae* (Ranson *et al*., 2001 and Ortelli *et al*., 2003).

INSECTICIDES AND OXIDATIVE STRESS

Oxidative stress caused by the exposure of insecticides (Abdollahi *et al*., 2004) and insect GSTs play important role in anti-oxidant defence through preventing the Glutathione peroxidase (GPx) activity and repairing the secondary products damaging that are formed by ROS and direct conjugation of trans 4 hydroxy 2 nonenal (HNE) which is end product of lipid peroxidation (Singh *et al*., 2001, Sawicki *et al*., 2003 and Ding *et al*., 2005). From the delta, epsilon and sigma types of GSH, GPx activity has been detected in insect GST,s. Induction of GST expression caused by the oxidative stress produced by the putative binding sites and regulatory elements in epsilon and delta type of gene promoters from Anophilines, and has been found supporting the anti-oxidant physiological role of some GSTs (Ding *et al*., 2005 and Lumjuan *et al*., 2005).

INSECT CYTOCHROME P450

Cytochrome P450 is hemoprotein and in monooxigenase system, it act as terminal oxidases. Its name arises from its own absorbance peak characteristic at 450nm. Several names are exist of the P450 enzyme i.e. Cytochrome P450 monooxygenase, Mixed function oxidases, heme thiolate proteins etc. in the metabolism of exogenous and endogenous compounds, P450 play important role and also involve in necessary processes like growth development, plant toxins tolerance, feeding etc (Scott *et al*., 1998 and Scott, 1999). It also involve in degradation and synthesis of insect hormone and pheromones (Feyereisen, 1999).

ROLE OF CYTOCHROME P450 IN INSECTICIDE RESISTANCE

Cytochrome P450 annotated are present in the genome of *Anopheles gambiae* (Ranson *et al*., 2002). It is reported that in past *Anopheles gambiae* cytochrome P450 involved in resistance of insecticides. In *Anopheles gambiae*, P450s involvement in pyrethroid resistance to be described, specific P450 inhibitors uses in synergestic studies and in resistant mosquitoes the increased heme levels also given (Vulule *et al*.,1999). In pyrethroid resistant strain of *Anopheles gambiae* the cytochrome P450 over expression was reported (Nikou *et al*., 2003). In *Anopheles gambiae*, for further

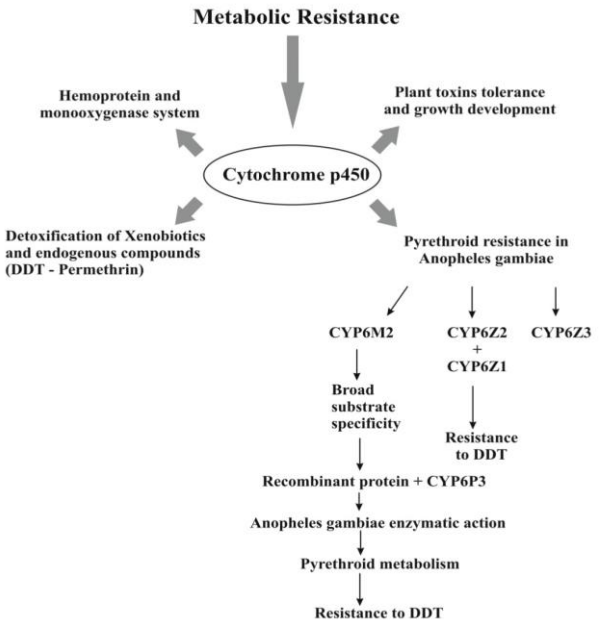
study of metabolic based insecticides, microarray chip was developed which contain fragments from 230 genes which associated with detoxification (David *et al*., 2005). In nigerian pyrethroid resistant strain of *Anopheles gambiae*, CYP325A3 was over-expressed constitutively (Awolola *et al*., 2009).

The gene which expression levels were related to pyrethroid resistance identified from Ghana. Out of these three were Cytochrome P450 (CYP6M2, CYP6Z2, CYP6Z3) (Muller *et al*., 2007). CYP6Z2 showed broad substrate specificity that may be related to xenobiotics detoxification and metabolism (McLaughlin *et al*., 2008). Although CYP6Z2 able to bind with permethrin and cypermethrin but it does not metabolize one of such insecticides (McLaughlin *et al*., 2008). Over-expression of several cytochrome P450 was identified in *Anopheles gambiae* pyrethroid resistant population (Djouaka *et al*., 2008). Recombinant proteins CYP6M2 (Stevenson *et al*., 2011) and CYP6P3 (Muller *et al*., 2008) of *Anopheles gambiae* studies described that pyrethroid could metabolize by the enzymes. In DDT resistant strain of *Anopheles gambiae*, another type (CYP314A1) was found (Vontas *et al*., 2005). In DDT resistant strain of *Anopheles gambiae*, CYP6Z1 and CYP6Z2 was over expressed (David *et al*., 2005).

CYTOCHROMES P450 AND MALARIA INFECTION

Injury responding genes, bacterial challenge and malarial infection responding genes was identified by analysis of genomic expression of *Anopheles gambiae* (Dimopoulos *et al*., 2002). Three cytochrome P450s were included, Out of these three, one is related to infection, oxidative stress and microbial challenge, second was related to septic infection response and third was related to malarial infection response and Lipopolysaccharide presence (Dimopoulos *et al*., 2002).

When CYP4C27 and CYP306A1 were expressed different ways s in the presence of gram negative (*Salmonella thyphimurium*) and gram positive (*Staphylococcus aeurus*),



SPECIFIC CYTOCHROME P450 RESISTANCE MECHANISM IN ANOPHELES GAMBIAE

the cytochrome P450 invovlement was exist into microbial challenge response (Aguilar *et al*., 2005). CYP4H17, CYP6M3, CYP6AG1, CYPaj5 were differently expressed cytochrome P450s; CYP49A1 and CYP12F4 were mitochondrial cytochromes (Dong *et al*., 2009). During different stages of invasion of mid gut, the cytochrome P450s expressed differentially and during plasmodium wild type infection comparison with plasmodium (Vlachou *et al*., 2005).

Response of *Anopheles gambiae* to different parasites i.e. *Plasmodium falciparum* and *Plasmodium berghei*, slightly immune response induced by mosquitoes to each parasite and mosquitoes able to sensed infected blood constituents, even that attacking ookinetes are not present (Dong *et al*., 2006). In the study, blood meal infected with chloroquine and blood meal infected with plasmodium was taken and gene expression of chloroquine and plasmodium for the presence of malaria was investigated. The results indicates that a variety of transcript encoded proteins involved in various processes of cytochrome P450 were infected by chloroquine whereas *Plasmodium berghei* present in the blood meal presented a different gene expression i.e. CYP9L1, CYP304B1, CYP305A1(Abrantes *et al*., 2008).

During the study of malarial infection, it was analyzed that the influence of the infection of

Plasmodium berghei is present at two time points i.e. one day blood meal and Eleven days after blood meal. During one day blood meal, the parasites attack the midgut epithelium while during eleven days after blood meal releasing of sporozoites were started to the hemolymph, in the mid gut and fat body. During one day time, 17 down-regulated and 5 up-regulated cytochromes P450 were reported in the mid gut when blood meal infected by the plasmodium. Up-regulated contain (CYP9L1, CYP304B1, CYP325H1, CYP6M2 and CYP6Z2). While in the fat body, 1 down-regulated and 5 up-regulated cytochrome P450s were found. In case of 11 days after blood mealtime, 3 down-regulated and 2 up-regulated cytochrome P450s were found in the mid gut, and in the fat body 1 up-regulated and 1 down-regulated cytochrome P450s were found. More significantly, cytochrome P450 might play direct role in plasmodium response when the parasites attack the epithelium of midgut. The cytochrome P450s over-expression could be part of response mechanism of mosquitoes to the parasite attack takes place in the mid gut. It might be possible that in the rearrangement of cytoskeleton, P450s play a important role (Vlachou *et al*., 2005 and Vlachou and Kafatos, 2005), or on the other hand, in the production of nitric oxide and other reactive oxygen redicals, P450s could be involved (Han *et al*., 2000 and Luckhart *et al*., 1998).

MOSQUITO RESISTANCE GENES

There are certain enzymes that are responsible for inducing resistance in mosquitoes, these enzymes up-regulated by some genes. Due to mutations in the mosquito proteins, resistance may produce directly. Many insecticides hit or target to the nervous system of the insect. Acetylcholinesterase affected by these insecticides. The genetic changes which occur due to insecticides even a small change, the target shape can alter due to these changes. That's why proteins do not consider susceptible to the insecticide poisoning (Brun *et al*., 1996).

Another way to mosquito resistance to insecticides is in which the insecticides do not reach to the target point or any system of mosquito. Due to the production of Glutathion-S-transferase (GSTs), Mono-oxygenase and Esterase in the gut, salivary glands and sub cuticular layer of mosquitoes, the insecticides or other repellents can soak up or break down. That's why insecticides do not effect to the mosquitoes. Some proteins are present in the salivary glands or gut which detect the presence of insecticides which mosquitoes inhaled through the cutical or swallowing. These proteins screen out the insecticides and due to the presence of screening proteins, resistance may produce in mosquitoes. These proteins produced in large quantity i.e. some insects possesses 80 times more esterase genes in all cells. Due to the presence of this large quantity of esterase proteins, the insecticides have no effect to the mosquitoes or any other insects (Cygler *et al*.,1993).

CONCLUSION

Cytochrome P450s have a strong connection with *Anopheles gambiae* response to malarial as cytochrome P450 provides insecticides resistance. So, production of cytochrome P450 is of utmost importance to combat malarial transmission. This review aims to identify specific cytochromes in the cell which are specifically involved in producing insecticide resistance. One of the cytochrome P450 which are highly expressed in a pyrethroid resistant strain of *Anopheles gambiae* is CYP6M2 and also over expressed in response to plasmodium infection in mid gut and fat body only 1 day after an infected blood meal showing that CYP6M2 simultaneously be involved in the two processes described above which play a key role in the activation mechanism of CYP6M2 expression. Similar mechanism is found in other cytochromes e.g. CYP6Z1 which is highly expressed in pyrethroid resistant strains of *Anopheles gambiae* and also over expressed in response to plasmodium infection. The increase in the expression of cytochrome P450 is mediated endogeneously by parasite infection and insecticide exposure. But CYP6Z2 is somewhat different as compared to CYP6M2 as it is down regulated in contrast to CYP6M2 in the midgut of *Anopheles gambiae* at day 1 and day 11 after an infected blood meal. CYP6Z2 is activated by binding with

permethrin and cypermethrin. However, CYP6Z2 is not able to metabolize these compounds. This result indicate that CYP6Z2 is a pyrethroid resistant strain when is over-expressed stimulating not only the insecticide resistance but also other processes. Hence it is concluded that the problem of insecticide resistance is mitigated by grasping the over-expression of cytochrome P450s.

Although GSTs attained a great attention from last few years but some aspects of GSTs remain unknown. The GST diversity in some mosquitoes species like *Culex quinquefasciatus* who has highly polluted breeding site, expected that higher diversity of GSTs. In anopheline population i.e. *Anopheles albimanus* and in *Anopheles gambiae*. Only DDT resistance was confirmed which related to GST-based mechanism in *Aedes aegypti* and in *Anopheles gambiae*, Cytochrome P450 monooxygenase may be involved in resistance to DDT. GSTs which have the Glutathione peroxidase (GPx) activity are also related to the pyrethroid resistance. The higher GST expression with the GPx activity increases the pyrethroid tolerance instead of the confirmation of resistance against such insecticide. The implementation of efficient malarial and vector control programme to combat malaria and other human diseases requires complete knowledge about the mechanistic role of cytochromes in providing parasite and insecticide resistance which is still not completely understood.

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