Review Article

Glutathione S-transferase Activity in Diagnostic Pathology

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Abstract

Glutathione S-transferase (GST) is a generic term that describes a class of proteins involved in phase-II detoxification of endogenous compounds and xenobiotics. Regulation and function of GSTs have influences on cell growth, oxidative stress, as well as disease progression and prevention. Although not routinely assayed in clinical laboratories, the present review highlighted the application of GST activity in diagnostic pathology. Human GSTs are divided into three main families, namely, the cytosolic, mitochondrial and membrane-bound microsomal GSTs. The expression of GST isoforms in biologic systems may well be of relevance from a clinical and toxicity standpoints and applies in the renewed efforts in eradication and control of parasitic infections. A survey of frequency of polymorphism and measure of GST activity among human population groups are reliable and valuable diagnostic tools. The reliability of GST activity in diagnostic pathology is underscored in pathologic conditions/disorders whose etiologies are associated with overwhelming levels of oxidative stress and failure of GST-mediated detoxification mechanisms.

Keywords: Diagnosis; Glutathione S-transferases; Isoforms; Pathology; Polymorphisms

Introduction

Glutathione S-transferase (GST; EC: 2.5.1.18) is a generic term that describes a class of proteins involved in phase-II detoxification of endogenous compounds and xenobiotics [1]. GST isoenzymes exhibit differential but overlapping substrate specificity, in which the catalytic efficiency of the individual isoenzyme dictates the extent to which biotransformation of the substrate occurs [2-5]. The GST-mediated detoxification pathway ensures cellular protection from environmental insults and oxidative stress, though it has also been implicated in cellular resistance to drugs [5-11]. For instance, overexpression of GSTs in the endothelium serve to protect soft tissues against oxidative damage from aldehydes such as 4-hydroxynonenal (4-HNE) [12] and neuroprotection of photoreceptors is connected with GST-mediated reduction of oxidative stress in retinal explants from *rd1/rd1* mice [13].

Much attention was focused on thiol-mediated antioxidant/detoxification proteins since the 1970s [14]. Early reports showed that GSTs isolated and characterized from rat and human liver [3,15-18], pigeon, locust gut, housefly and other sources [16] have common properties to bind to reduced glutathione (GSH) and wide variety of hydrophobic compounds. The structural dimensions of the GSTs have been exhaustively described [14]. Overall, regulation and function of GSTs have influences on cell growth, oxidative stress, as well as disease progression and prevention [5]. GSTs are divided into two distinct family members, the membrane-bound microsomal and cytosolic family members. The expression or activities of specific GST isoforms in various biological systems are closely associated with different clinical conditions and toxicity outcomes. Thus, surveying the frequency of polymorphism or measurement of GST activity among human population groups are reliable and valuable diagnostic tools. Although not routinely assayed in clinical laboratories, the present review highlighted the application of GST activity in diagnostic pathology because of the indispensable role of the enzyme in cellular functional integrity.

Evidence acquisition

Scientific search engines such as PubMed, Pubget, Medline, EMBASE, Google Scholar, ScienceDirect and SpringerLink were used to retrieve online publications from 1969 to 2015. Keywords such as 'Plasmodium', 'malaria', 'glutathione detoxification', 'ligandins' 'oncology' 'polymorphisms', 'metabolic disorders', 'hepatobiliary'

etc. were used to collate relevant articles. The results were then cross-referenced to generate a total number of 125 references cited in this review.

Functions of glutathione S-transferases

The functions of GSTs have been classified into two general categories [19,20]. As intracellular binding proteins [2,21,22], GSTs function on a broad scale in solubilizing and transport of substances much as the extracellular functions of albumin described elsewhere [23,24]. The GST from rat liver, designated as transferase B, has been shown to be identical to the bilirubin binding protein or 'ligandins' [25]. Although ligandins have high affinity for endogenous compounds such as bile acids, haemin, bilirubin, fatty acids and steroids [16,18,22], whose conjugates are eventually sequestered [26], the bound GSTs are devoid of catalytic processing and do not form glutathione conjugates with their substrates [18,27]. Another specific protective role of GST as ligandin is the specific binding of intra-erythrocyte GSTP1-1 isoform to Jun-kinase, a pro-apoptotic enzyme that becomes inactive when bound to GST [26,28].

The second major function is the protection of cellular components [29,30] by the preferential reaction of electrophilic agents with GSH through the enzymatic action of GSTs, and thereby prevents the reaction of electrophiles with cellular nucleophiles. The enzyme may also detoxify certain extremely reactive substances by direct covalent binding to electrophilic agents [1,22,31]. For the most part, GSTs catalyze the conjugation of electrophilic groups of hydrophobic drugs and xenobiotics to form glutathione-thioethers [32]. These thioethers are converted to mercapturic acid by the sequential actions of γ-glutamyl transpeptidase, depeptidase and N-acetylase [2,15,33] prior to the eventual elimination of the hydrophilic conjugates.

Reactive oxygen and nitrogen species (ROS/RNS) can alter the

structure and/or function of number proteins by their selective modification of proteins such as enzymes, receptors, structural proteins, transcription factors and transport proteins and may also alter a variety of protein-protein interactions. Additionally, raised cellular ROS/RNS levels promote peroxidation of structural and particulate lipids such as those present in biomembranes and lipoproteins, which form the molecular basis of several pathologic conditions. The GSTs participate in protecting the cell against deleterious actions of ROS/RNS by promoting redox homeostasis through neutralization of excess reactive electrophiles, whose chemical actions elicit numerous signaling cascades associated with cell proliferation, inflammatory responses, apoptosis and senescence [14]. In parasitic infections, e.g. malaria, GST is a component of the thiol-mediated antioxidant detoxification systems of Plasmodium that are required for survival of the malarial parasite in hyperoxidative intracellular environment it encounters during its development in mammalian and insect hosts [7,34-36]. Also, physiochemical analyses of the gastrointestinal blood-feeding nematode *Haemonchus contortus* showed that the parasite harbors GST of high-affinity for hematin, which represented the parasite adaptation to blood or tissue feeding from the host [37]. Helminth GSTs participate in detoxification of lipid hydroperoxides and carbonyl cytotoxins produced by oxygen-reactive intermediates [38]. Accordingly, *Plasmodium falciparum, H. contortus* and helminth GSTs have been suggested to be attractive targets for new anti-parasitic drugs and vaccine discovery [37-39].

Molecular dimensions of glutathione *S*-transferases

Human GSTs are divided into three main families, namely, the cytosolic, mitochondrial and membrane-bound microsomal GSTs [5,8]. For instance, the cytosolic human *GSTP1-1* isoform (*h*GSTP1-1), which is a major intra-erythrocyte transferase- representing 95% of its entire GST pool [20,40], is a homodimeric intracellular protein of about 46 kDa expressed in different organs and cell types [26]. Specifically, the molecular mass of the cytosolic GSTs monomers are in the range of 22-29 KDa and exhibit activity over a wide variety of substrates with considerable overlap [1,2,4]. Each monomer contains an active site with two sub-sites: a less conserved H site for binding to varieties of hydrophobic substrates and a highly conserved G site for GSH binding [14]. The general mammalian cytosolic GSTs encompass dissimilar dimeric isoenzymes within molecular mass of 45-55 KDa, which have been assigned to at least four generic classes, namely, α-, μ-, π-, and Ɵ-GSTs [2,3,26,41,42] in addition to the K- Ω-, δ-GSTs [5,43], whereas the membrane bound microsomal GSTs are component of another classified proteins, the so called membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) [44-46]. The MAPEG entities are involved in endogenous metabolism of leukotrienes- and prostaglandins-derived mediators of pain, fever, and inflammation as well as in biotransformation and detoxification of electrophilic substances [14,46]. Using electron crystallography, the molecular dimensions of microsomal GST has been established as well as identification of critical amino acid residues that are responsible for intramolecular or intermolecular contacts in stabilizing the active site, which applies in interpreting the structure–function relationship for similar MAPEG entities [46].

Isoforms

The human liver cytosolic GST classes are α-, β-, γ-, δ-, ξ- [25] and Ź-GSTs [47]. These six classes of GST originate from six different chromosomes but share ~ 30% sequence identity [14]. GST isoforms have cell specific distribution (e.g. *GSTA1* in hepatocytes and *GSTP1* in

the biliary tract of the human liver) [48]. The *GSTP1* isoform exists in the human erythrocytes [49], which is different from the GSTs present in the human liver [1,17]. Although, all GST isozymes have two domains and similar topologies, <10% of the protein is strictly conserved [14]. The occurrence of genetic polymorphisms in the various classes of cytosolic GSTs have been described and linked to the prevalence of incidences of cancers, parasitic infections, diverse pathologic conditions and disorders [8,10,14,50-52]. For instance, polymorphisms in the *GSTP1* gene arise from nucleotide transitions that change codon 105 from Ile to Val and codon 114 from Ala to Val, thus generating four *GSTP1* alleles: wild-type *GSTP1*A* (Ile105→Ala114), *GSTP1*B* (Val105→Ala114), *GSTP1*C* (Val105→Val114) and *GSTP1*D* (Ile105→Val114) [14,53]. Evaluation of three GST isoforms-*GSTM1*, *GSTP1* and *GSTT1* in patients presenting acute respiratory distress syndrome showed an association between *GSTM1*-null, *GSTM1*- and *GSTT1*-double null polymorphisms with increased incidence of mortality [10]. Additionally, individuals with the *GSTM1*-null or *GSTT1*-null genotypes display absence of enzymatic activity and are believed to be at higher risk to cytotoxic effects of a wide spectrum of xenobiotics and carcinogens [54]. Structural analyses of these GST variants showed steric alteration at the substrate-binding site of the enzyme without affecting the GSH binding site, which correspondingly implies that GSH binding affinity are not affected, whereas significant alterations at the substrate binding domain occur [55]. As a consequence of GST polymorphisms, enzymatic activities of the GST variants are significantly altered.

Parasitic infections

The level of parasite GST activity serves to define the capability of the organism to withstand anti-parasitic drugs. For instance, in chloroquine-resistant strains of rodent (*Plasmodium berghei, P. yoelii*) and human malarial parasites, GST activity was significantly raised when compared with the sensitive strains and was directly related to drug pressure on resistant parasite [56]. Alterations in serum or plasma levels of GST activity and its GSH cofactor are diagnostic of parasitic infection in susceptible individuals [57,58]. According to Sohail et al., [57] the mean activity of GST in serum and plasma of patients infected with *P. vivax* were less (6.43 and 5.65 IU/L respectively) than the corresponding healthy subjects (11.65 and 10.09 IU/L respectively). They further noted that the decreased GST activity served to protect the host erythrocytes against the invading malarial parasite by up-regulating oxidative defense mechanisms. In the same vein, previous reports have shown that antioxidants such as GSH, catalase and α-tocopherol were lower in patients with malaria [51,59] or visceral Leishmaniasis [58] than the control groups. Consequently, low levels of plasma GST activity may elicit the accumulation of ROS/RNS because of decreased antioxidant scavenging capacities with attendant membrane lipid peroxidation and oxidation of haemoglobin to methaemoglobin [58]. According to Chikezie et al., [60], outcome of comparative study of GST activity of human erythrocyte genotypes infected with *P. falciparum* suggested that GST activity was a reliable biomarker and possesses promising rational for diagnostic potential in malaria.

In another investigation, Kavishe et al., [51] reported that polymorphisms in GST genes were associated with susceptibility and severity *P. falciparum* malaria in Cameroon population. In their study, the frequencies of polymorphisms in *GSTM1*, *GSTT1*, and *GSTP1* in DNA of 138 children from Cameroon were analyzed using multiplex polymerase chain reaction (mPCR) assay, whereas that of *GSTP1* was done using polymerase chain reaction-restriction fragment

length polymorphism (PCR-RFLP) assay. The findings indicated that malarious subjects with complications were more often of the *GSTM1*-null genotype (58–64%) as against those with uncomplicated malaria (32%), a difference that was statistically significant (x^2=6.7; p=0.05: x^2=3.5; p=0.031). Thus, there was an established relationship between the frequencies of GST polymorphisms and levels of severity and complication of malaria in children.

Oncology

The GST isoforms conjugate GSH to electrophilic carcinogens and there are incontrovertible evidence that underscores the relationship between GST polymorphism and incidence of development of cancers of the gastrointestinal tract, ovaries, prostate, and esophagus in selected mammalian population [9,61-67]. Furthermore, the level of expression of GST could provide useful diagnostic parameter in carcinoma of the breast [68] and bladder [69,70]. Evidence from molecular epidemiological studies showed that individual susceptibility to cancer is mediated by both genetic and environmental factors. For instance, individuals who have both *GSTT1*- and *GSTM1*-null genotypes are more predisposed to acute myeloid leukemia [71] as well as oral leukoplakia risk as a result of carcinogenic intermediates derived from or generated during habitual chewing of betel quid/tobacco [72,73].

In oncology, the *GSTP1* isoform, which is the most ubiquitous and prevalent GST in non-hepatic tissues, are present in raised levels in many tumors, particularly, that of the ovarian, non-small cell lung, breast, colon, pancreas and lymphomas as well as in wide range of drug resistant cell lines [14,74]. In specific terms, Bostwick et al., [9] reported differential expression of *GSTA1*, *GSTM1* and *GSTP1* isoforms in benign prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma. The study observed that consistent reduction or loss of expression of all subclasses of GST could engender the progression of prostatic neoplasia from benign epithelium to high-grade prostatic intraepithelial neoplasia and carcinoma. Another investigation according to Chen and Lin, [63] showed that raised levels of *GSTA1*, *GSTM1* and *GSTP1* activities confirmed oral epithelial dysplasias (OEDs) and squamous cell carcinomas in human. Specifically, total GST activities of the three isoforms were significantly elevated in mild OED, moderate OED, severe OED and squamous cell carcinoma when compared with that from normal buccal mucosa [63]. They further noted that *GSTP1* was the major isoform in the cytosolic fraction of oral mucosa and severity of OED was connected with the development squamous cell carcinoma, which appeared to increase correspondingly with increased level of *GSTP1* activity.

In another study, Naidu et al., [64] described the implication of total GST activity levels and *GSTP1* protein expression in paired samples of colorectal cancer, adenoma and normal mucosa from a total of thirteen patients using spectrophotometric methods. The study showed that GST activity was significantly raised in both colorectal cancer and adenomas when compared with normal colonic tissue-an indication that raised levels of GST activity may serve as a useful diagnostic index for colonic neoplasia in humans. Conversely, in the reports of Szarka et al., [61] significant low levels of GST activity was noted in blood lymphocytes from high-risk colorectal cancer individuals when compared with blood lymphocytes from control individuals (p < or = 0.004). However, no association was observed between the frequency of *GSTM1* phenotype and risk of colorectal cancer and high-risk individuals unable to express *GSTM1* had lower levels of GST activity than those from control subjects (p < or = 0.006) [61]. There are evidence to suggest an association between *GSTM1*-null genotype and increased risk of gastric cancer [67]. Using case-control study approach

involving PCR-based assays, Casson et al., [66] hypothesized that polymorphisms of microsomal epoxide hydroxylase and GST genes modulated the susceptibility to esophageal adenocarcinoma (EADC) associated with smoking, which showed a strong statistical association between smoking and risk for EADC in individuals.

There are contradictory reports on the use of GST activity as a diagnostic index in the monitor and ascertaining therapeutic benefits following treatment of ovarian cancer patients. Wrigley et al., [62] used immunohistochemistry and Western blot methods to evaluate the correlation between *GSTA1*, *GSTM1* and *GSTP1* isoforms activities in association with clinicopathological features and response to treatment in ovarian cancer, in which they noted that none of the GST isoenzyme levels were significantly correlated with response to treatment. In a related study, serum GST activity of untreated patients with malignant ovarian tumors was significantly raised when compared with those of healthy individuals and patients with benign ovarian tumors [65]. From their study, Akçay et al., [65] further suggested that a monitor of GST activity was important in diagnostic and therapeutic approach to detection and treatment of ovarian cancer.

Hepatobiliary damage

Patients with hepatocellular damage exhibit elevated plasma GST activity [75,76]. Although serum *GSTA1* activity is a sensitive biomarker of liver injury [77,78], studies according to Thorburn et al., [79] showed that it was not useful in ascertaining the level of liver inflammation in chronic hepatitis C infection, though the data may be of more value than ALT in monitoring response to treatment with α-interferon. Nevertheless, reports of Yukihiko et al., [77] noted that the degree of correlation between serum GST and ALT or AST was high in acute hepatitis, with ALT or AST exceeding 200 IU/L in fulminant hepatitis, primary hepatoma and gall stones, whereas in chronic hepatitis and liver cirrhosis the correlation was low. Furthermore, serum GST exhibit shooter half-life than ALT or AST in the blood, which suggest new and unique information for the diagnosis of acute liver diseases. A recent clinical survey by Weng et al., [80] established the association between drug hepatotoxicity and daily dose, liver metabolism and lipophilicity of oral medications. They noted that high oral dose of drugs and extensive hepatic GST mediated drug metabolism are independent but not synergistic risk factor for oral drug to induce hepatic injury, which correlated with significant plasma ALT/AST elevation. The findings have further confirmed the potentials of applying GST activity in prediction and monitoring of drug toxicity. Nevertheless, earlier reports by Rattenbury et al., [81] had noted that measurement of *GSTB1* isoform in serum using radioimmunoassay technique may be a better predictor of hepatic dysfunction in cystic fibrosis than conventional liver function tests.

Diabetes mellitus and metabolic disorders

The GSTs, in concert with antioxidant systems, modulate oxidative stress associated with diabetes mellitus (DM). For instance, mitochondria-specific *GSTK* isoform has also been implicated in obesity, diabetes and related metabolic disorders [5]. Conversely, *GSTP1*-isoleucine/valine and valine/valine alleles, alone or in association with *GSTM1*-null and *GSTT1*-present genotypes, does not influence the risk of susceptibility to development of metabolic disorders [82]. Although the studies according to Santl et al., [83] did not find a significant association between *GSTM1* and *GSTP1* polymorphisms and carotid atherosclerosis, the *GSTT1*-null genotype and *GSTT1*-null/*GSTM1*-null haplotype might be potential determinants of susceptibility to advanced atherosclerosis in patients with T2DM.

Using a 2-year change of the common carotid intima media thickness (CCA-IMT), which was measured using B-mode ultrasonography, de Waart et al., [84] suggested that smokers who expressed the *GSTM1*-null genotype, and thus lacking this functional detoxifying enzyme, developed progression of atherosclerosis in a significantly ($p=0.02$) increased rate when compared with smokers with *GSTM1*-positive genotype. A related study by Park et al., [85] showed that *GSTT1*–null genotype might be connected with carotid atherosclerosis related to rheumatoid arthritis in Korean postmenopausal women without histories of smoking. Furthermore, the protective role of GST activity against vascular cell injury was reported by Xu et al., [86], in which they established that *h*GSTA4-4 overexpression protected the integrity of vessel wall epithelium from oxidative injury, as earlier mentioned [12], and attenuated transplant arteriosclerosis.

Studies have shown that silencing the *GSTA4* gene resulted in mitochondrial dysfunction, as was also observed in mice that exhibited *GSTA4*-null genotype, which contributed to insulin resistance in Type 2 DM (T2DM) [5]. Accordingly, mPCR assay of GST isoforms has established a relationship between GST gene polymorphism and DM [87-89]. In specific instance, the *GSTM1*-null/*GSTT1*-null genotypes contributed to the clinical course of T2DM patients and increased susceptibility to T2DM in Brazilian population [88], whereas *GSTT1*-present genotype conferred protection against the development of a T2DM [90-92]. Another study by Amer et al., [87] showed that the frequency of the Val allele in exon 5 of the *GSTP1* gene in patients with T2DM was 15.2% as against that observed in healthy controls (9.6%); and was statistically significant ($p=0.03$) when compared with Ile allele carriers. They further noted that the presence of *GSTP1* heterozygous mutant allele Ile/Val in Egyptian population was more common in subjects with T2DM than in the control group (30.4% and 19.2%, respectively; $p=0.02$). Recent findings of Rasheed et al., [89] have corroborated the association of *GSTM1* and *GSTT1* gene polymorphisms with T2DM in Iraqi patients.

Cross-sectional study by Velladath et al., [93] showed a weak positive correlation between erythrocyte GST activity and HbA$_{1c}$ concentration in diabetic patients ($r=0.239$, $p=0.089$). Although the study appeared to suggest that GST activity is associated with formation of HbA$_{1c}$ in diabetic patients, chronicity of the disease along with treatment modalities that might have played a significant role in the outcome of the study [93].

Reproductive system disorders

The *GSTM1*- and *GSTT1*-null genotypes have been associated with male infertility [94] and female infertility [95,96]. The high frequency of homozygotes *GSTM1* gene deletion among patients with endometriosis suggests a possible contribution of environmental toxins in the pathogenesis of this disease due to the absence or low activity of *GSTM1* [50]. Many environmental and genetic etiological factors that are responsible for the occurrence of early pregnancy loss have been controversially discussed. However, the frequencies of polymorphisms in phase-I drug metabolizing enzymes (cytochrome P450 genes) and phase-II detoxification enzymes (e.g., GST genes) may contribute to the development of pre-eclampsia [96]. The occurrence of the *GSTP1b-1b* genotype –a non-functional *GSTM1* or *GSTT1* allele has been linked to relatively lower *GSTP1* activity with consequential impaired placental detoxification capability, which represents a risk factor for recurrent early pregnancy loss [97]. In concord with earlier investigation by Suryanarayana et al., [98], studies according to Polimanti et al., [99] and Nair et al., [100] reported that the expression of *GSTA1*- and *GSTM1*-positive/null genotypes in Italian women and

GSTT1- and *GSTM1*-null genotypes in Indian women, respectively were connected with the incidence of reoccurring pregnancy loss. The findings particularly noted that the -69T allele in the *GSTA1* gene may be considered as a predisposing factor of recurrent miscarriage.

Chandra et al., [101] recently reported that there was a definite association between maternal *GSTT1*- and *GSTM1*-null genotypes activities and higher incidence ($p=0.001$) of fetal growth restriction (IFGR), which correlated with fetal weight. They further noted that cumulative levels of GST activity in mothers giving birth to IFGR babies was about 50% of the values found in the control group ($p<0.001$). Measurement of GST activity in neonates is a useful biomarker of oxidative stress and evaluation of protective treatment trials at birth [102].

Measurement of human placental GST activity served to ascertain general health status of newborn in radioactivity-contaminated and chemically polluted areas of Ukraine and Belarus [103]. The findings showed that newborns with the most compromised health status displayed the greatest down-regulation of GST activity=144–162 mU mg protein^{-1} as against newborn GST activity=258–395 mU mg protein^{-1} in uncontaminated and pollution free areas.

Finally, GST activity is a reliable biomarker of oxidative stress in liver, kidney and testes, and more specifically, a measure to ascertain acute toxic effects of zearalenone, which is a non-steroidal estrogenic mycotoxin produced by several species of Fusarium, on reproductive system of adult male Swiss albino mice [104]. A related study by Quinn et al., [105] showed that resistance of mouse liver to aflatoxin B1 (AFB1) could be traced to single constitutive GST isoenzyme with a relatively high activity toward DNA-binding metabolites of AFB1.

Alcoholism and substance abuse

GSTM1 genotype in human population may be associated with a greater susceptibility to alcohol-induced spermatogenesis disorders, whereas the occurrence of *GST M1*-null and *GSTP1*-null genotypes are associated with alcoholic liver disease [106-109]. Expectedly, alcohol-dependent subjects exhibited significantly lower ($p<0.001$) GST activity in blood and saliva when compared with control subjects due to elevated oxidative stress and impairment of antioxidants in alcoholism [110]. Nevertheless, the investigation revealed a significant increase ($p<0.001$) in GST activity, with near control values, following alcohol withdrawal.

According to the reports of La Vignera et al., [111], the frequency of *GSTM1* genotype in heavy drinkers with normal spermatogenesis differed from that of corresponding moderate drinkers, whereas the frequency of *GSTM1* genotype in heavy drinkers with disorders of spermatogenesis was similar to moderate drinkers with or without disorders of spermatogenesis. Additionally, since the study of Pajarinen et al., [106] noted that >20% of heavy drinkers had normal spermatogenesis; it was an indication that the *GSTM1* genotype exerts a protective effects on alcohol-induced spermatogenesis disorders.

Studies involving the role GSH and redox signaling in substance abuse showed that chronic use of drugs, such as cocaine and methamphetamine lead to the formation of ROS/RNS and alterations in GSH and redox homeostasis [112]. As expected, GST activity exerted protective role during substance abuse and intoxication. The hazardous effect of heroin intoxication on different regions of the brain was evaluated using real time polymerase chain reaction (RT-PCR) in the reports of Gutowicz et al., [113], in which they noted that the protective effect of GST was observed to be lower in brain stem than in brain cortex or hippocampus.

Renal injury and uremia

Localization of *GSTA1* isoform in specific parts of the proximal renal tubule, which is readily released into the urine following injury, is used an excellent biomarker for proteinuria [114]. Over-expression of GST by >50% in erythrocytes of patients with chronic renal failure and uremia [20,26,115,116] and early diagnosis for uremia-related complications [117], which have received immense attention and documentations. Galli et al., [20] reported that over-expression of GST activity was the consequence of an increased expression of the protein rather than a kinetic modulation of the enzyme. In these studies, the significant increase in erythrocyte GST activity in pre-dialysis patients showed a positive correlation with the disease severity. Furthermore, the stable level of erythrocyte GST activity, during the life span of the erythrocyte, provides a suitable yardstick for assessing the adequacy of different dialytic techniques [20,26]. One of such evaluation was carried out in complementary to a mathematical model that takes into account the urea clearance in a single hemodialysis session {Kt/V_{urea}: parameter for classification of kidney disease outcomes quality initiative [118]; where K = dialyzer clearance of urea, t=dialysis time and V=volume of distribution of urea} in the course of depuration against large and small toxins in uremic patients [26].

Rheumatoid arthritis

Meta-analysis of epidemiological surveys involving PCR based assays showed that there was a relationship between *GSTM1, GSTT1* and *GSTP1* polymorphisms and pathogenesis of rheumatoid arthritis in Asian or European population [119-122]. On the contrary, case-control study approach using amplification refractory mutation system-PCR and mPCR assays revealed that only the *GSTM1* polymorphism was associated with rheumatoid arthritis risk in a sample of the Iranian population [123]. Toxicological assessments showed that *GSTM1*-null and *GSTT1*-null genotypes alongside *GSTP1* (Ile105→Val114) polymorphism display low GST detoxification capacity, which are potential risk factors that influence susceptibility to rheumatoid arthritis and impact on the outcome of the disease [124]. The therapeutic response to non-steroidal anti-inflammatory drugs (e.g., D-penicillamine) in rheumatoid arthritis may be influenced by GST polymorphisms. Studies according to Layton et al., [125] showed that patients who possess the *GSTM1*0-GSTM3*A* haplotype are significantly less likely to show beneficial response to D-penicillamine.

Conclusion

The expression of various GST isoforms in biologic systems is of relevance from clinical and toxicity standpoints and applies in renewed efforts in the eradication and control of parasitic infections. The present review showed that a survey of frequency of polymorphism and a measure of GST activity among human population groups are reliable and valuable diagnostic tools in ascertaining the prevalence and susceptibility to various forms of pathology by individuals who express such protein genotypes. Additionally, the reliability of GST activity in diagnostic pathology is underscored in pathologic conditions/disorders whose etiologies are associated with overwhelming levels of oxidative stress and failure of GST-mediated detoxification mechanisms.

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