

INVESTIGATION OF GLUTATHIONE S-TRANSFERASE (GST)
EXPRESSION AND ACTIVITY IN MOUSE WITH MULTIPLE SCLEROSIS
(MS)

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**INVESTIGATION OF GLUTATHIONE S-TRANSFERASE (GST)
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SCLEROSIS (MS)**

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

INVESTIGATION OF GLUTATHIONE S-TRANSFERASE (GST) EXPRESSION AND ACTIVITY IN MOUSE WITH MULTIPLE SCLEROSIS (MS)

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Multiple Sclerosis (MS) of unknown etiopathogenesis is a chronic demyelinating disease of the central nervous system. It mainly destroys myelin in the brain and spinal cord. Non-traumatic injuries have been observed in this disease for young adults. Various factors affect MS, but oxidative stress is one of the most important causes of demyelination. Glutathione S- Transferases (GSTs) can be described as a versatile enzyme family of eukaryotic and prokaryotic phase II metabolic isoenzymes. They have enzymatic or non-enzymatic functions in the body. Their main task is to conjugate GSH to endogenous and exogenous electrophilic compounds for the detoxification process. In this study, it was aimed to reveal the relationship between the activity and protein expression of the Glutathione S-Transferase (GST) enzyme family and MS disease in a female C57BL/6 mouse autoimmune encephalomyelitis (EAE) model. There was no statistically significant difference ($p \leq 0.05$) in mouse liver GST protein expression between the two groups of animals, the MS patient model and the control group. However, higher GST enzyme activity was detected in the MS group compared to the control group. In

conclusion, considering the post-translational modifications affecting GST members in some pathways, this study could lead to the development of a new drug metabolized by GST that can be used in the treatment of MS and studied in detail in the future.

Keywords: Multiple Sclerosis, Glutathione S-Transferase, Experimental Autoimmune Encephalomyelitis, Mouse



ÖZ

GLUTATYON S-TRANSFERAZ'IN (GST) EKSPRESYONU VE AKTİVİTESİNİN MULTİPL SKLEROZLU (MS) FAREDE İNCELENMESİ

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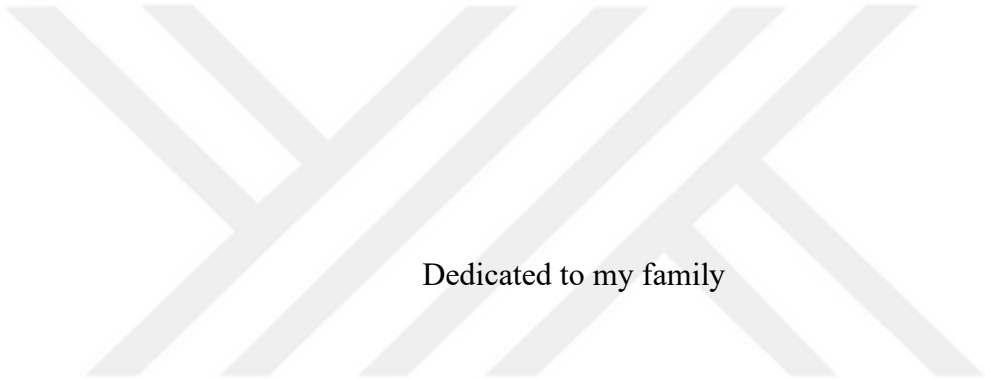
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Etiyopatogenezi bilinmeyen Multipl Skleroz (MS), merkezi sinir sisteminin kronik demiyelinizan bir hastalığıdır. Esas olarak beyin ve omurilikteki miyelini yok eder. Bu hastalıkta travmatik olmayan sakatlıklar genç yetişkinler için gözlenlenmiştir. MS'i çeşitli faktörler etkiler, ancak oksidatif stres demiyelinizasyon oluşumunun en önemli nedenlerinden biridir. Glutasyon S-Transferazlar (GST'ler), ökaryotik ve prokaryotik faz II metabolik izoenzimlerin çok yönlü bir enzim ailesi olarak tanımlanabilir. Vücutta enzimatik veya enzimatik olmayan fonksiyonlara sahiptirler. Ana görevleri, detoksifikasyon işlemi için GSH'nin endojen ve eksojen elektrofilik bileşiklere konjugasyonunu sağlamaktır. Bu çalışmada, dişi C57BL/6 fare otoimmün ensefalomyelit (EAE) modelinde, Glutasyon S-Transferaz (GST) enzim ailesinin aktivitesi ve protein ekspresyonu ile MS hastalığının ilişkisi ortaya konmak istenmiştir. MS hasta modeli ve kontrol grubu olmak üzere iki grup hayvan arasında fare karaciğer GST protein ekspresyonunda istatistiksel olarak anlamlı bir fark ($p \leq 0.05$) bulunmamıştır. Ancak MS grubunda, kontrol grubuna göre daha yüksek protein aktivitesi tespit edilmiştir. Sonuç olarak, bazı yolaklarda yer alan GST

üyelerini etkileyen translasyon sonrası modifikasyonlar göz önünde bulundurulduğunda, bu çalışma GST tarafından metabolize edilen yeni bir ilacın geliştirilmesine öncülük edebilir ve MS tedavisinde kullanılabilir ve gelecekte ayrıntılı olarak çalışılabilir.

Anahtar Kelimeler: Multipl Skleroz, Glutasyon S-Transferaz, Deneysel Otoimmün Ensefalomyelit, Fare





Dedicated to my family

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LIST OF ABBREVIATIONS

ABBREVIATIONS

APC	Antigen Presenting Cells
BBB	Blood-Brain Barrier
BCA	Bicinchoninic Acid
BSA	Bovine serum albumin
CD	Celiac Disease
CIS	Clinically Isolated Syndrome
CNS	Central Nervous System
COX2	Cyclooxygenase 2
CSF	Cerebrospinal Fluid
EAE	Experimental Autoimmune Encephalomyelitis
EBV	Epstein–Barr Virus
ERB	Elektronik Running Buffer
GST	Glutathione S-transferase
MAPEG	Membrane-Associated Proteins in Eicosanoid and Glutathione Metabolism
MBP	Myelin basic protein
MHC	Major Histocompatibility Complex
MOG	Myelin Oligodendrocyte Glycoprotein
MS	Multiple Sclerosis
NO	Nitric Oxide

PGD ₂	Prostaglandin D ₂
PGH ₂	Prostaglandin H ₂
PLP	Proteolipid protein
RIS	Radiologically Isolated Syndrome
TCR	T Cell Receptor
Th	T helper cell
T _{REG}	Regulatory T Cell



CHAPTER 1

INTRODUCTION

1.1 Multiple Sclerosis

Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) affected by the combination of both genetic predisposition and environmental factors (Patsopoulos, 2018). It is a type of chronic disease that is thought to have an autoimmune origin and comorbidities with another autoimmunity like Epstein–Barr Virus [EBV] infection (Constantinescu & Gran, 2010). MS affects approximately 2.3 million people globally, specifically young adults (Doshi & Chataway, 2017). Women in the world encounter two-fold higher risk in MS when compared with men (Ferreira et al., 2013). Myelin sheath around axons in the brain and spinal cord is destructed in the course of MS (Depaz et al., 2011). Spasticity, fatigue, bladder, and cognitive dysfunction can be notable for the common symptoms of MS, each of which has specific treatments (Thelen et al., 2021). To clarify disease etiology, an improved mechanistic understanding of the genetic background of neurological disease is of utmost importance. The disease-related Major Histocompatibility Complex (MHC) Class II molecules play a crucial role at this stage in a way that they enable foreign or autoantigenic peptides to be recognized by T cell receptors (TCRs) on autoreactive CD4⁺ T cells (Gregersen et al., 2004). When an unknown antigen is recognized, autoreactive cells T Helper1 (Th) and Th17 are activated to produce pro-inflammatory cytokines; interleukin 1 (IL-1) & interferon-gamma (IFN- γ) and interleukin 17 (IL-17) (Klineova & Lublin, 2018). Their production leads to more Th cells getting in and deterioration of blood-brain barrier (BBB), which gives rise to Th cells to move on to CNS and evoking another inflammation with microglia activation, oxidative damage, energy failure resulted from a mitochondrial injury as

shown in Figure 1.1. Ultimately, they cause the formation of plaque or sclera. Besides Th cells, B cells are involved in the pathogenesis of MS, as well. In addition to demyelination, neuronal and axonal injury, inflammation and oxidative stress are also involved in advancement of MS (Parchami Barjui et al., 2017).

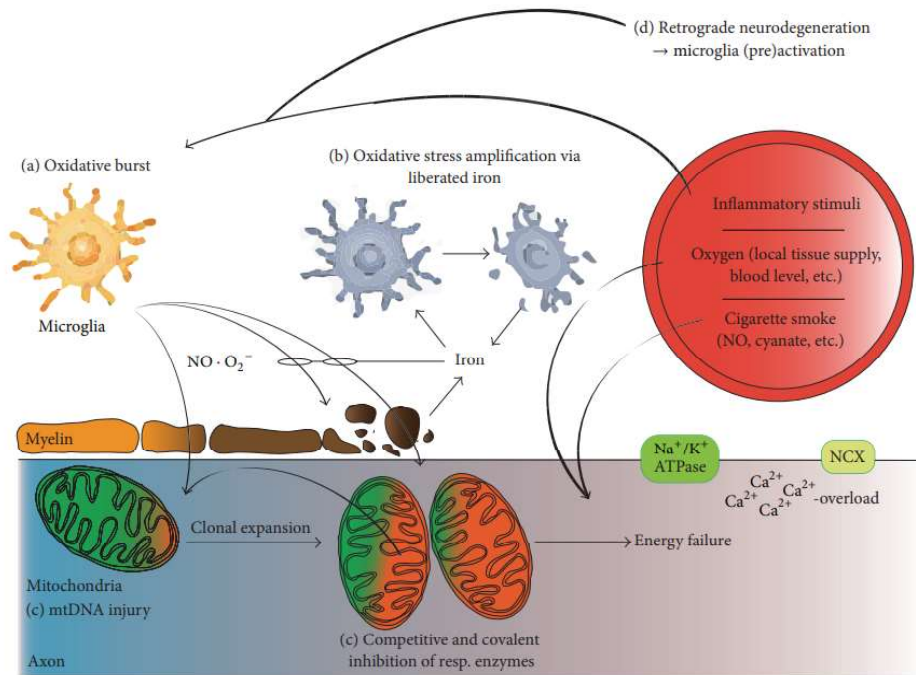


Figure 1.1 Some mechanisms leading to tissue injury in Multiple Sclerosis (Lutsikii & Esaulenko, 2007).

Cytoskeletal rearrangements in endothelial cells and loss of tight junctions resulting from locally produced ROS lead to a change in BBB permeability, promoting myelin phagocytosis, which brings about axonal injury, mitochondrial dysfunction, and OD cell death (Carvalho et al., 2014). For MS patients, the body's immune system senses the myelin as a foreign substance like bacteria, attacking and stripping the myelin sheath off the nerve fibers either entirely or partially, forming scars called lesions or plaques. After that damage to the myelin, messages coming from the brain can ease off, broken or not get through at all.

There are two forms of MS, an aggressive form that causes severe disability or death and a benign state. Latter leads to minor disability after a long disease duration. However, in both cases for young adults, non-traumatic disabilities have been observed. In addition to patients' medical history and conducting a physical examination, two clinical tools, Magnetic Resonance Imaging (MRI) and Lumbar Puncture (LP) are the tests that can be used to diagnose MS. After revealing the importance of axonal loss related to disability in MS, MRI studies have become more crucial since tissue disruption has been shown via T1-weighted images, and spinal cord atrophy can be a vital sign for disability (Mann et al., 2000). Besides, paramagnetic substances like free radical nitric oxide (NO) in and around active plaques has been revealed with the help of MRI. Depending on the places where the damage occurs, several symptoms can be seen, such as fatigue, problems with walking, balance or coordination, blurred or double vision, loss of muscle strength, cognitive and speech problems, etc. There are three different types of MS proposed in 1996 by the U.S. National Multiple Sclerosis Society (NMSS) Advisory Committee on Clinical Trials in Multiple Sclerosis, which are Relapsing-Remitting MS (RRMS), Secondary Progressive MS (SPMS), and Primary Progressive MS (PPMS) as shown in Figure 1.2.

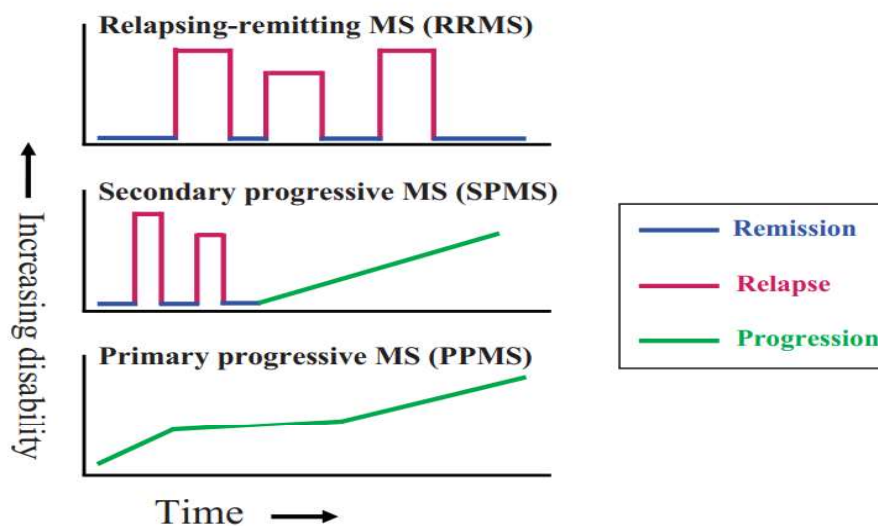


Figure 1.2 The types of MS (Depaz et al., 2011).

RRMS is typically defined as the type of MS that most patients show (Sand, 2015). They can recover entirely or incompletely between attacks or clinically named relapses that occur due to demyelination of focal area lasting over 24 hours and extending days or weeks before improving. The time when the relapses occur is unpredictable, which can last for days, weeks, or months and can appear with new symptoms or worsening of previous symptoms. SPMS can be seen later in the people with RRMS previously when their condition worsens consistently. The patients with SPMS show a gradual decline in neurological functioning; in other words, decrease in brain volume and develop further axonal loss (Parchami Barjui et al., 2017). For PPMS, the patients do not go through the relapsing-remitting stage, and the patient's clinical deterioration has become much worse. Like SPMS, these patients with PPMS show neurodegeneration, including mild to severe inflammation. Besides this, minimal inflammation on MRI occurs in minority patients with SPMS (Mann et al., 2000). So, the transition from RRMS to progressive MS is associated with elongated chronic inflammation (Lassmann et al., 2012). There is two other nomenclature for phenotypes of MS. In most MS cases, the initial symptoms would cause acute clinical attack, converting to RRMS, called Clinically Isolated Syndrome (CIS) added to MS nomenclature in 2012 (Sand, 2015). Another one is Radiologically Isolated Syndrome (RIS), introduced in 2009, defined as demyelination in the absence of clinical symptoms. Oxidative stress is one of the most critical causes for the formation of demyelination due to reactive oxygen species (ROS), which harms macromolecules like DNA (Parchami Barjui et al., 2017). Therefore, the ability to diminish toxic residuals of oxidative stress away from the body is crucial for protecting against the increased risk of neurodegenerative diseases, such as MS (Stavropoulou et al., 2007). The balance between the endogenous antioxidant system and the level of cellular ROS can fall into decay because of the increase in ROS production and the decrease in antioxidant protection, ultimately leading to damage to lipid and DNA (Carvalho et al., 2014).

Glutathione (GSH) participates in the detoxification process as a cofactor for Glutathione S-Transferase (GST) to protect nerve cells (Parchami Barjui et al.,

2017). Of these GSTs, GSTM and GSTT1 draw attention because they are associated with loss of function because of homozygous deletion. In the Iranian population, Barjui and their colleagues have sited that there would be relationship between decreasing enzymatic activity and higher risk of being MS (Parchami Barjui et al., 2017). Another important point highlighted in the same study that GSTM1 null phenotype was found to be more frequent in females with MS which might be related with estrogen metabolism and detoxification. Additional studies are needed to highlight the relationship between GSTs and MS disease.

As a source of toxicants, air pollution has been getting attention since some pro-inflammatory markers in the brain like cyclooxygenase 2 (COX2), and CD14 have increased when exposed to chronic air pollution. These type of pro-inflammatory cytokines would lead to neuroinflammation and neurodegeneration, which are associated with MS progression through directly affecting CNS by crossing BBB or triggering epigenetic changes (Abbaszadeh et al., 2021). In addition to air pollution, toxicity and oxidative stress can also be caused by exposure to heavy metals. The study carried out by Aliomrani, and colleagues had revealed that when healthy and MS individuals were compared, their arsenic and cadmium blood concentrations were high in persons with MS. Also, GSTM1 null genotype can cause to increase in inclination to cadmium toxicity, especially in patients with smoking habits (Aliomrani et al., 2017). Another accentuated subject is the association between MS and exposure to organic solvent and how it affects GSTM1. Landtblom and colleagues have reported that the patients with GSTM1 null genotype, when exposed to organic solvents, showed a two-fold increase in the risk of MS development compared to the control group (Landtblom et al., 2003).

1.2 Experimental Autoimmune Encephalomyelitis

MS is unique to humans, and no animal model imitates the features of that disease faithfully (Pachner, 2011). However, for years, many animal species like mice have been used to find out underlying causes, particularly clarifying the mechanism

behind neurological diseases, such as MS and Celiac disease (CD). Such animals have been brought into the structure that encodes the desired genes, such as CD4, TCR, and autoantigens, in a way that makes them transgenic so that scientists can study relevant human diseases like MS on those mice immunized with disease-associated antigen (Gregersen et al., 2004). The difficulty in the sampling of autoreactive T cells and the strong medication received by the patient during later stages of the disease necessitate the use of the animal model.

Experimental Autoimmune Encephalomyelitis (EAE) is the first identified and best-characterized animal model for human autoimmune disease. EAE was discovered to shed light on the origin of neuromyelitis optica (Ransohoff, 2012). This animal model is similar to MS in many ways, including axonal loss, the presence of multiple CNS lesions, and the temporal maturation of lesions from inflammation through demyelination (Baxter, 2007). The EAE model has been also used to clarify the biology of cytokine mechanisms (Ransohoff, 2012). Besides mice, EAE has been developed in many species, such as goats, dogs, sheep, and rats. The development of EAE enables researchers to understand the disease mechanisms and test novel immunotherapies. However, over time, instead of larger rodents, mice have been used for EAE research to develop transgenic and knockout genetics (Ransohoff, 2012). More specifically for mice, the C57BL/6 mouse model has been preferred to induce the disease through immunization with myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) peptide, emulsified in Freund's adjuvant boosted with either heat-killed mycobacteria enhancing immune response by stimulating TLR or heat-killed *Mycobacterium tuberculosis* extract inducing CD4⁺ Th₁ response through the activation of TLR (Pachner, 2011).

Although the effect of pertussis toxin on the EAE model is not clear, it has been used either to augment the improvement of weakness in EAE or to diminish blood-brain barrier (BBB). Although this protocol is reusable, this model has some limitations; for instance, the C57BL/6 model is monophasic, which means there are no relapses. Another mouse model which is used to generate the RRMS EAE model, the SJL/J mouse strain is immunized with myelin proteolipid protein (PLP₁₃₉₋₁₅₁) peptide

(Ransohoff, 2012). PLP is a highly conserved and hydrophobic transmembrane protein (Boyden et al., 2020). In addition to these EAE models, there are many other model variants, such as genetically altered mutant mice (Pachner, 2011). Collectively, in vivo models consist of the autoimmune model, transgenic mice, viral model, and chemically-induced model, highlighting different pathophysiological features of MS disease (Torre-Fuentes et al., 2020).

In addition to divergent type of MS model, there are some types of myelin-based antigens investigated so far, such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), and proteolipid protein (PLP), are the main autoreactive antigenic components recognized by draining circulating CD4⁺ T cells in both healthy and MS patients (Koehler et al., 2002). It has been known that myelin proteins are one of the most known autoantigens in MS (Hellings et al., 2001). Myelin oligodendrocyte glycoprotein found on the outer surface of myelin sheaths is one of the most studied autoantigens for MS (Peschl et al., 2017). MOG peptide brings about enduring demyelinating lesions and activates microglia and encephalitogenic T-cell response in susceptible species (Torre-Fuentes et al., 2020). Furthermore, several studies have shown that demyelinating anti-MOG antibodies enhance disease severity, causing extensive demyelination in T cell-activated brain inflammation in mouse and primate models of EAE (Gold et al., 2006). Immunization of mice with one of these peptides enables the generation of reactive T cells in the periphery to enter CNS, which gives a start for autoimmune inflammation (Noorbakhsh et al., 2006). Over time, with the help of advances in imaging and next-generation transgenic & genetic engineering technologies, the use of the EAE model has disseminated in time (Dendrou et al., 2015).

1.3 Glutathione S-transferase

The Glutathione transferases, also known as Glutathione S-transferase (GSTs), can be defined as a highly versatile detoxification enzyme superfamily of eukaryotic and prokaryotic phase II drug-metabolizing isozymes (Sheehan et al., 2001). They have

a dimeric structure composed of identical or different chains and globular protein with N-terminal mixed helical (alpha/beta domain) or G domain and all alpha-helical domain or H domain. G sites are highly conserved and specific for binding glutathione cofactor to GST enzymes; however, the H site is highly variable and binds hydrophobic substrates (Wu & Dong, 2012). GSTs are categorized based on their cellular localization in eukaryotes into three distinct groups: cytosolic, microsomal, and mitochondrial or, by another name, membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) as shown in Figure 1.3 (Allocati et al., 2018).

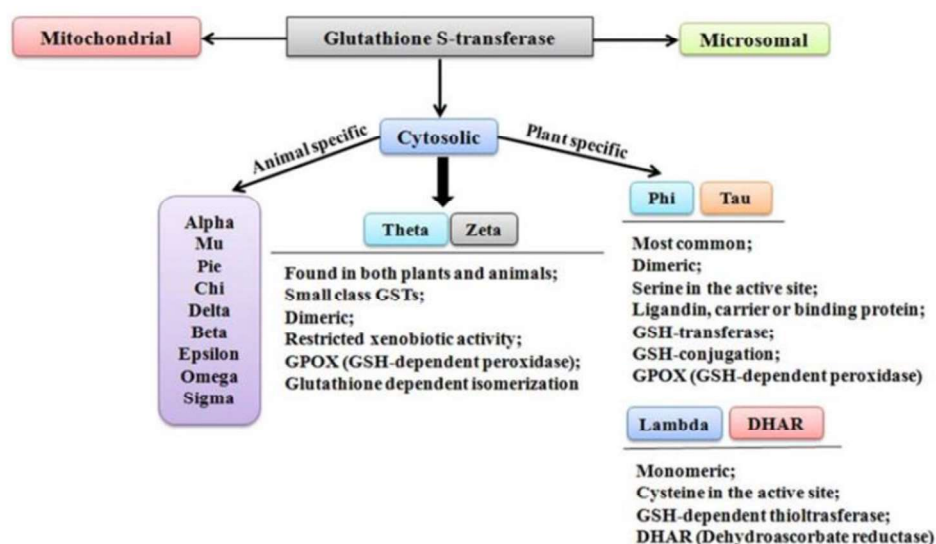


Figure 1.3 The family of GSTs (Kumar & Trivedi, 2018).

Microsomal GSTs are involved in the metabolism of endogenous compounds such as leukotrienes and prostaglandins. The pi and mu class of GST family, on the other hand, participate in the MAP kinase pathway responsible for cellular proliferation, apoptosis, and also differentiation in mammalian cells (Townsend & Tew, 2003). In addition to protecting the cells against oxidative metabolite-induced damage, GSTs also participate in tyrosine catabolism and dehydroascorbate reduction (Wu & Dong, 2012). Immunogenicity of DNA is linked to oxidation of nucleotides since when oxidized epitopes encompass the myelin and oligodendrocytes, it affects the development of MS disease. In this context, the GST family plays a vital role in

protecting the cells from the products of oxidative stress (Lee et al., 2015). Besides, they are responsible for removing reactive oxygen species (ROS), resulting from oxidative stress, catalysis of conjugations with endogenous ligands, and non-substrate ligand activity. From environmental exposures, mitochondrial dysfunction to reactive metals affect the production of ROS. As shown in Figure 1.4, ROS produced from these factors causes the formation of myelin phagocytosis, which in turn affects the development of neurological disease, such as MS.

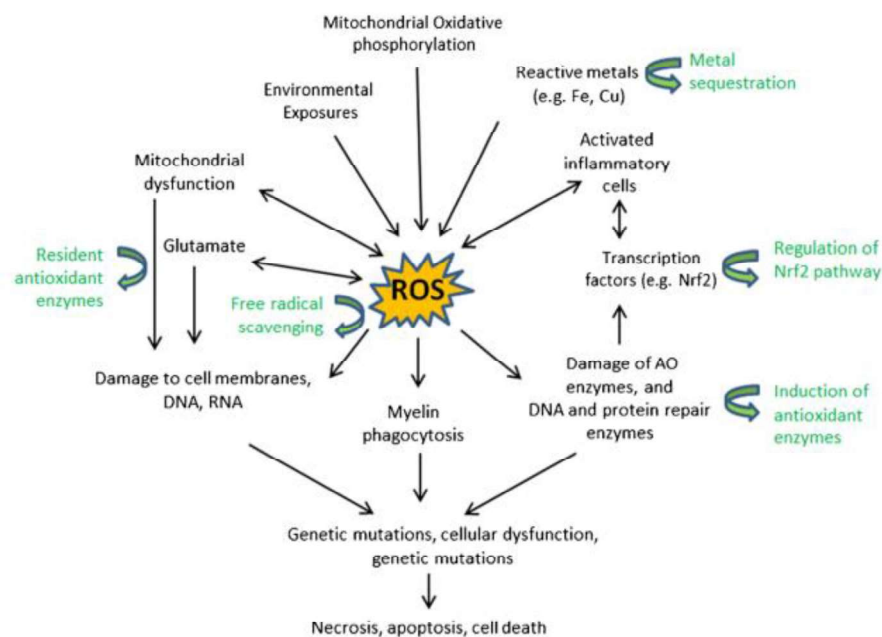


Figure 1.4 ROS resulting from many factors affects MS disease (Waslo et al., 2019). For the detoxification process, some harmful molecules like xenobiotics can pass the cell membrane freely, becoming substrate for the enzyme called Phase I reaction metabolism enzymes or drug-metabolizing enzymes like UDP-glucuronosyltransferase and sulfotransferase as shown in Figure 1.5. After phase I reaction, GSH is conjugated to xenobiotics by GSTs which makes xenobiotics more hydrophilic and less toxic.

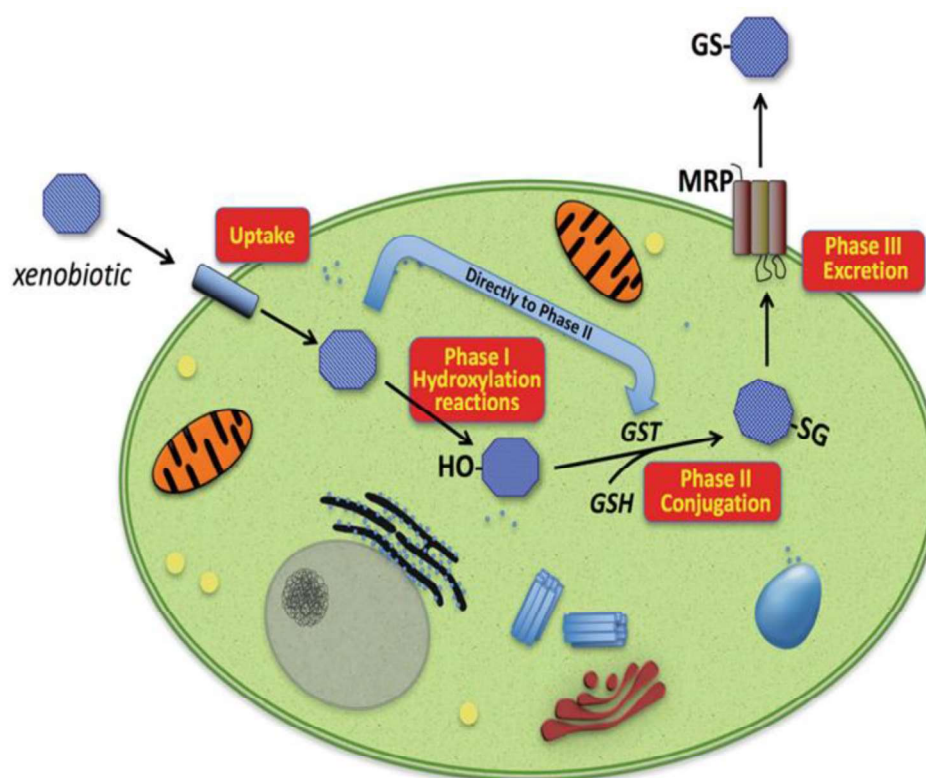


Figure 1.5 Detoxification process of xenobiotic (Allocati et al., 2018).

It has been reported that specific isozymes of GSTs are overexpressed in some tumours and may be related to asthma and multiple sclerosis making GSTs a candidate target for promising therapeutic agents for several neurodegenerative diseases (Townsend & Tew, 2003). One of the eight distinct gene family members GST Mu on chromosome 1 in humans, encodes soluble GSTs (Strange et al., 2001). GSTM1 is expressed in several tissues, such as the liver, brain, and stomach (Parchami Barjui et al., 2017). Like particular GST loci, mu family are polymorphic; for instance, there are three GSTM1 alleles, which are GSTM1*0, GSTM1*A, and GSTM1*B (Mann et al., 2000). The study conducted by Arakawa to evaluate absorption, distribution, metabolism, excretion, and toxicity showed that *Gstm1*- and *Gstm1*-null mice can be used as human-animal models since GST genes exhibit polymorphisms. Also, functional similarity between human GSTM1 and mouse GSTM1 has been found in the liver (Arakawa, 2013). Another experiment carried out by Song and colleagues was conducted to find out the relationship between

genetic polymorphism of GSTM1, GSTT1, and hepatocellular carcinoma (HCC) disease. They revealed that GSTM1-1 and GSTT1-1 null polymorphism is related to developing HCC (Song et al., 2012). The study focusing on another GST type, GST π , has gained attention after years of work due to its involvement in resistance to chemotherapy drugs through MAPK-pathway inhibition to block apoptosis in tumors (Dong et al., 2018). In addition, GSTP1-1 interrupts apoptosis via involvement in the JNK pathway via directly detoxifying and eliminating anti-cancer drugs. A specific inhibitor of GSTP1-1, TLK117, has been developed to increase the efficacy of chemotherapeutic drugs (Wu & Dong, 2012). Similarly, Nocodazole, a novel inhibitor of GST S1-1, is a promising agent for allergy and inflammation since GST S1-1 catalyzes prostaglandin H₂ (PGH₂) to prostaglandin D₂ (PGD₂).

1.4 Aim of the Study

Multiple sclerosis is a chronic, autoimmune disease with yet unknown etiology. Due to the presence of oxidative damage in the initial and chronic phase of MS, at this stage, antioxidant defence becomes of tremendous and undeniable importance for returning the disease onset and its progression to a previous normal condition. The immune cells infiltrated into CNS are the main target of immunomodulatory drugs to decrease immune cell activity, enter them into the CNS, and attack frequency. However, when looked at the immunomodulatory drugs developed to date and current treatments, their mechanism of action includes only the early stages of the disease; unfortunately, there is no effect on sequelae. Regarding the most crucial cause of MS, which is antioxidant defence, the GST family is vital and would carry out diverse cellular processes. The relationship between the course of MS disease and GSTs detoxification pathway has not been clearly understood. An improved mechanistic understanding of their correlation is of utmost importance to highlight the one part of its underlying mechanism that precise nature of it remains enigmatic. In the literature, there is not enough study to clarify the relationship between MS

disease and expression and activity of GST enzymes by using the Experimental Autoimmune Encephalomyelitis (EAE) mouse model.

In this thesis study, it was aimed to reveal the association between the protein expression and enzyme activity of GSTs and Multiple Sclerosis disease in progressive MS model of C57BL/6 mice. The findings of this research may unearth a new pathway to be target for new drugs in the treatment of MS.



CHAPTER 2

MATERIALS AND METHODS

2.1 Chemicals and Materials

BCIP[®]/NBT liquid substrate (B1911), cholecalciferol (C1357), bovine serum albumin (BSA; A7511), 2-amino-2(hydroxymethyl)-1,3-propanediol (Tris; T1378), ammonium persulfate (APS; A-3678), bromophenol blue (B5525), glycerol (G5516), glycine (G-7126), β -mercaptoethanol (M6250), hydrochloric acid 37% (HCl; 07101), methanol (34885), ethanol (24105), sodium dodecyl sulfate (SDS; L4390) and tween 20 (P1379) were purchased from Sigma-Aldrich Chemical Company, Saint Louis, Missouri, USA.

Hooke Kit[™] MOG₃₅₋₅₅/CFA emulsion with pertussis toxin (EK-2110) was purchased from Hooke Laboratories, Lawrence, Missouri, USA.

Potassium dihydrogen phosphate (KH₂PO₄; 04871), di-potassium hydrogen phosphate (K₂HPO₄; 05101), sodium hydroxide (06462), sodium chloride (NaCl; 1.06400), sodium hydroxide (NaOH; 06462) were the products of E. Merck, Darmstadt, Germany.

Halt[™] protease inhibitor cocktail (87786), PageRuler plus prestained protein ladder (26619), Pierce[™] BCA protein assay kit (23225), Pierce[™] ECL Western Blotting Substrate (32106) were purchased from Thermo Fisher Scientific, Waltham, Massachusetts, USA.

50 bp DNA ladder (N3236S) was the product of the New England Biolabs, Ipswich, Massachusetts, USA

Non-fat dry milk (170-6404), Extra Thick Blot Filter Paper (1703966), and tetramethyl ethylene diamine (TEMED; 161-0801) were the products of Bio-Rad Laboratories, Richmond, California, USA.

Goat anti-rabbit alkaline phosphatase-conjugated secondary antibody (ab6722) and recombinant anti-GAPDH antibody (ab181602) were the product of Abcam, Cambridge, United Kingdom.

The Anti-GST Antibody (sc-80998) and anti-mouse IgG-AP (sc-2008) antibodies were purchased from Santa Cruz (Santa Cruz, CA).

Absolute ethanol (LR0090605AGQ) and Glycine (LS07028004AJW) were obtained from ISOLAB, Wertheim, GERMANY.

Acetone (67-64-1) was obtained from DOP Organik Kimya, Ankara, Turkey.

Isopropanol (AS040-L50) was the product of Atabay, Istanbul, Turkey.

2.2 Animal Experiment

All procedures of animal studies of this work were approved by Bilkent University Animal Experimentation Ethics Committee. Animal experiments were carried out using 10-12 weeks old female C57BL/6 mice weighing 20-25 g. Mice were produced and housed at the Animal Experimental Unit in Bilkent University. Animals were randomly assorted into two groups as given in Table 2.1 and placed in individually ventilated cages.

Table 2.1 Experimental groups of the female C57BL/6 mice.

	<i>Groups</i>	<i>Number of Mice</i>
Control	Group 1 (Healthy)	5
MS Patient	Group 2 (EAE)	11

2.2.1 Experimental Autoimmune Encephalomyelitis (EAE) Immunization

C57BL/6J (10-12 weeks old) mice were immunized with Hooke Labs' Kit (Hooke Labs Inc., #EK-2110) according to the manufacturer's instructions. 100 μ L of myelin oligodendrocyte glycoprotein/complete Freund's adjuvant (MOG₃₅₋₅₅/CFA) emulsion, total 200 μ L, was injected subcutaneously into each mouse at two sites (lower and upper back of the mouse). Then, pertussis toxin (80 ng in 100 μ L PBS/animal) was injected intraperitoneally into each mouse two hours and twenty-four hours after the immunization. Ear tags were used to monitor mice individually, and the mice were observed daily after immunization for 30 days. The clinical score of the disease is assigned according to the manufacturer's scoring chart (Table 2.2). After 30 days of the EAE immunization, under anesthesia, the mice were sacrificed by perfusion with PBS. The liver of the animal was isolated and stored at -80 °C until further analysis.

Table 2.2 Clinical observations and mouse EAE scoring.

Score	<i>Clinical Observation</i>
0	The tail has tension and is erect.
0.5	The tip of the tail is limp.
1	The tail is limp
1.5	The tail is limp, and the hind leg is inhibited.
2	The tail is, and the hind legs are weak.
2.5	The tail is limp, and the mouse is dragging hind legs.
3	The tail is limp, and the hind legs are completely paralyzed.
3.5	In addition to score 3, it cannot right itself when the mouse is placed on its side.
4	In addition to scoring 3.5, there is partial front leg paralysis.
4.5	In addition to scoring 4, the mouse is not alert.
5	Death

2.3 Total Protein Extraction

The liver organ was taken out after perfusion with PBS solution. The cryogenic grinding method was used for homogenization of mouse liver. The organ was put into a ceramic mortar, including liquid nitrogen, and ground with a pestle in this method. The 1% HaltTM protease inhibitor cocktail and the T-PERTM tissue protein extraction reagent were combined before usage. After the homogenization process, approximately 50 mg of the homogenate was weighed, and 500 μ L of the T-PERTM reagent was added in a microcentrifuge tube. The homogenate-reagent mixture obtained was centrifuged at 10,000 x g for 5 minutes. The supernatant comprising total protein was taken and stored at -80 °C until use.

2.4 Determination of Protein Concentration

Protein concentrations of total protein extract acquired from the mice liver samples were determined by the BCA (Bicinchoninic Acid) method using crystalline bovine serum albumin as a standard (Wiechelman et al., 1988). BCA method relies on the formation of a Cu^{+2} protein complex in a basic environment, then reduction of the Cu^{+2} to Cu^{+} in a process that the reaction leads to forming a purple color that absorbs light at a wavelength of 562 nm. At the end of the procedure, the amount of Cu^{+2} reduced is proportional to the amount of protein present in solution.

As the manufacturers' manual was followed, PierceTM BCA protein assay kit was used to quantify protein concentrations of the samples. Bovine serum albumin (BSA) was prepared and used as the standard with series of different concentrations (25, 125, 250, 500, 750, 1000, 1500, 2000 μ g/mL). Blank, standards, and the samples were performed as duplicates. 25 μ L of standards and the samples were put into the wells of 96 well plate. Then, 200 μ L of working reagent (reagent A: B, 50:1) was added into each well and mixed on a plate shaker for 30 seconds. The plate was covered with foil and incubated for 30 minutes at 37 °C. At the end of the incubation process, the absorbances of samples were measured at 562 nm with MultiskanTM GO

Microplate Spectrophotometer from Thermo Fisher Scientific, Waltham, Massachusetts, USA.

2.5 Determination of Protein Expression by Western Blotting Technique

Effects of EAE immunization on protein expression of Glutathione S-transferase (GST) enzyme in the liver of the mice was analyzed by the Western blot method (Mahmood & Yang, 2012). Before immunoblotting, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins. To do this, 4% stacking gel and 10% separating gel in a discontinuous buffer system were used. The gels were prepared freshly one day before the experiment, as described in table 2.3 below.

Table 2.3 Components of separating and stacking gel solutions.

Components	Separating Gel Solution	Stacking Gel Solution
Monomer Concentration	10 %	4%
Gel Solution	5 mL	650 µL
dH ₂ O	6,02 mL	3,05 mL
Separating Buffer	3,75 mL	---
Stacking Buffer	---	1,25 mL
10% SDS	150 µL	50 µL
10%APS	75 µL	25 µL
TEMED	15 µL	5 µL
Total Volume	15 mL	5 mL

The gel sandwich unit was prepared in such a way that there was a space of one cm in between. After the solution was prepared in the above quantities, the 4250 µL of

10% separating gel solution was poured into the glass plates on the casting stand. At this point, 1000 μ L of butanol was added immediately to cut the interaction with oxygen to obtain a smooth gel surface of separating gel and accelerate the polymerization process. After polymerization of the separating gel, the butanol was poured out and 1500 μ L of 4% stacking gel solution was dispensed onto the separating gel, and then the 15 wells comb was placed immediately. After the polymerization was completed, the comb was removed, and 1X Electrophoresis Running Buffer (ERB) was added. Each well was cleaned with a syringe to eliminate any air bubbles and gel particles if left for the samples to run smoothly. Vertical gel electrophoresis was performed using Mini-PROTEAN tetra cell mini trans blot module (Bio-Rad, Richmond, CA).

The samples were diluted with dH₂O according to the following formula to get 4 mg/mL final protein concentration;

$$V = \frac{[\text{Conc. of protein}]}{5.32} \times 20 - 20$$

V is the volume of dH₂O to be added to dissolve 20 μ L of the sample.

The samples were diluted with the 4X sample dilution buffer (pH 6.8, 0.25M Tris-HCl, 8% SDS, 40% glycerol, 20 % β -mercaptoethanol, and 0.01% bromophenol blue). They were incubated for 3 minutes at 100 °C heat block. After incubation, they were put into ice immediately. 10 μ g of the samples were loaded into wells, and 5 μ l of protein ladder was loaded as a marker. Then, the gel running module was incorporated into the main buffer tank, which is filled up with 1X Electrophoresis Running Buffer. The tank was linked up with the Bio-Rad power supply, and electrophoresis was started at 150V, and when comes to stacking gel, it was continued with 200V. When the electrophoresis was completed, the glass was placed into the transfer buffer (25 mM Tris, 192 mM Glycine) for 5 minutes. Meanwhile, the PVDF membrane was put into 100% methanol for one minute to open the pores. Then the membrane was equilibrated via incubating in the transfer buffer for 5 minutes. The gel was taken from the glass and transferred into a transfer buffer for

10 minutes to equilibrate the gel and get rid of SDS. After that, the gel, the PVDF membrane, and Whatman papers were placed on the preparing sandwich, as shown in Figure 2.1.

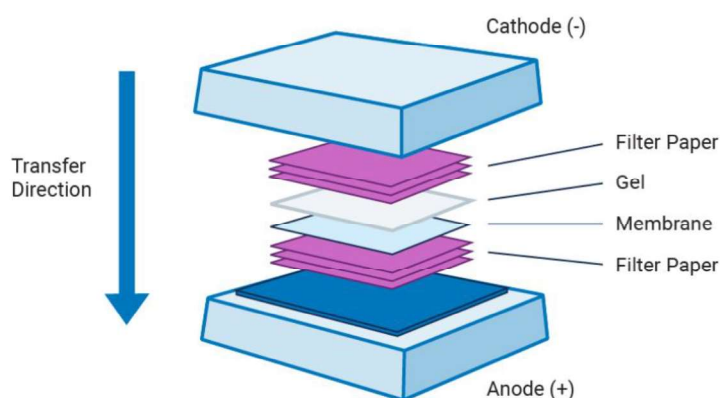


Figure 2.1 Western Blot Sandwich.

The transfer sandwich was inserted between the top and the bottom cassettes of the Trans-Blot® Turbo® semi-dry transfer system (Bio-Rad Laboratories, Richmond, CA, USA). The transfer was performed at a constant 25 V and up to 1 A for 30 minutes.

Reagents:

Gel Solution

14.6 g acrylamide and 0.4 g N'-N'-bis-methylene-acrylamide were dissolved separately with dH₂O then mixed and filtered through filter paper. The final volume was completed to 50 mL.

Separating Buffer (1.5 M Tris-HCl, pH 8.8)

18.15 g of tris-base was dissolved with 50 mL dH₂O, and titrated with 10 M HCl to pH 8.8. The volume was completed to 100 mL. The pH was checked at the end.

Stacking Buffer (0.5 M Tris-HCl, pH 6.8)

6 g of tris-base was dissolved with 60 mL dH₂O, and titrated with 10 M HCl to pH 6.8. The volume was completed to 100 mL. The pH was checked at the end.

Sodium Dodecyl Sulfate - SDS (10%)

1 g of SDS was dissolved with dH₂O, and the volume was completed to 10 mL.

Ammonium Persulfate - APS (10%, Fresh)

40 mg of APS was dissolved in 400 μ L distilled water.

Tetramethylethylenediamine - TEMED (Commercial)

Sample Dilution Buffer-SDB (4X)

2.5 mL of 1 M tris-HCl buffer (pH 6.8), 4 mL glycerol, 0.8 g SDS, 2 mL β -mercaptoethanol and 0.001 g bromophenol blue were used and the volume was completed to 10 mL with dH₂O.

Electrophoretic Running Buffer - ERB:

0.25 M Tris, 1.92 M glycine (10X Stock, diluted to 1X before use by adding 0.1% SDS)

15 g tris-base was dissolved with 350 mL dH₂O, then 72 g glycine was added. The volume of the mixture was completed to 500 mL.

It was prepared as 10X stock solution and it was diluted to 1X. 1 g of SDS was added per liter of 1X buffer before use.

Once the transfer was finished, the membrane was washed with TBST (20 mM Tris-HCl pH 7.4, 500 mM NaCl, 825 μ L Tween20 (5X)) for 10 minutes. Then, the membrane was incubated with blocking solution (5% non-fat dry milk in TBS) at room temperature for an hour on a shaker. After that, without washing with TBST, the membrane was incubated with the primary antibody of protein of interest for 2 hours at room temperature on a shaker. When the incubation was over, the membrane

was washed with TBST (20 mM Tris-HCl pH 7.4, 500 mM NaCl, 0.05% Tween 20) 3 times for 5 minutes each. After the washing, the membrane was incubated with secondary antibodies for an hour on a shaker. Then, the membrane was washed with TBST 3 times, each of which was 5 minutes. At the end, the membrane was incubated with the BCIP[®]/NBT alkaline phosphatase substrate. The antibodies and their dilutions are given in Table 2.4. Chemidoc XRS+ (Bio-Rad, USA) was used to take images. The band intensities were analyzed by Image J visualization software developed by NIH.

Table 2.4 Primary and secondary antibody dilutions.

Protein	1 st Antibody	2 nd Antibody
GAPDH	1/1000	1/1000
GST	1/500	1/500

2.7 Determination of Total Glutathione S Transferase (GST) Activities

For determination of total Glutathione S-Transferase activity of mouse liver total protein extract, 1-Chloro-2,4-dinitrobenzene (CDNB) was used as a substrate. The rate of Glutathione conjugate (1-(S-Glutathionyl)-2,4-dinitrobenzene (DNB-SG)) formation is observed by following the rate of increase in absorbance at 340 nm. The reaction catalyzed by glutathione S-transferase is shown in Figure 2.2.

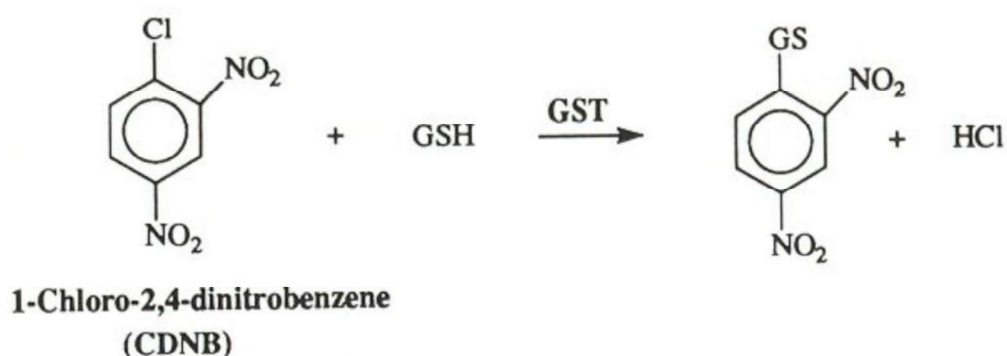


Figure 2.2 Reaction catalyzed by GST.

Reagents:

1. Potassium Phosphate Buffer: 50 mM, pH 7.0
2. Reduced Glutathione (GSH): 20 mM (Fresh)
3. CDNB: 20 mM (Fresh, Light Sensitive)

CDNB was dissolved in absolute ethanol and then the volume was completed with distilled water. Ethanol should not exceed to 3% of assay mixture. CDNB solution must be prepared freshly and should be stored in a dark bottle. .

2500 μ L 50 mM Potassium Phosphate buffer pH 7.0, 200 μ L 20 mM GSH and 150 μ L of total protein extract and 150 μ L of 20 mM 1-Chloro-2,4-dinitrobenzene (CDNB) as a substrate was included a typical assay mixture in a final volume of 3 ml and mixed well. A blank cuvette was used in each time. The reaction was started after addition of 150 μ L of 20 mM 1-Chloro-2,4-dinitrobenzene (CDNB), followed by thioether formation at 340 nm for 2 minutes continuously by using Shimadzu UV-160A UV-visible spectrophotometer (Schimadzu Corporation, Analytical Instruments Divisions, Kyoto, Japan). The absorbance obtained from blank cuvette was subtracted from absorbance of enzymatic reaction to eliminate non-enzymatic product formation.

The enzyme activity was calculated using $0.0096 \mu\text{M}^{-1} \times \text{cm}^{-1}$ as a coefficient of thioether formed by GST.

GST activity was determined according to the following formula;

Activity(nmol/min/mg)

$$= \frac{\Delta OD_{340}}{0.0096 \mu\text{M}^{-1} \text{cm}^{-1}} \times \frac{\text{Total Volume}}{\text{Volume of Sample}} \times \text{Dilution Factor}$$

2.8 Statistical Analysis

Statistical analyses were performed by using the GraphPad Prism version 6 statistical software package for Windows. The student's t-test was used for the comparison of two groups. All results were expressed as means with their Standard Deviation (mean $\pm$ SD), and $p < 0.05$ was chosen as the level for significance.



CHAPTER 3

RESULTS

3.1 Protein Concentration of Mouse Liver Homogenate

Pierce™ BCA protein assay kit was used to measure protein concentrations of the samples by following the manufacturers' manual. Bovine serum albumin was used as the standard with different concentrations (25, 125, 250, 500, 750, 1000, 1500, 2000 µg/mL). All the measurements, including blank, standards, and the samples, were performed as duplicates. The results of total protein concentrations in mouse liver tissue homogenates were given in Figure 3.1.

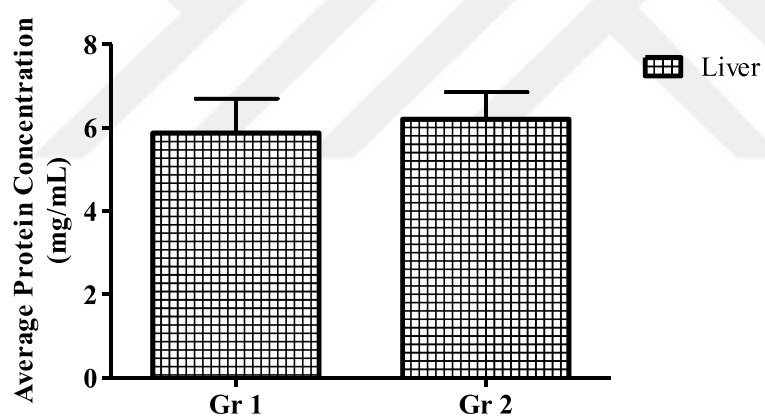


Figure 3.1 Protein concentrations in mouse liver homogenate.

Average protein concentration of Group 1 (Control) is measured as 5,9 mg/ml, whereas average protein concentration of Group 2 (EAE) is measured as 6,2 mg/ml. The error bars represent $\pm$ SD.

3.2 Clinical Scoring

In this study, there were two groups, which are Gr 1(Control) and Gr 2(EAE) that is immunized with the emulsion of MOG₃₅₋₅₅ peptide. Daily clinical scoring has been done after the first week of immunization as shown in the Figure 3.2. The first symptoms started to appear after the 10th day of the injection and as shown in the figure, the mice have arrived to the maximum score at 15-16 days.

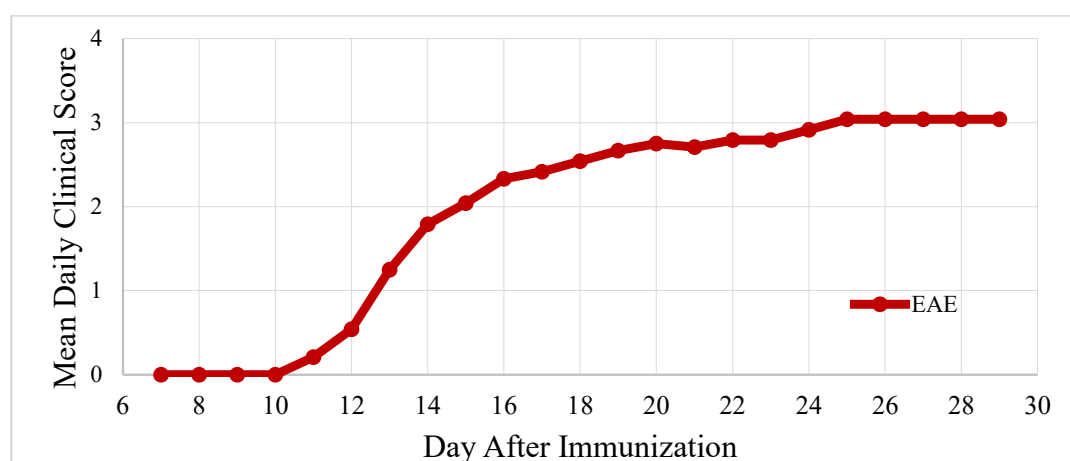


Figure 3.2 Daily clinical observation of immunized mice.

3.3 Effects of EAE Immunization on GST Protein Expressions

Effects of EAE immunization on GST protein expressions of female C57BL/6 mice in liver were determined by Western blotting *via* specific antibodies. GAPDH (37 kDa) was used as the protein loading control. GST protein is expected to be 26 kDa. The representative immunoblot of liver GST protein expression in Gr 1 (Control), and Gr 2 (EAE) is shown in Figure 3-3. Primary rabbit monoclonal anti-GAPDH (1/1000 dilution) and mouse monoclonal anti-GST (1/500) and monoclonal anti-rabbit alkaline phosphatase conjugated secondary (1/1000 dilution for GAPDH) and monoclonal anti-mouse alkaline phosphatase conjugated secondary (1/500 for GST) antibodies were used for immunochemical detection of GAPDH and GST protein. The intensity of each band was quantified as an arbitrary unit, relative peak area

(RPA) by Image J software. This RPA was relatively set to 1.00 for Gr 1 (control), and the protein expression of the other group was calculated relatively to Gr 1. The quantifications were expressed as the mean $\pm$ SD of the relative protein expression from three independent experiments and the level of significance was chosen as $p < 0.05$. The result of EAE immunization on GST protein expression in mouse liver and the comparison of the protein expression differences between the two groups were shown in Figure 3-3 and Figure 3-4, respectively. The relative protein expression of control group Gr 1 and EAE immunized group Gr 2 were 1.0 ± 0.2 and 0.9 ± 0.30 , $p \geq 0.05$, respectively. There was no significant difference between GST expression of control and EAE immunized group of animals.

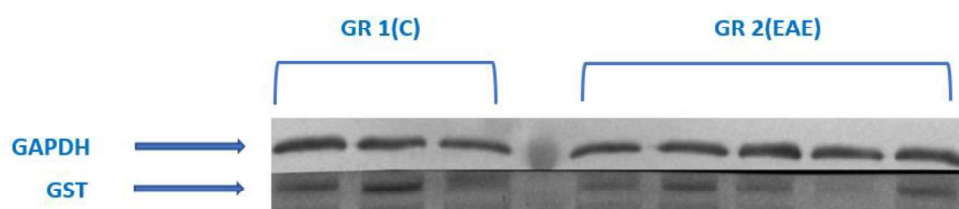


Figure 3.3 Effects of EAE immunization on GST protein expression in mouse liver.

Representative immunoblot of liver GST protein in Gr 1 (Control), and Gr 2 (EAE). Experiments were repeated at least three times.

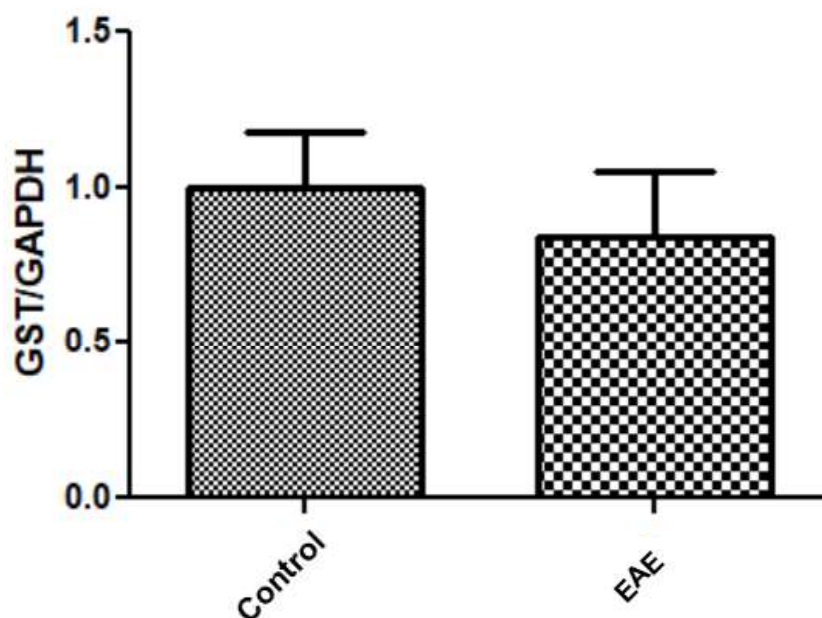


Figure 3.4 Comparison of liver GST protein expression in control and EAE immunized animal groups.

Experiments were repeated at least three times.

3.4 Effects of EAE Immunization on Total Glutathione S -Transferase (GST) Activities

Total Glutathione S-Transferase activity of mouse liver homogenate was determined by using, 1-Chloro-2,4-dinitrobenzene (CDNB) as a substrate. The rate of Glutathione conjugate (1-(S-Glutathionyl)-2,4-dinitrobenzene (DNB-SG)) formation was measured by following the rate of increase in absorbance at 340 nm. The comparison of liver GST activity of Control Gr 1 (3 mice) and EAE immunized Gr 2 (6 mice) animals were given in Figure 3.5. There was a statistically significant difference between these two groups ($p \leq 0.05$).

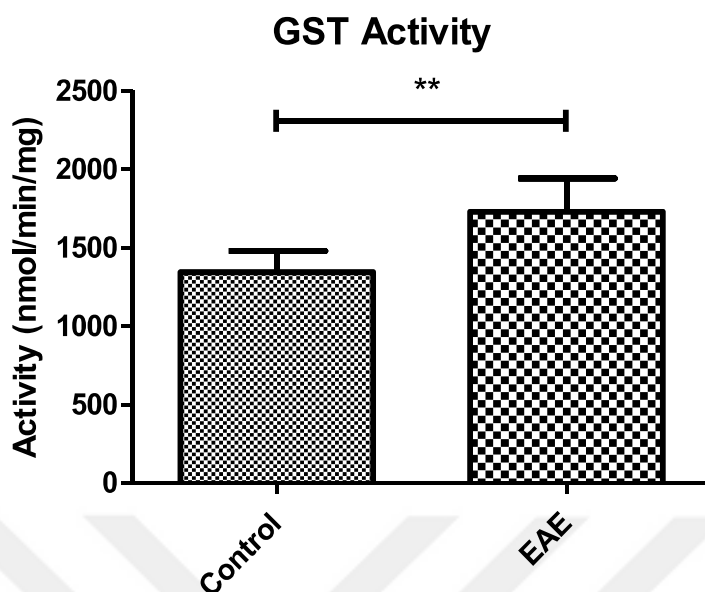


Figure 3.5 The comparison of liver GST activity of Control Gr 1 (5 mice) and EAE immunized Gr 2 (11 mice) animals.

The quantifications were expressed as the mean $\pm$ SD.

All measurements were done as triplicate. **Significantly different ($p \leq 0.01$).

The average activity of control group was found as 1343,01 (nmol/min/mg), whereas the average activity of EAE group was measured as 1728,80 (nmol/min/mg). So, the average activity of Gr2 (EAE) is higher than the control group. As the bars represent, there was a statistically significant difference between liver GST activity of Control Gr 1 (5 mice) and EAE immunized Gr 2 (11 mice) animals.

CHAPTER 4

DISCUSSION

Multiple Sclerosis is a disease characterized by inflammation, destruction and extensive loss of myelin sheath (Hassani & Khan, 2019). Although the autoimmune origin and etiology have not been fully clarified, but genetics and environmental factors trigger the formation of the disease. In addition, several provocative factors should be considered to clarify the genetic background of neurological disorders, such as Multiple Sclerosis. Oxidative stress is one of the most critical factors that affects the progression of the disease because neurons are highly sensitive to this stress. Indeed, pathophysiology of MS is related with oxidative stress due to the fact that during acute relapses and chronic plaques, the presence of destruction caused by oxidative stress has been proven in many studies. The Glutathione transferases can be regarded as a detoxification enzyme superfamily of eukaryotic and prokaryotic phase II drug-metabolizing isozymes (Sheehan et al., 2001). This group of enzyme families performs peroxidase and isomerase activity in the cell. Up to now, with developing technology and increasing knowledge about triggering factors, there have been limited studies conducted to shed light on the relationship between the MS and the GST enzyme family. It has long been known that GSTs have been involved in resistance to some chemotherapy agents. GST-mediated drug resistance occurs two ways; one of them is via direct detoxification, another is by affecting mitogen-activated protein kinase pathway that responsible for cellular survival and death signalling. What we know is GSTs have been using as a therapeutic target since some isozymes are overexpressed in some types of tumours, which can be linked to other neurodegenerative diseases, such as MS. For the cancer and tumour therapy, prodrugs have been preferred for the long time since it causes to decrease the possibility of toxicity towards normal tissues, in other words, minimizing off-target effects. For instance, *cis*-3-(9H-purin-6-ylthio) acrylic acid (PTA) has been

used for prodrug of antitumour 6-mercaptopurine needed GSH conjugation for its activation (Townsend & Tew, 2003). Another example is related with increase in oral absorption of the drugs. Metformin prodrug namely as cyclohexyl sulfonamide derivative has been developed. The scientist has obtained the result that the derivative has been converted to parent drug successfully (Allocati et al., 2018).

Indeed, to investigate and understand the relationship between MS and GST enzyme family, it is crucial to study the protein expression and enzyme activity of GSTs by using the EAE mouse model. Several different models recognize different peptides and trigger different types of MS. For instance, the SJL model recognizes PLP peptides, commonly used for RRMS studies. Even though many rules have to be managed when working with the EAE model in mice, it is the most common animal model generated via myelin antigens with attention to animals and the type of MS. EAE model shows similarity to MS in many ways: axonal loss, the presence of multiple CNS lesions, and the temporal maturation of lesions from inflammation through demyelination (Baxter, 2007). Indeed, the C57BL/6 mouse strain has been used to study progressive MS, and the widely used mouse myelin antigen is MOG₃₅₋₅₅ peptide. Although EAE is the commonly used animal model to study MS, several elements should be considered when generating this model. Basically, EAE is induced in C57BL/6 mice by immunization with an emulsion of MOG₃₅₋₅₅ incomplete Freund's adjuvant (CFA), followed by administration of pertussis toxin in PBS first on the day of immunization and then again, the following day. To fully understand the disease's onset and development, it is necessary to pay attention to the factors affecting the severity of EAE to achieve reliable and uniform EAE development. For instance, eliminating factors that can cause stress before EAE onset improves the success of model generation. Age, gender selection, and pertussis toxin dose are also crucial because female mice show more consistent disease. In addition, the pertussis toxin dose should be adjusted carefully. Also, the environment throughout animal studies should be sterile to prevent any other antigen involvement and should be quiet environment (Constantinescu et al., 2011). Collectively, low-

stress mouse handling, good injection technique, proper antigen emulsion, optimum amount of pertussis toxin and sterile conditions are required for successful injection.

This study investigated the relationship between the mouse liver GST enzyme activity and GST protein expression with the MS disease using enzyme assay and Western blotting technique, respectively. There was no statistically significant difference between the control and MS model group animals in terms of liver GST protein expression level. Further investigation was carried out to reveal whether post translational modifications participate in the GST pathway. To achieve that, total GST activity was measured spectrophotometrically. The result showed that there was a statistically significant difference between control and MS model group animals in liver GST enzyme activity. Enzyme and substrate concentrations, temperature, pH, and presence of activators and inhibitors are all grouped of factors affecting enzyme activity. Lack of correlation between protein expression of GST gene and GST enzyme activity in mouse liver may be due to post-translational regulations in EAE conditions. For instance, GST- π with influencing cellular signals including apoptosis and cell growth has the ability to block tumour cell apoptosis. In one study, it was demonstrated that one of the endogenous lipid mediators 15d-pg $_2$ can cause post translational alkylation of cysteine residue of GST- π polypeptide chain, that leads to enzyme inactivation (Ściskalska & Milnerowicz, 2020). Subsequently, several other studies worked on GSTP1 have demonstrated that GSTP1 plays a crucial role in both stress response and cellular proliferation pathway via blocking c-Jun N Terminal Kinase. Ranganathan and co-workers have figured out that phosphorylation of serine residue of GSTP1 where c-Jun N Terminal Kinase binds was found in human gastric cancer cells Kato III and they concluded that GSTP1 may have a regulatory function in the apoptosis and cell proliferation. Because all the post-translational modifications described so far have been shown in the malignant cell line, this phosphorylation may be responsible for cellular signalling pathway that is specific to cancer (Ranganathan et al., 2005). Further studies are needed to clarify how post-translational modifications may affect the pathway related to Multiple Sclerosis. It can be said that a relative increase in GST activity

when compared to the control group can be evaluated positively because this enzyme family is involved in the detoxification process. However, since they also participate in phase II drug metabolism, they can render active metabolites to inactive form, which leads to the formation of drug resistance. In addition to that, an increase in GST enzyme activity may decrease GSH concentration over time, so the pathways in which GSH are involved can be affected negatively because substrate concentration is one of the crucial factors determining the quality of enzyme activity. After a certain time, due to the fact that the level of GSH is going to decline, it causes increased toxicity. As mentioned about the role of the overexpression of GSTP1 in many cancer types leads to the occurrence of multidrug resistance by conjugating chemotherapeutics. It has been known that GSTP1 protects tumour cells by involving detoxification of anti-cancer drugs and by preventing apoptosis via c-Jun N Terminal Kinase pathway (Wu & Dong, 2012). Some well-known chemotherapeutic agents have toxic effects, some of which can show cardiotoxicity and immunosuppression effect. When GST π prodrugs are undergone breakdown, they release cytotoxic metabolites. One of the GST π prodrugs is TLK286 which is GSH derivative. It leads to production alkylating agent which blocks some molecules stimulating drug resistance (Dong et al., 2018). Combining current therapies with neuroprotective, remyelinating, or regenerative immunotherapies should be carefully reviewed and studied because these therapies do not still prevent those that cause progressive MS. At the molecular level, it was known that miRNA expression is dramatically changed in different diseases, like MS but the exact mechanism of how it affects the MS has not been clearly understood (Hassani & Khan, 2019). So, cellular miRNA profiles need to be investigated deeply. To prevent increase in the risk of new relapses, the numerous mechanisms for therapeutic targeting and the identification of new predictive biomarkers and related pathways and the new candidates for the immunomodulatory drugs should be worked on. Furthermore, understanding oligodendrocytes, their precursors, and the mechanism of action on remyelinating therapies must be considered. Taken into consideration all of these, it can be concluded that more studies are needed to investigate the association between GST

enzyme activity, GST expression and Multiple Sclerosis. In addition, the importance and effects of post translational modifications in the GST protein and therefore GST enzyme activity should be studied.





CONCLUSION

Multiple Sclerosis is a potentially disabling and inflammatory disease of the central nervous system. MS is affected by genetics and environment but its exact mechanism remains only partially understood. In this disease, the central attack zone is the myelin sheath, which eventually deteriorates, and permanent damage happens. In this regard, the most crucial part of circumvention is antioxidant defence. The GST family, which is previously known as ligandin, is a prokaryotic and eukaryotic phase II metabolic isozyme. This enzyme family is vital and can be divided into an ever-increasing number of classes based on a combination of criteria and carry out diverse cellular processes, such as resistance to some agents used for chemotherapy. The immune cells infiltrated into CNS are the main target of immunomodulatory drugs to decrease immune cell activity, enter them into the CNS, and attack frequency. However, when we look at the immunomodulatory drugs developed to date and current treatments, their mechanism of action includes only the early stages of the disease; unfortunately, there is no effect on sequelae. This study was aimed to investigate the relation of the protein expression and enzyme activity of the GST family with multiple sclerosis using the C57BL/6 mouse model immunized with MOG. Our results showed that although there is no difference between the liver GST protein expression level of the control animals and MS mouse model, the GST enzyme activity is different between groups. MS model group animals have significantly higher enzyme activity compared to control group animals. The findings of this research may unearth a new pathway for the development of new drugs for MS. In the light of these findings, each GSTs family member that have the possibility to affect the MS pathway can be examined in detail in terms of protein expression and isozyme activity. GST isozyme activity studies can be done to determine the effectiveness on MS disease and MS related pathways. Besides, the results obtained from this study may be affected by post-translational modifications; that is why these GST family enzyme pathways can be considered as a future study.



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