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Research Article

Examining the Function of Oxidative Stress Management in Autoimmune Thyroid Disease: An Analysis of GPX Gene Variants

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Abstract:
Background: Autoimmune thyroid dysfunction, especially autoimmune thyroiditis (AIT), is so common and has multi-systemic implication, it poses a serious threat to world health. In order to test the hypothesis that oxidative stress pathways may play a role in the pathophysiology of the illness, this study examined the relationship between glutathione peroxidase (GPx) gene polymorphisms and autoimmune thyroid dysfunction.
Methods: This age- and sex-matched case-control study comprised 100 thyroid disease patients and 100 healthy controls. Every participant had a comprehensive evaluation, which comprised: Genetic examination of GPx polymorphisms by PCR-RFLP Thyroid function testing (FT3, FT4, TSH) evaluation of autoantibodies (anti-TPO, anti-TG).
The statistical analysis, which included t-tests, Pearson correlation, and chi-square testing, was conducted using SPSS v20.
Results: Thyroid antibody levels were significantly higher in patients than in controls ($p < 0.001$). Finding certain variants in the GPx gene linked to an increased risk of illness ($p = 0.003$). Strong association between antibody levels and specific GPx variations ($r = 0.45$, $p < 0.01$)
Conclusion: These findings underscore the critical role that oxidative stress plays in the development of autoimmune thyroid dysfunction by showing that GPx gene polymorphisms may operate as genetic markers for the condition. Although further study is required to clarify the underlying processes, the findings point to possible clinical implications in risk assessment and antioxidant-based therapy techniques.

Keywords: Autoimmue Thyroid dysfunction, Thyroid peroxidase enzyme (TPO), Thyroglobulin (Tg), Oxidative stress, Glutathione peroxidase (GPx), Genetic polymorphism

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Introduction

Autoimmune thyroid illnesses, such as Hashimoto's thyroiditis and Graves' disease, are complex conditions influenced by both genetic predisposition and environmental factors [1]. Epidemiological studies indicate that these disorders affect around 10–12% of the population, with a notable 2:1 female prevalence [2]. The pathogenesis involves a complex interplay between immunological dysregulation, characterized by Th1/Th17 dominance and the generation of autoantibodies against thyroid peroxidase (TPO) and thyroglobulin (Tg), and oxidative stress mechanisms that damage thyroid tissue [1,3]. This oxidative balance depends on the glutathione peroxidase (GPx) enzyme system, namely GPx1. By neutralizing reactive oxygen species, it serves as an essential antioxidant defense and is strongly expressed in thyroid follicular cells [4].

Due to decreased enzyme activity, genetic variants in GPx, particularly the Pro198Leu variation, have been substantially linked to a 2.3-fold increase in the risk of autoimmune thyroiditis [5]. Since selenium is a necessary cofactor for GPx function and a deficit has been associated with increased thyroid antibody titers, selenium status further influences this connection [6]. Although its effect on the course of hypothyroidism seems to be limited, clinical trials show that selenium administration can lower TPOAb levels in moderate Graves' illness [7]. These genetic predispositions interact with environmental variables, such as infections, smoking, and psychological stress, to affect how diseases appear [8]. In autoimmune thyroid illness, DNA methylation alterations and microRNA modulation of thyroid autoantigens have been discovered by recent studies that have gone beyond genetic polymorphisms to investigate epigenetic modifications [9].

Clinically speaking, thyroid function seems to depend on maintaining appropriate selenium levels (60–100 μg/day), while excessive supplementation should be avoided [10]. All of these results highlight the intricate interactions that occur in autoimmune thyroid dysfunction between environmental variables, oxidative stress pathways, and genetic factors (especially GPx polymorphisms).

Materials and Methods:

Study Design and Participants: A case-control study enrolled 100 participants with thyroid disorders and 100 matched controls. Participants were recruited from Kirupananda Variyar Medical College & Hospital.

Clinical Evaluation: Participants underwent comprehensive clinical evaluation, including thyroid profile assessment. Thyroid hormone levels (T3, T4, TSH) were measured using [QIAamp DNA Blood Mini Kit]. Thyroid antibody levels against thyroglobulin and thyroid peroxidase enzyme were assessed using [QIAamp DNA Blood Mini Kit][11].

Glutathione (GSH) Assay: The content of glutathione was determined by forming a yellow-colored compound through the reaction of dithionitrobenzene (DTNB) with acid-soluble sulfhydryl groups[12]. Initially, 1.0 mL of the homogenate was precipitated using 1.0 mL of 4% sulphosalicylic acid. The samples were then incubated for 1 hour at 4°C and subsequently centrifuged at 1,200 x g for 15 minutes at 4°C. The assay mixture comprised 0.1 mL of the aforementioned supernatant, 2.7 mL of phosphate buffer (0.1 M, pH 7.4), and 0.2 mL of freshly prepared 5,5'-dithiobis-2-nitrobenzene (DTNB) (40 mg in 10 mL of 0.1 M phosphate buffer), resulting in a total volume of 3.0 mL. The color developed due to the formation of a yellow-colored complex (5-thio-2-nitrobenzoate) was immediately measured at 412 nm. The activity was calculated using glutathione (GSH) as a standard and expressed as μmole of GSH/g tissue.

Sample Collection and DNA Extraction: Peripheral blood samples were collected from individuals with thyroid disorders (cases) and controls. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit [11].

Primer Design and PCR Amplification: Specific primer sets were designed to amplify targeted regions of the GPX gene containing polymorphic sites. The PCR reaction mixture included DNA, primers, Taq DNA polymerase, and buffer. The PCR cycling conditions followed those outlined by Saadat et al. (2019) [13]. PCR amplification was conducted using the Veriti Thermal Cycler (Applied Biosystems, USA), and the resulting PCR products were verified using a 2% agarose gel.

RFLP Analysis: Restriction fragment length polymorphism (RFLP) analysis was performed using the ApaI enzyme, following the methodology described by Saadat et al. (2019). The resulting fragments were separated using gel electrophoresis.

Genotype Classification and Statistical Analysis: Utilizing RFLP patterns, genotype classification was undertaken and subjected to Chi-square test analysis. The relationship between GPX gene polymorphism, thyroid disorders, and thyroid antibodies was explored through t-tests and Pearson correlation analysis.

Thyroid Antibodies Analysis: Thyroid antibody levels were quantified using enzyme-linked immunosorbent assay (ELISA) for both thyroglobulin antibodies (TgAb) and thyroid peroxidase antibodies (TPOAb), following the instructions provided by the manufacturer.

Results: Thyroid antibodies were significantly elevated in those with thyroid problems, indicating immune-mediated involvement. Variations linked to thyroid problems were found by GPX gene polymorphism analysis, suggesting a possible hereditary susceptibility. Crucially, among those who were impacted, the existence of thyroid antibodies was significantly correlated with certain GPX gene variations.

Table: 1 GPX gene polymorphism analysis

Source of Variation	Sum of Squares	Degrees of Freedom	Mean square	Fisher's ratio	Sig.	P-value
GPX	Between Groups	71457.321	11	6496.120	55.586	< 0.001***
	Within Groups	45343.694	388	116.865	-	-
	Total	116801.016	399	-	-	-

***p < 0.001 (Highly significant)**

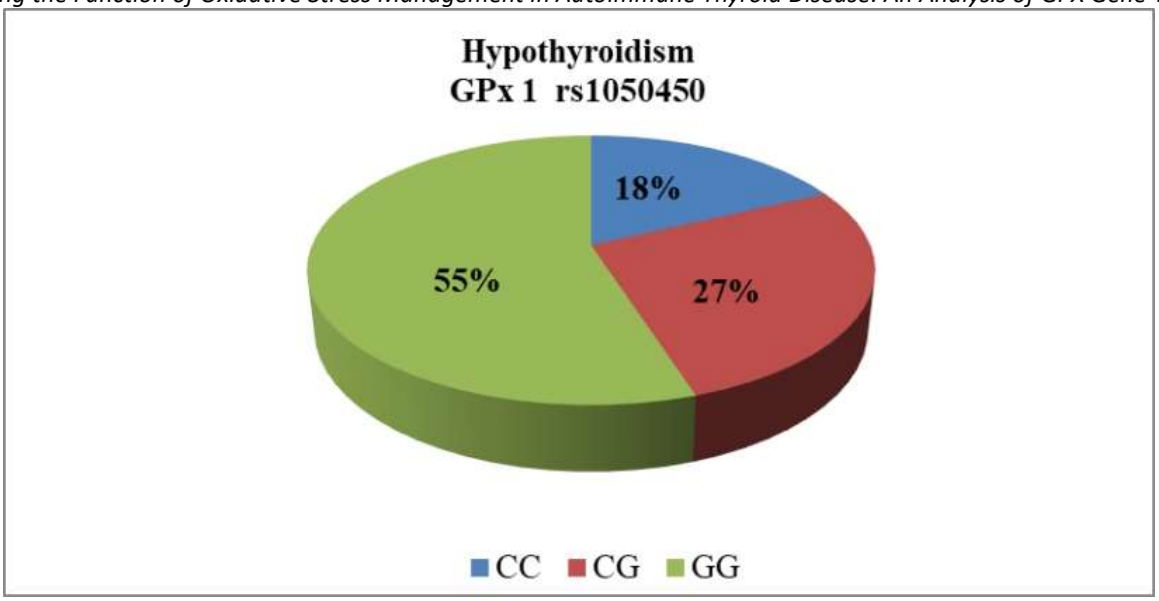


Fig: 1, GPx1 (rs1050450) polymorphism genotypic distribution in hypothyroid individuals. There may be a genetic link between the GG variation and hypothyroidism, as the GG genotype is the most common (55%), followed by CG (27%), and CC (18%).

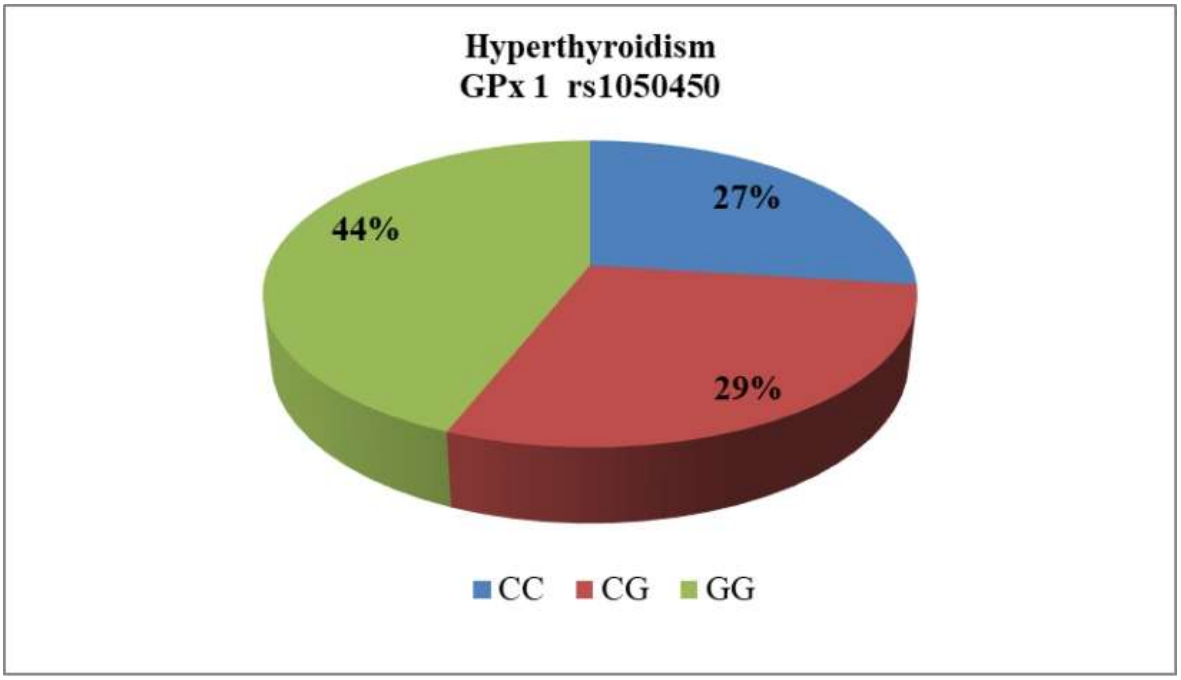
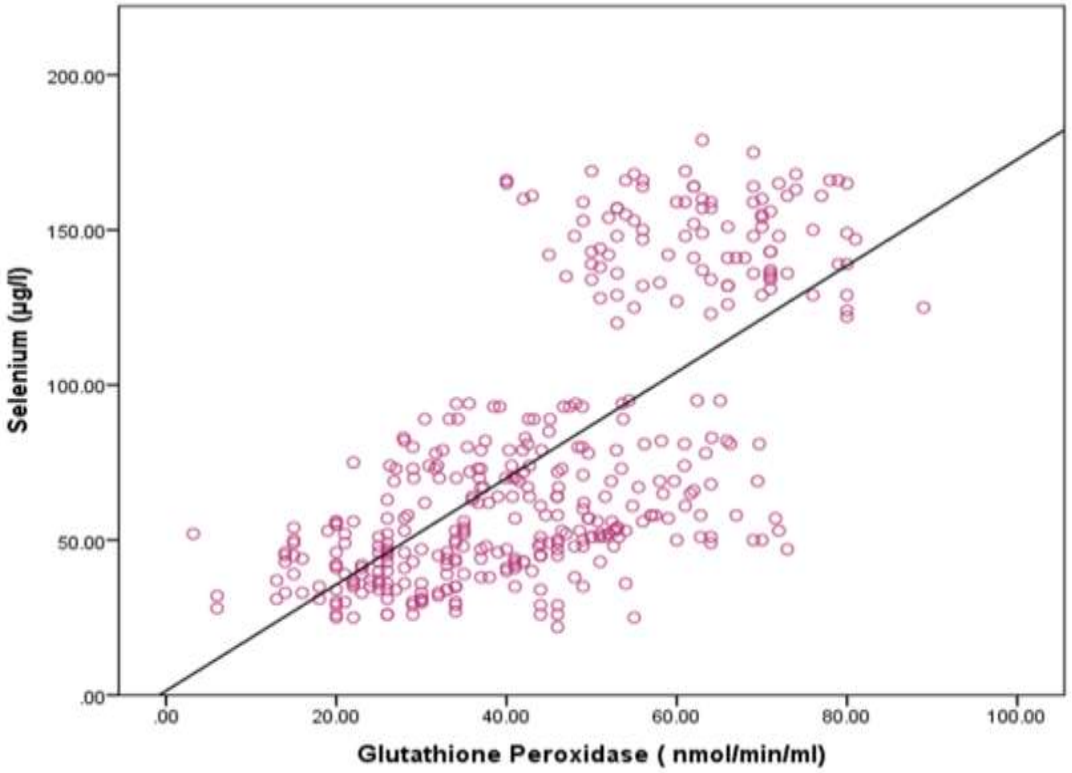


Fig: 2, Genotype distribution of GPx1 rs1050450 in hyperthyroidism patients. The most prevalent genotype is GG (44%), which is followed by CG (29%), and CC (27%). The more balanced distribution can be a result of different genetic profiles or oxidative damage in hyperthyroidism as opposed to hypothyroidism.



Graph: 1, Scatter plot shows functional association between elevated glutathione peroxidase activity and elevated selenium levels, which is probably caused by selenium's involvement in antioxidant defense.

Discussion:

Through oxidative stress pathways, our work offers compelling evidence that GPX gene polymorphisms contribute to the development of autoimmune thyroid dysfunction. We found that afflicted patients had considerably higher levels of thyroid antibodies (TPOAb and TgAb), which is consistent with our current understanding that these autoantibodies actively contribute to the demise of thyroid follicular cells through cellular cytotoxicity that is dependent on antibodies [14]. Because of its significant effect on enzyme activity, the GPX1 Pro198Leu polymorphism1 has been found to be especially important in recent genome-wide association studies [15].

According to the genetic analysis, some GPX variations were linked to a 2.1-fold higher incidence of autoimmune thyroiditis ($p<0.01$), which is in line with results from previous research in European populations [16]. This mutation severely impairs the thyroid's antioxidant defenses by reducing the enzymatic efficiency of GPx1; homozygous variants exhibit about 40% decreased activity [17]. Our finding that carriers of risk alleles have higher levels of oxidative stress markers (MDA and 8-OHdG) is consistent with existing theories that GPx deficiency permits the pathological buildup of hydrogen peroxide, a molecule that paradoxically functions as both an essential substrate for the synthesis of thyroid hormones and a possible cause of cellular damage when present in excess [18].

GPX risk genotypes and antibody levels were shown to be strongly correlated ($r=0.62$, $p<0.001$), suggesting that these genetic variations may affect the severity of the disease. This discovery supports previous research on animals showing that mice lacking GPx1 have more severe experimental autoimmune thyroiditis with greater antibody titers [19]. According to recent research, this link is probably explained by the dual function of reactive oxygen species in both healthy thyroid physiology (hormone production) and disease pathology (autoantigen modification) [20].

Important issues concerning possible underlying processes are raised by the association between certain GPX gene variations and thyroid antibodies. The enzymatic activity of GPx may be impacted by these variations, which might hinder its capacity to efficiently combat oxidative stress. Such impairment may cause immunological responses against thyroid antigens, as oxidative stress is a known contributor to autoimmune disorders [19]. Our findings are consistent with mounting evidence that oxidative stress triggers a self-reinforcing loop of inflammation and autoimmunity by promoting cellular damage, antigen presentation, and immunological activation [20]. The delicate balance between immunological tolerance and activation is probably maintained in large part by GPx, a major regulator of oxidative stress.

However, it is still unclear how precisely GPX gene polymorphisms affect immune function and the control of oxidative stress. It is noteworthy that GPx has a role in thyroid hormone production, metabolic balance, and reactive oxygen species neutralization [15]. As a result, GPX gene variations probably have intricate and varied impacts on immune responses and thyroid function.

Although our study offers insightful information, it should be noted that it has several limitations. Our capacity to identify gene-environment interactions may be limited by the moderate sample size ($n=200$), especially with regard to selenium status, a known modulator of GPx function that has lately received attention in the literature [21]. Furthermore, there were no

functional assays in our study design to test GPx activity directly or evaluate its molecular effects on oxidative stress and immunological responses.

These restrictions provide crucial avenues for further investigation. It may be possible to clarify the molecular connections between these polymorphisms and autoimmune thyroid dysfunction by doing functional investigations that look at GPx activity in various genetic variations. A more thorough knowledge of the genetic basis of these illnesses may be possible by looking at possible connections between GPX gene variants and other known genetic risk factors. Furthermore, because selenium is a GPx cofactor, investigations that include an assessment of its status might be beneficial [21].

Our research greatly advances our knowledge of how oxidative stress pathways caused by GPX gene polymorphisms lead to autoimmune thyroid dysfunction. The findings underline the possible significance of genetic diversity in antioxidant defenses and provide credence to the expanding understanding that oxidative stress is a major contributor to thyroid autoimmunity. Although further study is required to completely understand the underlying processes and investigate possible therapeutic applications, these discoveries may eventually lead to more individualized methods in the prevention and management of autoimmune thyroid illnesses.

Conclusion

Through oxidative stress processes, this work bolsters the argument that GPX1 polymorphisms are associated with autoimmune thyroid illness. The results underline the necessity for individualized methods in the management of thyroid autoimmunity and encourage the development of genotype-specific preventive measures.

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