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ORIGINAL RESEARCH PERIOPERATIVE MEDICINE

GSTM1 and GSTT1 null genotypes as risk factors for chronic kidney disease

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ABSTRACT

Background & objective: Chronic kidney disease (CKD) is considered one of the long-term diseases in which the kidneys fail to perform their normal functions adequately. It is a major medical and social problem that impacts about 10-13% of the population. Patients usually have to be put on life-long treatment, including frequent hemodialysis or even renal replacement. The current study was designed to reveal the relationship between genotypes of *GSTMI, GSTT1* and the risk factors for CKD, to understand the links with the pathogenesis.

Methodology: The current study included 50 CKD patients and 25 healthy individuals without health problems as a control group. Specimens were collected at the Al-Hussein Teaching Hospital from January to March 2024. Multiplex PCR technique was used to detect the genotypes of the studied genes. Besides personal and family history, relevant laboratory investigations were carried out.

Results: Family history was the common risk factor for CKD (80%). Urea, creatinine, and sugar levels were higher in CKD patients than in healthy individuals ($P < 0.05$). The majority of CKD patients (60%) had *GSTM1(-)* with significant differences compared to healthy patients (OR=1.90; 95%, 0.72-5.04). 48% of the patients had *GSTT1(-)* but only 32% of the healthy individuals had *GSTT1(-).* The frequencies of *GSTM (-)/ GSTT1(-)* were significantly higher among CKD patients (OR=2.26; 95%, 0.74-6.88).

Conclusion: The study reveals that the GSTM1(-) / GSTT1(-) genotypes increase the risk of CKD, possibly due to oxidative stress pathways, suggesting the need for targeted therapeutic strategies to mitigate oxidative damage in susceptible populations.

Key words: CKD; GSTM1; GSTT1

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1. INTRODUCTION

One of the major medical and social condition is the chronic kidney disease (CKD). Approximately 10-13% of the population is diagnosed with CKD which is often associated with cardiovascular risk and it is progressive and irreversible.[1] CKD presents one of the highest risks of mortality and complications, especially cardiovascular disease (CVD).[2] The major causes of CKD include hypertension, diabetes, pyelonephritis, chronic glomerulonephritis, autoimmune disease, polycystic kidney disease, prolonged acute renal disease thyroid disorder etc.[3,4] Despite the fact that risk factors including diabetes, hypertension, and dyslipidemia are obviously linked to cardiovascular in CKD patients, the Framingham prediction instrument indicates that these traditional risk variables are not very good at predicting coronary events in this population.[5] In addition, Atherosclerosis risk in communities study suggested that both novel and traditional risk factors are related with stage four of CKD.[6] Another Study suggested that CKD patients with heart disease have novel risk factors than the general population.[7] Oxidative stress (OS) is a key

driver has been proposed in the pathogenesis of kidney and heart disease.[8]

The overproduction of free radicals, especially reactive oxygen species (ROS) or a decrease in antioxidant defenses are states of oxidative stress.[9] Oxidant production primarily takes place in the mitochondria, facilitated by mitochondrial enzymes, including cytochrome P450. Overproduction of ROS may contribute to renal injury and atherosclerotic pathogenesis in CKD.[10] ROS are significantly implicated in uremia. It may play a role in the pathogenesis of atherosclerosis, cardiovascular issues, and other complications related to chronic kidney disease, including anemia and endothelial cell dysfunction.[9,11] The human body has several lines of defense against oxidants called antioxidants, including enzymatic and non-enzymatic defenses, which inhibit many oxidation reactions. Antioxidant systems operate through cascades of scavenging oxidants that block the production of free radicals. During these processes, oxidants are transformed to less harmful substances, and the production of secondary hazardous metabolites is inhibited. Then, the antioxidant system benefits the correct of the injuries and promotes an endogenous defense system.[12] Superoxide dismutase(SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GSTs) are the main enzymatic antioxidants which reduce the impact of oxidants.[13]

Glutathione S-transferases (*GSTs*) represent a superfamily of genes. It produces enzymes that promote the binding of glutathione molecules to electrophilic substrates. The enzymes of glutathione detoxify exogenous and endogenous electrophiles that react with essential biological molecules such as DNA.[14] Alpha (*GSTA*), tau (*GSTZ*), sigma (*GSTS*), omicron (*GSTO*), kappa (*GSTK*), pi (*GSTP*), mu (*GSTM*), and theta (*GSTT*) are among the isoforms of *GSTs* genes.[15] The most common isoforms are *GSTMI* and *GSTT1*, which have various genotypes. The null genotype of these genes has been correlated with increased cytogenetic damage and lack of enzymatic activity.[16] The *GSTM1* is located on chromosome 1p13.3, and encodes the mu class of enzymes, however the *GSTT1* gene located on chromosome 22q11.23 and codes of the theta class of enzymes.[17] Previous study suggested an relationship between *GSTM1(-)/GSTT1(-)* and development of CKD.[18] In Iraq, the association between null genotypes of *GSTM1(-)/GSTT1(-)* genes and CKD has not received much attention, so this study designed to reveal the relationship between the null genotypes of *GSTM1(-)/GSTT1(-)* genes and CKD.

2. METHODOLOGY

2.1. Sample collection and processing

Laboratory work was conducted at the molecular lab of Analytical Department, College of Science - University of Thi-Qar. Venous blood (2.5 mL) was collected in an EDTA anticoagulant vacutainer and stored in a refrigerator until DNA isolation.

2.2. DNA isolation

DNA was isolated using the gSYNCTMDNA mini kit based on manufacturer instructions. The purity and concentration of DNA were tested using a NanoDrop 1C spectrophotometer.

2.3. Multiplex PCR for GSTM1 and GSTT1 polymorphisms

Multiplex PCR technique was used to determine the genotypes of GSTM1 and GSTT1 polymorphisms. Multiple primers were used in the PCR reaction to produce amplicons of various sizes with different DNA sequences. The annealing temperature for each primer set was optimized to work appropriately within a single reaction, and we ensured that the amplicon lengths were sufficiently different to form distinguished bands when visualized on a UV light transluminator by gel electrophoresis. The following primers were used: the forward primer of GSTM1 gene is 5′- GAGGAACTCCCTGAAAAGCTAA AG -3′ and reverse 5′ CTCAAATATACGGTGGAGGTCAAG-3′, for GSTT1 gene, forward primer is 5′-TTCCTTACTGGTCCTCACATCTC-3′ and reverse5′-TCACCGGATCATGG CCAGCA-3′, as an internal control, albumin gene was adopted using

Table 1: The demographic distribution of healthy group and CKD patients

Demographic data	Healthy group (n = 25)	CKD group (n = 50)	P value
Age (years)	40.11 ± 16.03	50.09 ± 15.35	0.09
Gender			
• Male	12 (48%)	23 (46%)	0.87
• Female	13 (52%)	27 (54%)	
Smoking			
• Non-smokers	17 (68%)	35 (70%)	0.85
• Smokers	8 (32%)	15 (30%)	
Family history			
• Yes	4 (16%)	28 (56%)	0.0012 **
• No	21 (84%)	22 (44%)	

** P ≤ 0.05 considered significant; Data presented as mean ± SD or n (%)*

Table 2: Routine renal function parameters of healthy group and CKD patients

Laboratory tests	Healthy group (n = 25)	CKD group (n = 50)	P value
Blood urea (mg/dl)	37.32 ± 12.19	82.61 ± 20.10	0.003*
Serum creatinine (mg/dl)	1.06 ± 0.47	3.93 ± 0.98	0.029*
eGFR (mL/min/1)	79 ± 12.7	51.8 ± 7.4	<0.001 **

*Data presented as Mean ± SD; *P ≤ 0.05 considered significant*

Table 3: GSTM1 and GSTT1 genotypes among healthy group and CKD patients

Genotypes	Healthy group (n=25)	CKD patients (n=50)	OR	95%CI
GSTM1				
Present (+)	14 (56)	20 (40)	1.00	—
Null (-)	11(44)	30 (60)	1.90*	(0.72−5.04)
GSTT1				
Present (+)	17 (68)	26 (52)	1.00	—
Null (-)	8 (32)	24 (48)	1.96*	(0.71−5.36)

Data presented as n (%); OR - Odds ratio; CI - Confidence Interval

Table 4: Combined GSTM1 and GSTT1 genotypes among healthy group and CKD patients

Polymorphism	Healthy group (n = 25)	CKD Patients (n = 50)	OR	95%CI
GSTM1\GSTT1				
Present\Present	13 (52)	18 (36)	1.00	—
Null \ Null	7 (28)	22 (44)	2.26*	(0.74-6.88)
Present \ Null	1 (4)	2 (4)	1.43	(0.11-17.67)
Null \ Present	4 (16)	8 (16)	1.44	(0.35-5.83)

Data presented as n (%); OR - Odds ratio; CI - Confidence Interval

Forward primer 5′GCCCTCTGCTAACAAGTCCTAC-3′ and reverse primer 5′-GCCCTAAAAAGAAAA T CGCCAATC-3.[19] The PCR program involved a 10-minute initial denaturation at 95°C, 30 cycles of 1 minute each at 95°C, 58°C, and 72°C for denaturation, annealing and extension respectively , then a final 10-minute extension at 72°C. A UV light transluminator was used to visualized the PCR products from the co-amplification of GSTM1 (216 bp), GSTT1 (480 bp), and albumin (350 bp) using 2% agarose gel electrophoresis. A gel image is shown in Figure 1.

2.4. Statistical Analysis

Biochemical parameters and demographic data were compared among the patients and healthy using chi-square and independent T test. Statistical significance was set at p ≤0.05. To compare the frequencies of genotypes in patients and healthy group, the odds ratios (OR) were calculated and compared using version 20 of SPSS software. OR ≥ 1.5 means a genotype is risky.

3. RESULTS

The current study included 75 individuals (25 healthy individuals and 50 CKD patients) Table 1. The mean ages were 40.11±16.03 and 50.09±15.35 for the healthy and CKD patients, respectively. In this study, 48% of the healthy subjects were males and 52% were females. As for the CKD patients, 46% were males and 54% were females with non-significant differences between healthy subjects and patients (P = 0.87). Regarding smoking states, there were no significant statistical between CKD patients and healthy group (P = 0.85). The current study showed that the majority of the patients with CKD (56%) had a positive family history.

The results showed significant differences in renal function markers between healthy individuals and CKD patients. The mean blood urea level in patients with CKD was 82.61 ± 20.10 mg/dl which was significantly higher than that of healthy individuals 37.32 ± 12.19 mg/dl with a significance of p = 0.003). As for serum creatinine levels, they were highly elevated in the CKD group (3.93 ± 0.98 mg/dl) compared to SARS-CoV-2 negative healthy subjects (1.06 ± 0.47 mg/dl) (p-value 0.029) as expected due to kidney failure. And, eGFR was reduced in CKD patients (51.8 ± 7.4 mL/min/1.73m²) when compared to healthy individuals (79 ± 12.7 mL/min/1.73m²) with P < 0.001 as shown in Table 2.

3.1. Genotype Results

Figure 1 shows the various genotypes of *GSTM1* and *GSTT1* genes. The amplification at 216 bp indicated the *GSTM1(+)* genotype, while the presence of a band

at 480 bp indicated the *GSTT1(+)* genotype. The albumin gene, used as an internal control, appeared at 350 bp.

The polymorphisms of GSTM1 and GSTT1 genotypes in CKD patients and healthy individuals are shown in table 3. The frequencies of GSTM1(-) genotype were 44% and 30% in the healthy and CKD groups, respectively, with significant differences between the study groups (OR=1.90; 95%, CI=0.72-5.04). In addition, the ratio of

GSTT1(-) null genotype was higher in CKD patients (48%) than in the healthy group (32%), with an increased risk of CKD susceptibility (OR=1.96; 95% CI=0.71-5.36).

In this study, analysis of the combined effect of *GSTM1\GSTT1* genotypes showed that the GSTM*1(-) \GSTT1(-)* genotype was associated with the development of CKD (OR=2.26; 95%, CI=0.74-6.88).

4. DISCUSSION

The study revealed that most CKD patients fall within the age range, which aligns with broader research on CKD prevalence in older populations. Flaherty et al. (2024) reported a significant increase in the prevalence of CKD with advancing age in the United States, with estimates exceeding 50% for those aged ≥ 70 years.[20] This study represents a significant variation in family history in patients with CKD and highlights the substantial variability in family history among patients with CKD. Family health history is recognized as an important tool for evaluating the risk of prevalent chronic conditions such as kidney diseases. Additionally, there are notable limitations in the limited number of studies that have investigated the familial aggregation of CKD. [21–23] While these studies show varying percentages, they all indicate that a significant proportion of patients with CKD have a family history of kidney disease. The differences in reported rates may be due to variations in the study populations, definitions of family history, and the specific type of kidney disease considered.

The results indicated notable disparities in renal function tests between healthy individuals and patients. CKD studies have indicated notable disparities in renal function tests between healthy individuals and patients with CKD. Specifically, urea and creatinine levels in patients with CKD are significantly elevated compared with those in healthy individuals.[24] A meta-analysis of 12 studies with 44,721 patients observed substantial variability in GFR estimation equations between healthy individuals and those with kidney disease, which consistently demonstrated that individuals with CKD have significantly lower GFR values than healthy people, reflecting impaired kidney function in CKD patients.[25] Monitoring trends, particularly GFR, is often sufficient in clinical practice to inform treatment decisions and predict prognosis.

The present study observed notable differences between the null genotypes of GSTM1/GSTT1 and CKD. Numerous studies have examined the association between GSTM1/GSTT1 genotypes and CKD risk. A case-control study and meta-analysis indicated that the null genotypes of GSTM1 and GSTT1 are risk factors for CKD in China, with the GSTM1 null genotype being linked to an increased risk of CKD (OR = 1.62). The GSTT1 null genotype is associated with an elevated risk of chronic kidney disease (OR = 1.72).[26] A separate study indicated that the GSTM1 and GSTT1 null genotypes were associated with a 1.8-fold increase in the risk of end-stage renal disease (ESRD) ($P < 0.001$). Patients exhibiting combined null genotypes (GSTM1-null + GSTT1-null + GST-null) demonstrated a 3.3-fold increased risk of end-stage renal disease (ESRD) compared to individuals with normal genotypes.[27] The association between these genotypes and CKD risk appears to be mediated through oxidative stress, patients with GSTM1 or GSTT1 null genotypes exhibit greater susceptibility to oxidative stress than individuals with normal gene expression. [28] However, not all studies have found a significant association; a large cohort study of 46,983 participants found no association between GSTM1 copy number and kidney failure risk over a mean 9.2-year follow-up period,[29] although not all studies showed consistent results, and there is growing evidence that GSTM1 and GSTT1 genetic variants may influence CKD risk and progression, potentially through effects on oxidative stress pathways.[30]

5. CONCLUSION

The study concludes that GSTM1 and GSTT1 null genotypes associated with an increased risk of CKD, potentially mediated through oxidative stress pathways. These findings could have implications for identifying individuals at a higher risk of CKD and developing targeted therapeutic strategies to mitigate oxidative damage in susceptible populations.

6. Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request

7. Acknowledgments

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8. Ethical approval

Written informed consent was obtained from all participants. Ethical approval was granted by the ethical committee of College of Science - University of Thi-Qar. The

study followed the guidelines by Declaration of Helsinki for research involving human subjects.

9. Conflict of Interest Statement

The author declare that they have no competing interests

10. Funding Sources

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11. Author Contributions

The authors contributed equally in conceptualized the research, collected data, participated in data analysis and write-up, editing and review.

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