EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 2
50-52
Year: 2019

GLUTATHIONE S-TRANSFERASE M1 GENE DELETIONS AND THEIR EFFECT ON IRON STATUS IN RENAL FAILURE PATIENTS

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Article Received on 25/11/2018 **Article Revised on 15/12/2018** **Article Accepted on 05/01/2019**

ABSTRACT

Background: Renal failure is one of endemic disease in sudan characterized by chronic and acute renal failure. The renal failure disorder differs in etiology and symptoms and in the consequence of disease. Deletion in Glutathione S-transferase M_1 gene polymorphism in renal failure patient this problem may be cause of increase serum ferritin. **Objectives:** To detect Glutathione S-transferase M gene null genotype and their effect on iron status in patients with renal failure in Khartoum state. **Materials and Method:** A case control study was done in 50 renal failure patients and 40 normal controls. Included measurement of serum ferritin level by TOSO machine and Assessment of GST M_1 polymorphisms by allel specific PCR approach briefly. **Results:** The GSTM1null genotype was present in (0%) of the renal failure patients. There was an insignificant association of GSTM1null genotype (P = 0.004) with effect on iron status in patients with renal failure. **Conclusion:** The GSTM1null genotype was not associated with effect on iron status in patients with renal failure in Sudan- Khartoum state.

KEYWORDS: Renal failure, GSTM1 (Glutathione S-transferase), Ferritin, TOSO machine, allele specific PCR.

INTRODUCTION

Renal failure is one of endemic disease in sudan characterized by chronic and acute renal failure. The renal failure disorder differ in etiology and symptoms and in the consequence of disease. Deletion in Glutathione S-transferase M_1 gene polymorphism in renal failure patient this problem may be cause of increase serum ferritin.

Glutathione S-transferase works as antioxidant that catalyzes the conjugation of reduced glutathione through sulphydryl group to electrophilic centres.[1] Deficiency of Glutathione S-transferases M_1 and T_1 (GST M_1 and $GSTT_1$) enzymes activity is caused by the inherited homozygous absence of the GST M_1 or $GSTT_1$ gene, respectively (i.e., GST M_1 null or $GSTT_1$ null genotype). Mutation in the gene is known to cause oxidative damage.[1] It has been observed that GST M_1 which is the member of glutathione S-transferase family plays an important role in detoxification of metabolites of xenobiotics involved in cancer. This activity is responsible for detoxification of compounds like lipid peroxides.[2]

Iron deficiency may develop in hemodialysis patients, especially when erythropoietin is given. The role of iron deficiency in the anemia of predialysis chronic renal failure (CRF), however, is much less clear. We have intravenously (IV) administered iron as ferric saccharate in a total dose of 200 mg elemental iron monthly.

The concentration of ferritin in serum gives a quantitative measure of the amount of storage iron in normal subjects and those with iron deficiency or overload. The mean level in normal men is 69 ng/ml, compared with a mean of 35 ng/ml in normal women. A concentration below 10 ng/ml is associated with a low transferrin saturation and iron-deficient erythropoiesis. clinical manifestations of iron overload including hepatic, cardiac and endocrine dysfunctions (growth impairment, hypogonadism, hypothyroidism diabetes mellitus, andhypoparathyroidism).[3]

PATIENTS AND METHODS

The study was done in khartoum – Sudan –Salma renal dialysis centre. It is a case control study included (50) Sudanese patients suffering from acute renal failure as well as 40 healthy volunteers as control group to compare the frequency of GST M_1 null gene. Information was obtained from the patients and control before collection using questionnaire. Any patient suffering from cardiovascular disease, liver disease, or GIT bleeding was excluded.6ml of venous blood was collected into 2 containers (3ml in plain container and 3 ml in EDTA anticoagulant container) from each participant.

Laboratory investigations were included measurement of serum ferritin level by TOSO. DNA was extracted using salting out method.

Ethical considerations

This study approved by the faculty of medical laboratory sciences, AL Neelain University, and informed consent obtained from each participant before sample collection.

Molecular analysis

DNA extraction

Genomic DNA was extracted by using salting out method. DNA samples were stored at -30°C until analysis.

Detection of GSTM1 Null polymorphism

Allele specific polymerase chain reaction was used for the polymorphic deletion of the GSTT1.

A PCR was carried out in a total volume of 20 µl. It consists of 2µl of genomic DNA, 1 µl from each primer (Table 1), 4 µl of "5X FIREPoL" ready to load master mix (SOLIS BIODYNE, TARTU-ESTONIA) and 12 µl-distilled water. The amplification conditions were initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 59°C for 50 sec, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min.

5 µl of PCR product was electrophoresed on 2% agarose gel containing ethidium bromide. Three µl of 100 bp DNA ladder (Promega, USA) was applied with each batch of patients' samples. GSTM1 genotypes were determined by the presence and absence (null) of bands of 215 bp.

Table 1: Primer sequence for GSTM1.

Primer direction	Primer Sequence	Product Size bp	
		GSTM1	GSTM1 Null
Forward	5'-GAACTCCCTGAAAAGCTAAAGC-3'	215	Absence
Reverse	5'-GTTGG-GCTCAAATATACGGTGG-3'		

Statistical analysis

Data was analyzed manually and by using computer software(SPSS version 21) and the results was presented in graphs. Statistical values for $p < 0.05$ were considered significant, and>0.05 were considered insignificant.

RESULTS

A total of 50 renal failure patients samples were collected in this study (28) was male, (22) was female. The mean age of patients was (40 – 60) years old.

The serum ferritin in chronic renal failure patients (mean 573.4 ± SD 350.6) and control (mean 92.5 ± SD 50.1) (P = 0.000). The serum ferritin in male (56%) (Mean 531.4286 ± SD 358.58058) and Female (44%) (Mean 626.8636 ± SD 340.87539) (P = 0.335).

Table 1: Showing comparison of mean value of S.ferritin related to age among chronic Renal failure patients.

Parameter	Age	N	Mean	Std. Deviation	*p.value*
S. ferritin	15-30	8	631.7500	446.48780	0.403
	31-40	8	641.2500	333.00011	
	41-50	13	592.1538	357.03474	
	51-60	11	618.0909	361.28422	
	61-70	4	351.0000	206.45419	
	71-80	6	431.0000	309.63269	

The serum ferritin relaterd to duration among chronic renal failure patients was (7) patients (2 months - 1 year) (mean 400.4286 ± SD 248.59328), (13) patients (2 -6 year) (mean663.2308 ± SD 369.40203), (20) patients (7-12 year) (mean 583.2500± SD 368.25333) and (10) patients (13 - 22 year) (mean 558.1000 ± SD 352.84257) (P =0.067).

Table 2: Frequency of glutathione S-transferase M_1 gene null in chronic renal failure patients and normal control.

M1 Genotype	Group	Frequency	*p.value*
M_1gene-present	case	50(100%)	0.004
	control	39(78%)	
M_1gene null	Case	0 (0%)	0.004
	control	11(22%)	

Glutathione S-transferase M1 gene null among chronic renal failure patients in male (0) null and (28) present, female (0) null and (22)present (P =0.000).

Glutathione S-transferase M1 gene null among chronic renal failure patients was (7) patients (2 months - 1 year) present and (0) null, (13) patients (2 -6 year) present and (0) null, (20) patients (7- 12 year) present and (0) null, (10) patients (13 - 22 year) present and (0) null (P =0.000).

Table 3: Comparison of glutathione S-transferase M_1 gene null between age among chronic renal failure patients.

Age	N	M_1gene present	M_1 gene null	*p.value*
15-30	8	8	0	0.000
31-40	8	8	0	
41-50	13	13	0	
51-60	11	11	0	
61-70	4	4	0	
71-80	6	6	0	

DISCUSSION

Renal disease is associated with a graded increase in oxidative stress markers. This could be the consequence of an increase in reactive oxygen species as well as a decrease in antioxidant defense. This oxidative stress can accelerate renal injury progression. We examined whether genetic variants of GSTM1 gene, member of a super family of glutathione S transferases, influence the course of kidney disease progression.[4]

The present study is reported from Sudan Khartoum state regarding the role of glutathione S-transferase M_1 gene deletions and their effect on iron status in patients with renal failure. In the present study, we have observed that there was no significant difference of $GSTM_1$ on iron status in patients with renal failure.

The result in this study disagree with finding from Indian population which their result showed that the null polymorphism genotype of the detoxifying enzymes are associated with the risk of developing end stage renal disease.[5]

Hamid Nomani, Lida Hagh-Nazari, Ali Aidy, in their study showed that findings indicate that oxidative stress, impairment of the antioxidant system and abnormal lipid metabolism may play a role in the pathogenesis and progression of ESRD and its related complication.

The result in this study differ from other result due to vary greatly in different populations, sample size and methods.

In summary this study revealed that glutathione S-transferase M_1 null genotype was not associated with iron status in patients with renal failure. However, further investigations are needed to confirm these results in other larger populations.

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