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RESEARCH ARTICLE

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

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ABSTRACT

Background: Renal failure is one of endemic disease in Sudan characterized by acute and chronic renal failure. The renal failure disorder differ in etiology and symptoms and in the consequence of disease. Deletion in Glutathione S-transferaseT in renal failure patient this problem may be cause of increase serum ferritin.

Objectives: The study was done to measure iron in patients with renal failure by measure serum ferritin level using Toso machine and to detect glutathione S-transferase T gene polymorphism using allel specific PCR

Materials and Methods: A case control study was done in 50 renal failure patients and 50 normal control. Included measurement of serum ferritin level by TOSO machine and Assessment of GSTT1 poly morphisms by allel specific PCR approach briefly

Results: Serum ferritin levels in cases of chronic renal failure under dialysis were significantly higher than that of the healthy controls (p value = 0.000). Half the cases had the null genotype of GSTT1 compared to 11 (22%) of the healthy controls with a p value of 0.003. The GSTT1 was found to be present in 25 (50%) of cases and 39 (78%) of controls. Serum ferritin level in both cases and controls was found to be not statistically related to the GSTT1 genotype (p value =0.5, 0.07 respectively)

Conclusion: Serum ferritin level was high in cases compared to controls. GSTT1 null genotype was significantly higher in cases compared to controls. No statistically significant association was found between serum ferritin and GSTT1 null genotype.

INTRODUCTION

Chronic renal failure (CRF) also called chronic kidney failure, chronic renal insufficiency, or uremia is a slowly progressive loss of renal function over a period of months or year and defined as an abnormally low glomerular filtration rates (GFR). CRF that leads to severe illness and requires some form of renal replacement therapy such as dialysis is called end-stage renal disease (Shuwa, 2005). CRF occurs in 1.0 of every 5000 people, usually in middle-aged and older people, although children and pregnant women are also susceptible. CRF may be irreversible, and eventually leads to total kidney failure. Many people are unaware of the problem until more than 70% of kidney function has been lost (Issue, 2007). Renal failure is one of endemic disease in sudan characterized by acute and chronic renal failure. The renal failure disorder differs in etiology and symptoms and in the consequence of disease. Deletion in Glutathione S-transferase T 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 centers (David, 1999). Deficiency of Glutathione S-transferases M1 and T1 (GSTM1 and GSTT1) enzymes activity is caused by the inherited homozygous absence of the GSTM1 or GSTT1 gene, respectively (i.e., GSTM1 null or GSTT1 null genotype). Mutation in the gene is known to cause oxidative damage (David, 1999). It has been observed that GSTT1 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 (Dizdaroglu). Excess unbound iron can destroy parenchymal tissues through peroxidation of the mitochondria, microsomes, Golgi apparatus, DNA and RNA(Cunningham, 2004 and Borgna-Pignatti, 2004). 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 (Silverberg, 1996). 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 35 ng/ml in normal women. A concentration below 10 ng/ml is associated

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with a low transferrin saturation and iron-deficient erythropoiesis (Jacobs, 1972). clinical manifestations of iron overload including hepatic, cardiac and endocrine dysfunctions (growth impairment, hypogonadism, hypothyroidism diabetes mellitus, and hypoparathyroidism) (Borgna-Pignatti, 2014).

MATERIALS AND METHODS

The study was done in Khartoum – Sudan –Salma renal dialysis centre**.** The case control study included (50) Sudanese patients suffering from chronic renal failure. A control group of 50 healthy volunteers to compare the frequency of GST deletion was also included in the study. Six milliliter (ml) of venous blood were collected from each patient, 3.0 ml in ethylene diamine tretraacetic acid (E.D.T.A) container for molecular analysis and 3.0 ml in a plain container for measurement of serum Ferrirtin level. Information was obtained from the patients and control before sample collection. Any patient with cardiovascular disease, liver disease, GIT bleeding was excluded.

Serum Ferritin Assessment

Serum Ferritin level was measured for each subject using Tosoh automated immunoassay analyzer (Tosoh AIA-36-Japan).

DNA extraction

Genomic DNA was extracted from whole blood samples by using salting out protocol Miller *et al*. 1988. Extracted DNA stored below -20 c until analysis.

Genotyping of GSTT1 null polymorphism

Allele specific polymerase chain reaction was used for detection of the polymorphic deletion of GST1 null polymorphism.

Primer direction	Sequence	Product size (bp)
Forward primer	5'TTC CTT ACT GGT CCT CAC ATC TC3'	480
Reverse primer	5'TCA CCG GAT CAG GCC AGCA3'	

PCR was performed in total volume of 20µl containing 4µl of genomic DNA,1µl of each primer (Table 1),14µl of Distilled water all these ingredients were put in pre mixed tube(Maxime PCR premix kit(I-Taq). Thermocycling conditions includes denaturation step at 94^0C for 10 minute followed by 35 cycles of denaturation at 95^0C for 1 minute ,annealing at 62^0C for 1 minute and extension at 72^0C for 1 minute and followed by final extension at 72^0C for 10 minute.(R) After amplification in (TC-412, UK) thermocycler ; PCR products were analyzed using 2% agarose gel containing ethidium bromide and also 100 bp DNA ladder was run with each batch of patients samples and visualized under UV transilluminator by gel documentation system (SYNGENE, JAPAN). GSTT1 genotypes were determined by the presence and absence (null) of band of 480pb.

Statistical Analysis

Logistic regression was used to assess the risk between *GSTT1* null genotypes and serum ferritin level. Odds ratio (OR) with a confidence interval (CI) of 95% was calculated. The chi-square test was used to compare the genotype distribution between patients and control. *A p-value* less than 0.05 was considered as statistically significant. We have used the statistical package SPSS version 16 (SPSS Inc., Chicago, IL, USA)

RESULTS

A total of 50 renal failure patients samples are collected in this study. The mean age of patients was 48.4±16.2. The mean duration for renal failure among the cases was 8.02 years. As shown in table 1, serum ferritin levels in cases of chronic renal failure under dialysis were significantly higher than that of the healthy controls (p value = 0.000)

Tabel 1. Serum ferritin in chronic renal failure patients and normal control

	Group	N	Mean	Std. Deviation	*p.value*
S.ferritin	Case	50	573.4	350.6	0.000
	Control	40	92.5	50.1	

However, no statistically significant difference was found between serum ferritin levels and gender, age and duration of renal failure in the cases group (p value = 0.335, 0.403, 0.06 respectively). as shown in table ,2,3,4

Tabel 2. Showing comparison of mean value of S.ferritin between male and female among chronic Renal failure patients

	Gender	N	Mean	Std.Deviation	*p.value*
S. ferritin	Male(56%)	28	531.4286	358.58058	0.335
	Female(44%)	22	626.8636	340.87539	

Tabel 3. Showing comparison of mean value of S.ferritin related to age among chronic Renal failure patients

	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	

Tabel 4. Showing comparison of mean value of S.ferritin relaterd to duration among chronic Renal failure patients

	Duration	N	Mean	Std.Deviation	*p.value*
S. ferritin	2 months - 1 year	7	400.4286	248.59328	0.067
	2 -6 year	13	663.2308	369.40203	
	7- 12 year	20	583.2500	368.25333	
	13 - 22 year	10	558.1000	352.84257	

Of the cases, 25 (50%) had the null genotype of GSTT1 compared to 11 (22%) of the healthy controls with a p value of (0.003). The GSTT1 was found to be present in 25 (50%) of cases and 39 (78%) of controls.

Tabel 5. Frequancy of glutathione S-transferase deletions (GSTT1) in chronic renal failure patients and normal control

	Group		*p.value*
Tgene present	Case	25(50%)	0.003
	Control	39(78%)	
Tgene nill	Case	25(50%)	0.003
	Control	11(22%)	

The frequency of GSTT1 gene deletion among the cases was found to be not statisitically related to gender, age, or duration of the diseases (p values = 0.5, 0.5, 0.6).as shown in table 6.7.8.

Tabel 6. Comparison of glutathione S-transferase deletions in male and female among chronic renal failure patients

Gender	N	T gene present	T gene nill	*p.value*
Male	28(56%)	14	14	0.512
Female	22(44%)	11	11	

Tabel 7. Comparison of glutathione S-transferase deletions between duration among chronic renal failure patients

Duration	N	T gene present	Tgene nill	*p.value*
2 month1year	8	5	3	0.663
2-6 year	13	6	7	
7-12 year	18	7	11	
13-22 year	11	7	4	

Tabel 8. Comparison of glutathione S-transferase deletions between age among chronic renal failure patients

Age	N	Tgene present	T gene nill	*p.value*
15-30	8	3	5	0.511
31-40	8	5	3	
41-50	13	7	6	
51-60	11	5	6	
61-70	4	3	1	
71-80	6	2	4	

Lastly, Serum ferritin level in both cases and controls was found to be not statistically related to the GSTT1 null genotype (p value =0.5, 0.07 respectively).

GST genotype	Serum ferritin
Normal genotype	0.07
GSTT1 null	0.5

DISCUSSION

Chronic kidney disease (CKD) is an irreversible progressive reduction in renal function an important source of long term morbidity and mortality. It has been estimated that CKD affect more than 20 million people in the united estate (National Kidney Foundation, 2002). Anemia commonly occurs in people with CKD, when kidneys are damaged, they do not make enough EPO as a result the bone marrow makes fewer red cells, causing anemia. Other causes of anemia in CKD in include blood loss from steps of hemodialysis, to prevent of anemia to need frequent red blood cell transfusion and EPO therapy, due to blood transfused and EPO therapy to lead of iron over load (Eschbach, 1994). Glutathione S-transferases (GSTs) constitute multifunctional enzymes that detoxify reactive electrophiles and products of oxidative stress.

Glutathione S-transferase genes (GSTT1 and GSTM1) are well known detoxification agents, and any mutation in the gene is known to cause oxidative damage. Deficiency in the activity of GSTM1 and GSTT1 enzymes is caused by the inherited homozygous absence of the GSTM1 or GSTT1 gene, respectively (i.e., GSTM1 null or GSTT1 null genotype (Taspinar, 2008). In our study We have found that the mean serum ferritin level (573.4±350.6) was significantly higher in cases compared to controls (p value =0.000)., going in consistency with the findings of other studies of Sharma et.al and Sanjay et.al and thalassemia by Adb Elgawad et.al (Sharma, 2010; Sanjay, 2012; Italiana, 2014). In this study, we found the GSTT1 null genotype to be more prevelant among the cases of chronic renal failure patiens under dialysis compared to the healthy controls (p value = 0.003). This finding is consistent with several other studies that were done on thalassemia by Adb-Elgawad et.al and Sharma *et al* (Italiana, 2014 and Sharma, 2010), aplastic anemia by DIRKSEN *ET AL*, (Italiana, 2014), and sickle cell disease patients one by Sanjay et.al and another by Rabab MA, Bothina MH (Sanjay, 2012 and Rabab, 2013). Some of the studies have postulated the GSTT1 null genotype to be a risk factor for sickle cell disease and inceased risk for aplastic anemia (Sanjay, 2012 and Dirksen, 2004), however, to assess this genotype role in chronic renal failure, further in depth studies are needed. Presence of the GSTT1 null genotype wasn't statistically associated with gender, age or duration of the diseses in our study. This is in concordance with the study done by Sharma V *et.al* (Sharma, 2010) and DIRKSEN *ET AL* (Dirksen, 2004). In this study we found no significant between the relation GSST1 and the ferritin in other literature review (Sharma, 2010; Sanjay, 2012; Rabab, 2013) patients with GSTT1 genotype tended to have high serum ferritin. The main limitation of this study is the small sample size.

Conclusion

Serum ferritin level was high in cases compared to controls. GSTT1 null genotype was significantly higher in cases compared to controls. No statistically significant association was found between serum ferritin and GSTT1 null genotype.

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