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Effects of Glyoxalase 1 gene variations on the progression of diabetic nephropathy in diabetic patients in Taif, Saudi Arabia

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The complications of diabetes are mainly due to the increased blood glucose where high levels of methylglyoxal (MG). The metabolism of MG and some other reactive dicarbonyl metabolite are catalysed by Glyoxalase 1 (Glo1) of the glyoxalase system. This process avoids possible DNA and protein damaging glycation. It is hypothesised that Glo1 is a probable factor in the progression of diabetes vascular complication like diabetic nephropathy. Glo1 variations including amino acid substitution Ala111Glu of Glo1 and Glo1 copy number were predicted to be tolerant but not been confirmed yet. These variations might have a direct effect on Glo1 enzyme activity and on the progression of the patient who have diabetic nephropathy. This study was aimed to examine the Glo1 variations in the local community and find out the relationship of the progression of the patients who have diabetic nephropathy and Glo1 variations. Blood samples were collected from patients of end stage renal failure and controls. The Glo1 genotype was determined by PCR and RFLP and digested with SfaNI digestive enzyme while Glo1 copy number was analysed using TaqMan® Copy Number Assays. Glo1 (AA) SNP and Glo1 copy number was higher in ESRD patients compared with healthy control, this might be used as indicator of ESRD progression. This indication may improve the quality of the provided health care as well as enhance the personalized patient care.

Keywords: Glyoxalase 1, diabetic nephropathy, single nucleotide polymorphism, copy number variation.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease affecting about 35.4 million people, or 9.1% of adults aged 20-79 in North Africa and Middle East in 2015 (IDF Diabetes Atlas, 2015a). Diabetic nephropathy (DN) ,among chronic diabetic complications, is one of the leading causes of end stage renal disease (ESRD) (Alwakeel et al., 2011a). There are increasing trends in diabetes and DN incidence. This signal a medical catastrophe in dialysis units, which causes a greater consumption of economic resources. In Saudi Arabia, nearly 11,000 patients have ESRD, where 20% is due to diabetes (IDF Diabetes Atlas, 2015b). It was reported that the complication rate associated with type 2 diabetes was 23% for cardiovascular and 32% for renal complications (Alwakeel et al., 2011b).

The complications of diabetes are mainly due to the increased blood glucose where high levels of methylglyoxal (MG) are produced (Rabbani, Xue and Thornalley, 2014a). MG is a effective glycating agent, which modifies mainly the residues of arginine in proteins and deoxyguanosine residues in DNA to form hydroimidazolone and imidazopurinone advanced

glycation end products (AGEs), respectively (Rabbani and Thornalley, 2012). Modifications by MG can lead to loss of protein function and DNA strand breaks and mutations. Despite this detoxification activity, approximately 1 – 5 mol% of proteins and 1 - 10 adducts per 10^6 nucleotides in DNA are modified by MG in the steady-state in vivo. The metabolism of MG is catalysed mainly by the glyoxalase system which maintain it at low tolerable levels (Xue, Rabbani and Thornalley, 2011).

The glyoxalase system as a protective system prevents the accumulation of related dicarbonyls and MG in cells and fluids of the body to levels that possibly cause toxicity and dysfunction, which is called the metabolic state of dicarbonyl stress in the biological system (Xue et al., 2012a; Rabbani, Xue and Thornalley, 2014a). The affectivity of glyoxalase system is related to the activity of glyoxalase 1 enzyme. Multiple factors affect Glo1 enzyme activity including Glyoxalase 1 copy number variation and Glyoxalase 1 polymorphism (Wu et al., 2011a; Shafie et al., 2016).

Copy number variations (CNV) as a genetic diversity have been found in many mammalian species (Freeman et al., 2006a). In addition, it was confirmed that Glo1 CNV is present in human (Wong et al., 2007). Glyoxalase 1 copy number is associated with the expression of Glyoxalase 1 messenger RNA and hence enzymatic activity. In addition, Glo1 copy number increase has been proven in renal failure (Shafie et al., 2016). In renal failure, the plasma MG concentration increases 5 to 7 fold while in healthy subjects is about 100 nM, and therefore it is usually used as an example of severe metabolic state of dicarbonyl stress (Rabbani and Thornalley, 2015a). Also, chronic hypoxia is a common characteristic of renal failure (Fu, Colgan and Shelley, 2016a).

Glyoxalase 1 polymorphism leads to substitution of an amino acid (Ala111Glu). This polymorphism was predicted to be tolerant due to the amino acid association which may lead to protein modification. The association of Glo1 polymorphism with vascular complication of diabetes was not confirmed (McCann et al., 1981; McLellan et al., 1994) nor the association with autism (Junaid et al., 2004; Rehnström et al., 2008). However, the association between Glu111Glu homozygote and the increased prevalence of cardiovascular disease and peripheral vascular disease in the fifth stage of renal failure was suggested (Kalousová et al., 2008a). This study aimed to examine the distribution of Ala111Glu and Glo1 CNV in local community of Taif City and to find out the relationship of the progression of the patients who have diabetic nephropathy and Glo1 variations. This may improve the quality of the provided health care as well as enhance the personalized patient care.

MATERIALS AND METHODS

Subjects:

This study was approved by Taif University Ethical Committee and performed in communication with Taif Diabetes Center. The number of participants was 80 DN patients aged between 59-71 years following written informed consent, all those participants were diagnosed with ESRD and diabetes. Blood samples (80 samples) were obtained from the patients who have diabetes for more than 15 years and were at stage 5 renal failure on hemodialysis. Also, 80 blood samples were collected from healthy subjects which served as controls aged between 49-72. Three ml of venous blood were drawn from each individual of the two groups under complete aspect condition and collected in EDTA containing tube for separation of peripheral blood leucocytes and for DNA extraction.

Detection of Glo1 variations:

Peripheral whole blood was used for DNA extraction by the Thermo Scientific DNA isolation kit (Thermo Scientific, UK). Genotyping of Glo1 was determined by polymerase chain reaction (PCR) amplification and restriction length fragment polymorphisms (RFLP) as described in (Rinaldi et al., 2014). In brief, the extracted DNA from peripheral blood leucocytes was PCR. The reaction mixture contains water, Biomix red, 200 ng/µl DNA sample, C419A genotyping forward primer (5'-TCAGAGTGTGTGATTTCGTG-3') and reverse primer (5'-CATGGTGAGATGGTAAGTGT-3'). Each reaction of the final volume of 20 µl contained 0.2 pmol/µl: forward primer, 0.2 pmol/µl: reverse primer, 1 mM: dNTPs, Taq polymerase, 1.5 mM: $Mg2^+$, stabilizer and buffer. The protocol used for PCR started with denaturing DNA template at 95°C for 2 min. Total of 40 cycles of PCR were performed. Each cycle consisted of a denaturation step for 45 sec at 94°C, 45 sec at optimum temperature for annealing step, and 1 min at 72°C for extension reaction. 2 min at 72°C were added as a final extension step after the last PCR cycle. After amplification, the digestion of the products with

SfaNI digestive enzyme were performed for 1 hour at 37°C. The digested product were electrophoresed by 2% agarose gel and the fragments were visualized under ultra violet light after staining with ethidium bromide. In order to identify the single base pair change, the restriction digest revealed 713 bp fragment in the presence of E 111 allele and 453 bp and 260 bp fragments in the presence of A 111 allele.

Gene copy number

After the extraction and purification of DNA samples, TaqMan® Copy Number Assay was performed to detect Glo1 copy number with TERT (telomerase reverse transcriptase) as a reference gene according to the manufacturer's protocol (PN 4397425, Applied Biosystems, Paisley, UK). CopyCallerTM software was used for data analysed.

Statistical analysis

Data was analysed using SPSS program version 19. Kolmogorov-Smirnov test was used for data normality of distribution testing. Student's t-test was used for the significance difference of the means in two groups parametric data of independent samples, where difference in variance was determined by F-test. The correlations were tested by Spearman`s test. The correlations and the comparisons were considered as significant statistically when $P < 0.05$.

RESULTS

Demographic study:

This study included 160 Saudi persons classified as 80 healthy subjects and 80 ESRD with diabetes. The patients have been diagnosed in Taif Diabetes Center. In table 3.1, the demographic of participants is illustrated. The objective was to find out the relationship of the progression of the patients who have diabetic nephropathy and Glo1 variations which may improve the quality of the provided health care as well as enhance the personalized patient care.

Glo1 Polymorphism:

Genotypes of Glo1 gene results from SfaNI restriction enzyme in all subjects are shown in table 3.2. Homozygous genotypes (AA) showed a significant statistical difference between healthy group and ESRD patient group. Homozygous genotype (AA) was higher in ESRD patients compared with healthy control ($P<0.01$). In addition, wild type genotype (CC) showed a weak significant statistical difference between healthy group and ESRD patient group. Wild type genotype (CC) was slightly higher in healthy controls compared with ESRD patients ($P<0.041$). Allele (A) was detected more in the case of ESRD patients when compared with healthy donors ($P<0.047$).

Table 3.1; The demographic of participants in the study

	ESRD		HEALTHY	
	Male	Female	Male	Female
Number of participants	40	40	40	40
Minimum	59	60	49	49
Median	68.5	66	67	56
Maximum	71	69	70	72
Mean	65.4	64.6	60.6	61.9
Std. Deviation	5.34	4.81	9.84	9.43

Table 3.2 Ala111Glu SNP in all participants. (Student's t-test; *, P < 0.05; **, P < 0.01)

	Control 80 (%)	ESRD 80 (%)	P value
Wild type CC	36 (45)	24 (30)	0.041*
Heterozygous CA	26 (32.5)	24 (30)	0.101
Homozygous AA	18 (22.5)	32 (40)	0.009**
C Allele	98 (61.25)	72 (45)	0.102
A Allele	62 (38.75)	88 (55)	0.047*

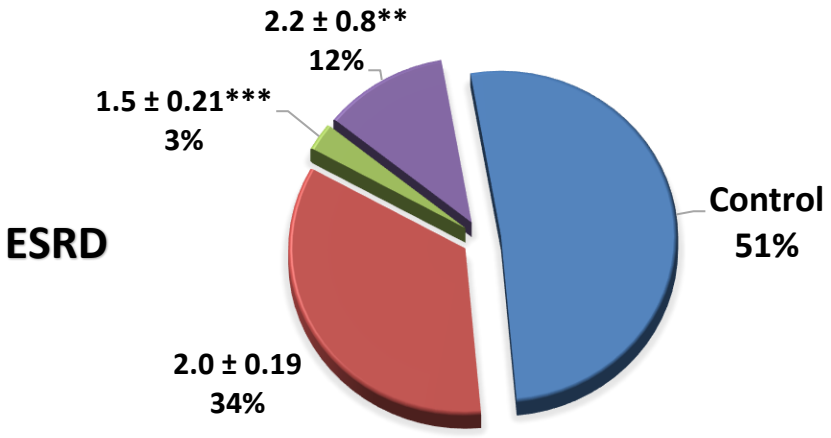

Figure 3.1 The relevance of Glo1 copy number in health controls and in EASD patients.
The figure shows grouped ESRD patients according to the number of Glo1 copy number and their percentage of all participants (Student's t-test; **, P < 0.01; ***, P < 0.001).

Glo1 copy number:

The relevance of Glo1 copy number (the other type of Glo1 variation) increase to human disease was assessed by the study of the association with severe metabolic state of dicarbonyl stress in EASD patients. In 76 healthy donors, the copy number of Glo1 was 2.00 ± 0.22. In 72 ESRD patients, 51 patients had Glo1 copy number indistinguishable statistically from the controls, 17 ESRD patients had Glo1 copy number higher than healthy controls (2.2 ± 0.08, P < 0.01), four had an abnormally low Glo1 copy number (1.52 ± 0.21, P < 0.001). Therefore, the prevalence of Glo1 copy number increase in the patients was 23.6% (Figure 1). The aetiology of ESRD patients and clinical chemistry characteristics of patients with ESRD were similar for those with or without increase Glo1 copy number in peripheral blood mononuclear cells.

In ESRD patients, the genotype distribution (CC, CA and AA) of Ala111Glu single nucleotide polymorphism (SNP) was: in the presence of Glo1 copy number increase, 2:6:9, where with no Glo1 copy number increase, 9:30:12 respectively. The change in genotype distribution was not significantly different.

DISCUSSION

Diabetic nephropathy is the leading cause of ESRD, which is one of the chronic diabetic complications. There are increased trends in the incidence of diabetes and DN (Alwakeel et al., 2011b). A direct relationship has been proven between the progression of the patients who have diabetic nephropathy and Glo1 activity (Giacco et al., 2014). The glyoxalase system prevents the accumulation of related dicarbonyls and MG in cells and fluids of the body to levels that possibly cause toxicity and dysfunction (Xue et al., 2012b; Rabbani, Xue and Thornalley, 2014b). As Multiple factors affect Glo1 enzyme activity including Glo1 copy number and Glo1 SNP (Wu et al., 2011b; Shafie et al., 2016), variations of Glo 1 gene were characterized in ESRD patients in this study. ESRD was studied as an example of severe metabolic state of dicarbonyl stress, as MG concentration in plasma of healthy subjects is approximately 100 nM while it increases 5 to 7 fold in ESRD patients (Rabbani and Thornalley, 2015b).

Genotypes of Glo1 gene results from SfaNI restriction enzyme showed as significant statistical difference Homozygous genotypes (AA) between healthy group and ESRD patient group (P<0.01). As Homozygous genotype (AA) was higher in ESRD patients compared with healthy control, this can give an indication of the ESRD progression. Previously, this genotype was associated with increased prevalence of peripheral vascular disease and cardiovascular disease in stage 5 renal failure on hemodialysis (Kalousová et al., 2008b).

The prevalence of increased copy number og Glo1 in ESRD was higher than the natural Glo1 duplication in the normal human population of about 2% (Freeman et al., 2006b). Chronic hypoxia is also a common characteristic of ESRD (Fu, Colgan and Shelley, 2016b). it can be

suggested that the increase of Glo1 copy number in ESRD patients is due to hypoxia and/or dicarbonyl stress. On the other hand, the decreased Glo1 copy number in four ESRD patients was possibly due to the damage of the DNA, which might be induced by uraemic toxins (Ersson et al., 2013).

In the study of the association between the increased copy number and Glo1 SNP, there was no association noticed. This is expected as it has been reported that the SNP gives rise to dimer allozymes with identical enzymatic activity (Kim et al., 1995); thereby, the exposure of both genotypes to the increased dicarbonyl stress in ESRD may be equal and thus both genotypes are similarly susceptible to the increase of Glo1 copy number.

CONCLUSION

This study aimed to find out the relationship of the progression of the patients who have ESRD and Glo1 variations in the local community. Glo1 (AA) SNP and Glo1 copy number was higher in ESRD patients compared with healthy control; this might be used as indicator of ESRD progression. The improvement of this indicator would be by identification of the correlation of Glo1 copy number and the stages of DN.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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