The study of Diabetic nephropathy disease and relashionshipe with GSTM1and GSTT1 genes and the risk factor of smoking of patients in the province of Thi-Qar

Hassan Ressan AL-Rikabi **Huda Taher Faisal**

Department of Biology – College of Education for Pure Science - University of Thi-Qar

Abstract:

The experimental group of this study contains 100 blood samples collected from patients with diabetic nephropathy infraction admitted to AL-Hussein Teaching Hospital and the educational center of the kidney industrial and civil medical laboratories in the province of Thi-Qar who ranged in age between 9-89 years old and 90 samples for people who are not infected with diabetic nephropathy infraction as a comparison group . the samples have been preserved in tube containers with EDTA under tempreture (-20^{o}) DNA was extracted and then GSTM1 and GSTT1 were amplified which are responsible for detoxification and Albumin as an internal control.

The results showed that 71% of the patients were living urban areas while 29% of the inhabitants of rural areas, the results also showed the proportion of patients who are smokers 34% and the non- smokers are 66% and patients who have family history are 51% compare to 49% for those who does not have family history and the results showed 39% of infected males and 61% infected females.

The results showed that the existence of the effect of smoking and lost GSTT1 gene is increasing the risk of diabetic nephropathy infraction by five times (OR= 5.037;95%CI= 1.242-20.426) as well as at the loss of two genes together GSTM1and GSTT1 as much as four times (OR= 4.308;95%CI= 0.444- 41.817) compared with the comparison group and the loss of together genes GSTM1,GSTT1 has contributed to increase the risk by (OR= 1.982;95%CI= 0.625-6.297).

Key word : Diabetic nephropathy, Genes, GSTM1, GSTT1 , PCR

Introduction:

Diabetes mellitus (DM) The term describes a metabolic disorder due to many pathogens such as excessive sugar, chronic blood disorders in the metabolism of carbohydrates, fats and proteins resulting from a defect in insulin secretion or insulin action, or both that triggered diabetes in the long term include damage and failure in various tissues and patients diabetes appear to have characteristic symptoms such as thirst and frequent urine and blurred vision, loss of weight, and when you get a coma disease progression or death in the absence of effective treatment. [(1)]

DM prevalence increases continually around the world ; it became one of the major global problems for the developing as well as the developed countries .It is affecting millions of peoples, about 6-7% of the world's population [(2) (3) (4)].

Diabetic nephropathy (DN) is defined as urinary albumin excretion equals to or more than 300 mg/24hr and more commonly represented by persistent albuminuria which is detected by various dipsticks [5].

DN with diabetic chronic renal insufficiency (CRI) is a leading cause of end stage renal disease worldwide [6]. The genesis of DN involves myriad of factors including older age, sex, hyperglycemia, and hyperlipidaemia. Ethnicity is the other major risk factor, with African, Americans, Asians, and native Americans being more prone to develop DN than Caucasians.

Nearly 30% of the cases of end-stage renal disease in India are due to diabetes, and this group is more likely to develop this complication than the Caucasians [7] [8] [9]. is rapidly becoming the leading cause of end – stage renal disease (ESRD), Particularly in the industrialized countries of the world [10].

Genes GSTs Glutathione S-transferaseare genes that belong to the phase II gene super family encodes enzymes play an important role in cellular protection and resistance to cellular compounds Almtaadh of the drug, since these genes contribute to the detoxification of the outputs of enzymes catalyzed reactions Phase I and the Phase II other enzymes to facilitate disposal [11].

Material and Methods:

1. Specimens Collection :

100 blood samples of diabetes nephropathy patients were collected from Al-Hussien Hospital Education in Al-Nasiryiah city and Artificial Kidney Center and 90 blood samples of non-diabetes nephropathy and type 2 diabetes as control group were collected from volunteers , their age range between 9 to89 years old. Two ml of peripheral blood was drawn from the two groups then it have been kept in EDTA tubes at $4c^0$ or $-20c^0$ for DNA extraction.

Data were collected according to Questionnaire form which includes age, sex, place of residence , family history of case , diabetes and blood pressure.

2 . **DNA Extraction:**

Method was used (12) in DNA extraction from blood samples, according to the following steps:

Added 500 Maekerolatr of a blood sample in the pipes (Eppendorf tube) capacity (1.5 ml) and then added 600 Maekerolatr solution (RBC buffer) after the expulsion of centrally quickly 5000 cycle for 15 minutes to a centrifuge and then poured the filtrate was retained sludge and repeated the process more than once note until the white precipitate at the bottom of the tube.

3. **Elactrophoresis:**

The electrophoresis tank has filled with 1X- TBE buffer about 3mm above the gel.Added 9 Maekerolatr of DNA undiminished oxygen to 3 Maekerolatr of the Bromophenol blue dye, or by (1:3) then it was loaded in wells of agarose gel.

Electrophoresis was done at 60V and 120 mA then the loading dye was left to migrate from the wells toward the other side.After electrophoresis , the gel was placed on a UV light and a digital photograph of the fluorescent Ethidium Bromide-stained DNA separation pattern was taken.

4. Multiplex Polymerase Chain Reaction (PCR):

Three primer pairs were included in the multiplex PCR for simultaneous amplification of fragments in the GSTM1 and GSTT1 and Albumin genes according to the protocol of [13) (14).

Table (1): Oligonucleotide primer sequences used for amplification of GSTT1 & GSTM1 genes.

Primers		Primer sequences	Length	Tm	TA
GSTM1	*F	5-GAA CTC CCT GAA AAG CTA AAG C- -3	22	64°C	59°C
	*R	5- GTT GGG CTC AAA TAT ACG GTG G- 3	22	64°C	59°C
GSTT1	F	5- TTC CTT ACT GGT CCT CAC ATC TC- 3	23	64°C	59°C
	R	5- TCA CCG GAT CAT GGC CAG CA -3	20	64°C	59°C
Albumin	F	5-GCC CTC TGC TAA CAA GTC CTA C- 3	22	64°C	59°C
	R	5- GCC CTA AAA AGA AAA TCG CCA ATC- 3	24	64°C	59°C

Table (2): PCR reaction for amplication of GSTM1 & GSTT1 genes.

materials	Volume
Master Mix	5 µl
Primer Forward	1 µl for one gene
Primer Reverse	1 µl for one gene
DNA	5 µl
D.W.	8 µl
Total	20 µl

Table (3): PCR condition for amplication of GSTM1 ,GSTT1 genes.

No. of Steps	Steps	Temperature	Time	No. of Cycle
1	Denaturation 1	94 °C	3 min	1 Cycle
2	Denaturation 2	94 °C	1 min	30 Cycles
3	Annealing	58°C	1 min	
4	Extension 1	72°C	1 min	
5	Final Extension 2	72°C	5 min	1 cycle

5. Detection outputs of the technique (PCR):

Followedthe same method of deportation electrode for the detection of (DNA), but with the use of (DNAmarker) and Alagaros concentration (2%)

After the detection of packets to a UV results were recorded as follows: - The emergence of the package when the pair baseband bp 215 means the presence of the gene GSTM1 and the emergence of a package when the pair baseband 350 bp mean

the presence of the gene, which was used Albumin xatrh interior. While the existence of a package when the pair baseband 480 bp, it means the presence of GSTT1 gene after comparison with DNA Marker [13] [14].

6. Statistical Analysis:

Test was used (T) and (OR) using a statistical program (SPSS ver .8) and the level of probability $P < 0.05$ and confidence interval 95% CI for comparison between patients and control samples to study the effect of deletion mutations in the genes GSTM1 and GSTT1 in the incidence of the disease.

Results:

The image (1) shows the migration of packages the DNA extracted from samples compared to patients.

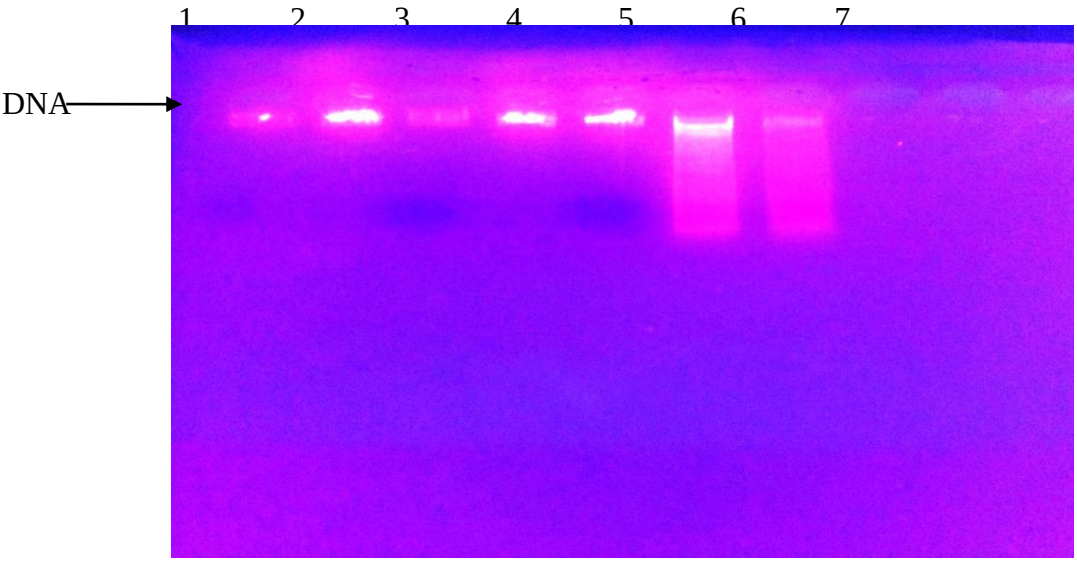

Figure (1): The Electrophoresie for DNA 0f agarose gel 0.8%

Lane 1,2,3 Patients samples of Diabetic Nephropathy

Lane 4,5 Patients samples of Diabetes Mellitus

Lane 6,7 Control samples .

The image (2) shows the electrophoresis for PCR results on agarose gel 2%

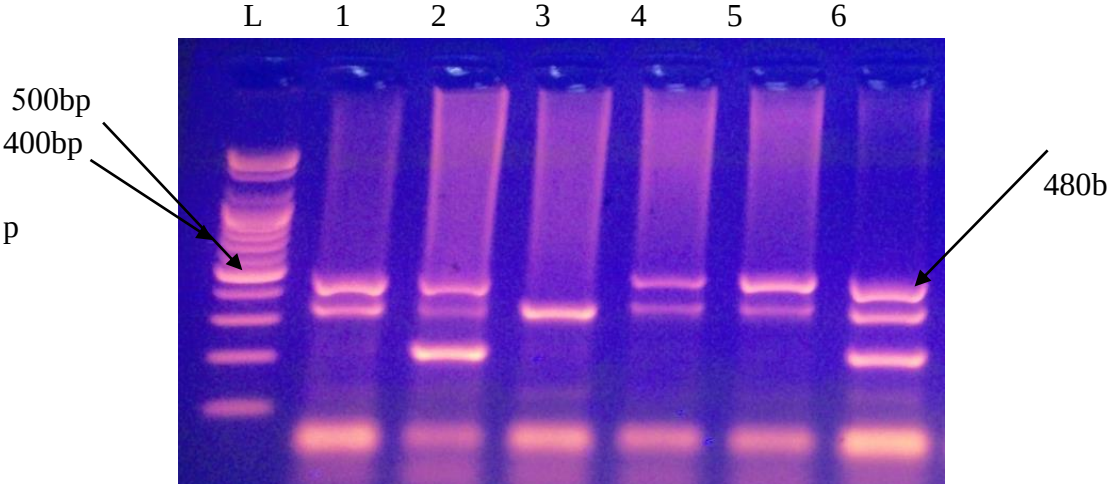

Figure (2): The Elactrophoresie for PCR technique results on Agarose gel 2%
L –measure DNA (1500 bp)
Lane 1,4,5 Loss of GSTM1 genes
Lane 2 Loss of GSTT1 genes
Lane 3 Loss of together genes GSTM1,GSTT1
Lane 6 Natural (contain the three genes).

This study showed:
1.1The loss of gene GSTM1 and risk of diabetic nephropathy disease.
Found that loss of the gene GSTM1 was (48.49%) in the control samples, while (31%) in patients samples, through statistical analysis showing no significant differences between the loss of the gene GSTM1 and the risk of diabetic nephropathy impairment compared to the control group in possession of this gene (OR= 0.469 ; 95%CI= 0.259 – 0.849).the table (4)
1.2 . The loss of gene GSTT1 and risk of diabetic nephropathy disease.
The percentage of risk for the disease by three times in patients when compared to the loss of gene GSTT1 control group with significant differences (OR= 3.017 ; 95%CI= 1.574 – 5.785).the table (4)
1.3. The loss of GSTM1, GSTT1 genes togathers and risk of diabetic nephropathy disease.
The percentage by almost twice the risk in patients when compared to the loss of the two genes together control group that they had a loss of genes at a rate of (18.19%) (OR= 1.883 ; 95%CI= 0.734 – 4.838) . As in the table below (4).

Table (4): combarison between models for genetic control samples and patients

Genetic models	Control groups%	Patients groups%	OR*	95% CI	P value*
GSTM1 (+)*	46 (51.11%)	69 (69%)			
GSTM1 (-)*	44 (48.49%)	31 (31%)	0.469	0.259 – 0.849	0.0123
GSTT1 (+)	72 (80%)	57 (57%)			
GSTT1 (-)	18 (20%)	43 (43%)	3.017	1.574 – 5.785	0.0009

GSTM1,GSTT1 (+)	36 (81.81%)	43 (70.49%)			
GSTM1, GSTT1 (-)	8 (18.19%)	18 (29.50%)	1.883	0.734 – 4.838	0.188

*(+)Presence of geenes *(-) loss of genes *P= 0.05
* OR Odd Ratio
*95%CI Confidence Interval

2. the impact of smoking and deletion of genes in the incidence of diabetic nephropathy for both sexes.

A-Smokers:

The results of the current study that there is the effect of smoking and the loss of the gene GSTT1 in patients with diabetic nephropathy as it was found that the loss of the gene GSTT1 in patients smoking contributes to the seriousness of the disease by five-fold compared to the control group (OR= 5.037 ; 95%CI= 1.242 – 20.426). While increasing risk of the disease by four times when the loss of the two genes together in patients smokers (OR=4.308 ; 95%CI= 0.444 – 41.817). The loss of the gene was increased by a few and did not have any significant effect on patients' risk of smokers develop the disease (OR= 0.409 ; 95%CI= 0.130 – 1.279). As in the table (5).

Table (5): Genetic models of GSTM1,GSTT1 genes of control and patients samples by Smoking for both sexes

Genetic models	Control groups%	Patients groups%	OR	95% CI	P value
GSTM1 (+)	9 (10%)	22 (22%)			
GSTM1 (-)	11 (12.22%)	11 (11%)	0.409	0.130 – 1.279	0.124
GSTT1 (+)	17 (18.89%)	18 (18%)			
GSTT1 (-)	3 (3.33%)	16 (16%)	5.037	1.242 – 20.426	0.024
GSTM1, GSTT1 (+)	7 (87.5%)	13 (61.90%)			
GSTM1, GSTT1 (-)	1 (12.5%)	8 (38.09%)	4.308	0.444 – 41.817	0.208

*(+)Presence of geenes *(-) loss of genes *P= 0.05
* OR Odd Ratio
*95%CI Confidence Interval

B – Non-smokers:

The results of the current study was that the case in patients than non-smokers increase by twice the loss of the gene GSTT1 when compared to the control group(OR= 2.302 ; 95%CI= 1.091 – 4.858), and the ratio of the risk by one-time loss when the two genes together (OR= 1.285 ; 95%CI = 0.422 – 3.916). as for the loss of the gene GSTM1 did not have any significant effect on the patients' non-smokers (OR=0.487 ; 95%CI= 0.241 – 0.986). The table (6).

Table (6):Genetic models of GSTM1,GSTT1 genes of control and patients samples by non-smoking for both sexes

Genetic models	Control groups%	Patients groups%	OR	95% CI	P value
GSTM1 (+)	37 (41.11%)	46 (46%)			
GSTM1 (-)	33 (36.66%)	20 (20%)	0.487	0.241 – 0.986	0.046

GSTT1 (+)	55 (61.11%)	43 (43%)			
GSTT1 (-)	15 (16.66%)	27 (27%)	2.302	1.091 – 4.858	0.029
GSTM1, GSTT1 (+)	29 (80.55%)	29 (76.31%)			
GSTM1, GSTT1 (-)	7 (19.45%)	9 (23.68%)	1.285	0.422 – 3.916	0.658

*(+)Presence of geenes *(-) loss of genes *P= 0.05
* OR Odd Ratio
*95%CI Confidence Interval

C-Comparison of genetic models for a group of patients only by smoking

The results showed when comparing genetic models for the two sets of patients from smokers and non-smokers no differences for the loss of the gene GSTM1 among patients smokers and non-smokers(OR= 1.150 ; 95%CI= 0.470 – 2.812) and also for gene GSTT1 (OR= 1.415 ; 95%CI= 0.619 – 3.239).as in the table (7)

Table (7):Genetic models of GSTM1,GSTT1 genes of control and patients samples by smoking for both sexes

Genetic models	Patients groups of smokers%	Patients groups of non- smokers%	OR	95% CI	P value
GSTM1 (+)	22 (22%)	46 (46%)			
GSTM1 (-)	11 (11%)	20 (20%)	1.150	0.470 – 2.812	0.759
GSTT1 (+)	18 (18%)	43 (43%)			
GSTT1 (-)	16 (16%)	27 (27%)	1.415	0.619 – 3.239	0.411
GSTM1, GSTT1 (+)	13 (61.90%)	29 (76.31%)			
GSTM1, GSTT1 (-)	8 (38.09%)	9 (23.68%)	1.982	0.625 – 6.297	0.246

*(+)Presence of geenes *(-) loss of genes *P= 0.05
* OR Odd Ratio
*95%CI Confidence Interval

Disscution:

The genes (GSTs) Glutathione S- transferase encoding the large gene family of enzymes that play an important role in protecting cells against free oxygenic species [(15)], there are many studies have pointed to the existence of a relationship between genes (GSTs) and deletions in the emergence of many diseases that are thought to be one of the causes is the oxidative stress such as cancer, [(13)] and bronchial asthma [(15),] vitiligo[(16)] and Myocardial infarction [(17).]

The results of the current study table (4) that for people with missing genes together GSTM1,GSTT1 and also with missing the gene GSTT1 are more susceptible to diabetes, renal impairment was found that the loss of the two genes together was increased by almost twice (OR= 1.883; 95%CI= 0.734-4.838) the loss of the gene was increased by three times (OR= 3.017; 95%CI= 1.574-5.785).

and (17) found , which indicated that the loss of Gene GSTT1 increase by three times (OR= 3.172; 95%CI= 1.595- 6.308), while the loss of the gene GSTM1 did not show

any significant difference (OR= 0.651; 95%CI= 0.341- 1.243) either loss of the two genes together GSTT1,GSTM1 did not show significant treatment effect (OR= 0.294; 95%CI= 0.160- 0.538).
(18), he pointed out that the loss of the two genes together (GSTM1andGSTT1) had increased six times (OR=6.80; 95%CI= 1.17- 29.5) and a half ago and the loss of the gene GSTT1 was increased by two and a half (OR= 2.70; 95%CI= 0.80- 9.19) while the gene GSTM1 was twice (OR= 2.06; 95%CI= 1.21- 3.46).
The reason may be due to increasing expression of GSTs in epithelial cells of the proximal tubule during the early stage of diabetes , likely in response to oxidative triggered by hyperglycemia or other toxic effects of glucose [(19).]

Present study showed significant association between GSTT1 null genotype and smoking in DN , that contributed to increasing the risk of infection five times (OR= 5.037;95%CI= 1.242- 20.426). and significant in GSTM1,GSTT1 null genotype that contributed to increasing the risk four times (OR=4.308;95%CI=0.444- 41.817). but no significant association between GSTM1andGSTT1 null genotype and GSTM1 null genotype and non-smokers in DN, but showed significant association between GSTT1 null genotype and non-smokers in DN that contributed to increasing the risk two times (OR= 2.302;95%CI=1.091- 4.858). that agreement with [(20)] and [(21)] . there was no evidence of association between GSTM1 and DN and Type 2 Diabetic mellitus , many factors may account for that, methodological issues should also be considered [(22).] GSTM1, GSTT1 null genotype showed a significant higher frequency that agreement with [(23).] This could be the consequence of an increase in reactive oxygen species as well as a decrease in antioxidant defense [(6)], Genetic variants that affect the capacity to handle oxidative stress may therefore influence the outcome of kidney disease [(24)] .

Conclusions:

Results of this study showed there are a significant relationship between smoking and loss of deletion GSTM1,GSTT1 genes with increase of diabetic nephropathy infection .

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دراسة مرض اعتلال الكلى السكري وعلاقته بفقد جينات الـGSTM1 and GSTT1 وعامل خطورة التدخين لدى المرضى في محافظة ذي قار

حسن ريسان الركابي هدى طاهر فيصل

قسم علوم الحياة – كلية التربيه للعلوم الصرفه – جامعة ذي قار

الخلاصة

جمعت 100 عينة دم بحجم 2,5 مل من اشخاص تتراوح اعمارهم بين 9-89 سنة من كلا الجنسين لآشخاص مصابين باعتلال الكلى السكري (مجموعة المرضى) يراجعون مستشفى الامام الحسين التعليمي – مركز الكلى الصناعيه والمختبرات الطبيه الاهليه في محافظة ذي قار .و 90عينة دم أعتمدت كمجموعة سيطره من غير المصابين بهذا المرض من مختلف الشرائح الاجتماعيه . حفظت العينات بانابيب حاويه على الـ EDTA وتم تضخيم الجينات أضافة الى تضخيم DNA بدرجة حراره (-20^0م) لحين استخلاص الـ DNA اضافة الى جين الالبومين كسيطرة داخليه بتقنية تفاعل البلمرة المتسلسل PCR .

أظهرت نتائج الدراسة أن 71% من المرضى كانوا من الذين يسكنون المناطق الحضريه بينما كانوا 29% من سكنة المناطق الريفيه , كذلك وجد أن 61% من المرضى كانوا اناث و 39% كانوا ذكور , أضافة الى التدخين حيث كان نسبة المدخنين 34% وغير المدخنين 66% , ونسبة المرضى الذين يملكون تأريخآ عائليا كانوا 51% والذين لايملكون تأريخا عائليا 49%.

كذلك اظهرت النتائج ارتباطآ بين فقدان الجينين معآ وخطر الاصابة عند المدخنين وغير المدخنين حيث كانت اربع مرات عند المدخنين (OR= 4.308;95%CI= 0.444-41.817)ومره لدى المرضى غير المدخنين (OR= 1.285;95%CI= 0.422-3.916) وكان الجين GSTT1 مؤثرا على المدخنين بخمسة اضعاف (OR= 5.037;95%CI= 1.242-20.426)ومرتين لدى المرضى غير المدخنين (OR=

(2.302;95%CI= 1.091-4.858) في حين لم يظهر اي فرق معنوي للمدخنين وغير المدخنين على التوالي عند فقدان الجين GSTM1.

الكلمات المفتاحية: اعتلال الكلى السكري , جينات.