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Association between polymorphisms of the DNA repair gene (OGG1) in Iraqi patients with type2 diabetes mellitus

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Abstract*:Chronic hyperglycemia in type 2 diabetes mellitus leads to elevated oxidative stress . As a consequence , the accumulation of reactive oxygen species (ROS)may cause additional damage to various biological macromolecules, including DNA. Several studies have demonstrated that oxidative stress plays an important role in the pathogenesis of cardiovascular alterations observed in diabetic patients and that hyperglycemia is the causal link between diabetes and increased oxidative stress.*
Objectives*:- The aim of the study was to compare the distribution of genotypes of DNA repair genes OGG1between type 2 diabetic patients and non-diabetic subjects and Study biochemical change and metabolism change in patients with DM Type2 .*
Methodology :- *Restriction fragment length polymorphism (RFLP) was used to determine the distribution of genotyping of codon 326 of OGG1 following primers are designed to encompass the ser 326 cys polymorphism sit. The study population included 100 subjects , including (60) patients with type 2 diabetes mellitus and (40) healthy control. The study was carried out in National Center for Diabetes Research/ (AL-Yarmouk teaching hospital) and Biology department laboratory/molecular lab. College of Science for Women in University of Baghdad from (December , 2014 to the April 2015).*
Result :- *The frequency of the Ser allele in OGG1 gene (0.62% in patients , 85% in control) The serine (wild-type) and cysteine (variant) allele frequencies were 0.62 and 0.38, respectively. The genotype and allele frequencies obtained from diabetic patients did not differ significantly from those found in control subjects with the OGG1 Ser326Cys polymorphism.*
Conclusion:- *From the present study the following points can be concluded :*

1. *The hyperglycemia, insulin resistance , abnormal change of lipoprotein , all these parameters are associated with T2DM.*
2. *The wild OGG1 Ser/Ser is more prevalence in control than patients.*
3. *The variant OGG1 Ser/Cys is more prevalence in patients than control.*
4. *The mutant genotype Cys/Cys may be conserved in patients and further study needs to elucidate that.*
5. *OGG1 genotypes do not have an effect on blood lipids given exposure to T2DM.*

Keywords: *OGG1, diabetes mellitus, Type II diabetes mellitus*

I. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both.(ADA2003)[(1)]. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source.

There are three main types of DM are recognized Type1 DM or (IDDM), Type2 DM or (NIDDM) and Gestational Diabetes Mellitus (GDM). Other specific types of diabetes are caused by specific genetic defects of beta cell function or insulin action, the pancreas diseases and drug or chemical induced diabetes mellitus (ADA, 2012).[(2)]

In type 1 diabetes, the body does not produce insulin, and daily insulin injections are required. Type 1 diabetes is usually diagnosed during childhood or early adolescence and it affects about 1 in every 600 children.(Nicki R. 2010) [(3)].

In type 2 diabetes is the result of failure to produce sufficient insulin and insulin resistance. Elevated blood glucose levels are managed with reduced food intake, increased physical activity, and eventually oral medications or insulin.(Brunner and Suddarths, 2008)[(4)].

Chronic hyperglycemia in type 2 diabetes mellitus leads to elevated oxidative stress . As a consequence , the accumulation of reactive oxygen species (ROS)may cause additional damage to various biological macromolecules, including DNA [(5)] . Among many genes the human 8-oxguanine DNA glygcosylase (OGG1) which encode by OGG1 gene which catalyzes the cleavage of the glycoside bone between the modified base and the sugar leaving a basic purine /Apyrimidine (AP)site in DNA. One of the most polymorphism in OGG1 is

(ser326 cys) which play a major roles in various disease. C/G transversion mutation (substitution) of serine with cytosine in codon 326 which lower the activity of the DNA repair enzyme. [6]

It is accepted that increased ROS generation is an important factor underlying the development of vascular complications in type 2 diabetes ,and possibly one of the factors responsible for an increased incidence of cancer in this group of patients.[7]

II. Methodology

The study population comprised (100) subject , including (60) with diabetes mellitus type2 and (40) with normal glucose metabolism and the Patients with renal , endocrine disease ,hepatic , uncontrolled hypertension, acute blood loss, alcohol intake, on medications for lowering lipid, and smokers were excluded from the study. The study was carried out in National Center for Diabetes Research/ (AL-Yarmouk teaching hospital) and Biology department laboratory/molecular lab. College of Science for Women in University of Baghdad from (December , 2014 to the April 2015).

Took samples from the plasma to measure the clinical indications for patients and control included the level of glucose in blood, insulin resistance, total cholesterol, triglycerides, high-density lipoprotein, low density lipoprotein, very low density lipoprotein(by use Abbot Kit) addition to link these factors to assess with the multiplicity of style genetic gene OGG1 .

They also took blood samples included molecular study to extract the DNA(use promega kit) and use the serial replication and the varying lengths of cutting restricted DNA to investigate the genetic diversity of the gene OGG1 of all members of this study.

In this study PCR- Restriction Fragment Length Polymorphism methods was used to detected the genotype of the Ser326 Cys Polymorphism. 15 µl aliquots of polymerase chain reactions (PCR) contained 5 ng genomic DNA, 15 µl Go Taq Hot Start Green Master Mix ,1 µl of Primer Forward, 1 µl of Primer Reverse and 8 µl Distilled water . Thermal cycling conditions for the OGG1were : initial denaturation 1 step for 5 minutes at 95°C followed by 1 cycles and {denaturation 2 step for 30 seconds at 94°C , Annealing step for 30 seconds at 55°C and extension 1 step for 30 at 72seconds°C } followed by 30 cycle. The final extension 2 step was performed at 72°C for 5 minutes. Primers sequences used to amplify the OGG1 fragment 200 bp where: F: 5 - ACT GTC ACT AGT CTC ACC AG – 3 and R: 5 -TGA ATT CGG AAG GTG CTT GGG GAA T - 3 (Jacek Kasznicki1 al., 2009)[8]. consequently, the ser/ser , ser/cys and cys/cys genotypes result in 200 bp ; 200 and 100bp ; 100bp digestion, respectively. Restriction fragment length polymorphism (RFLP) cut a DNA sequence by using restriction enzymes (Fun4H1)in to pieces , the action of this enzyme a specific places.

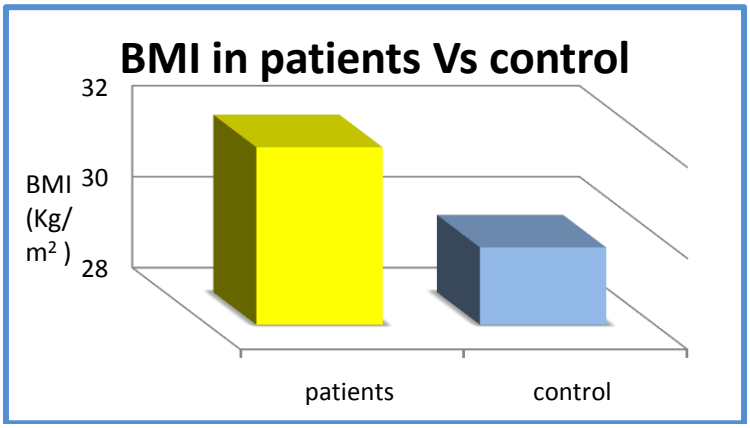
III. RESULTS

The results were carried out to indicate the relationships between the diabetes mellitustype2 (T2DM) patients and the genotyping of OGG1and measurement of some clinical parameters in patients group and healthy control group.

Table (1):BMI for the patients group and control group

BMI	Patient (n=60)	Control (n=40)
Mean ±SD (in Kg/m^2)	31.9±1.3	29.7±0.9
Normal (<25)	13(22%)	3(8%)
over weight (25- 29.9)	19(32%)	16(40%)
(Obese >30)	20(33%)	21(52%)
Morbidly obese >40	8(13%)	0(0%)

According to the BMI categories of the patients the results have shown (22%)normal, (32%)over weight, (33%) obese and (13%)morbidly obese. The result show's higher of BMI in patients (31.9±1.3 Kg/m^2)but not reach to level of significant(1.33) than of the control group (29.7±0.9), as shown in table (1)and figure (1)

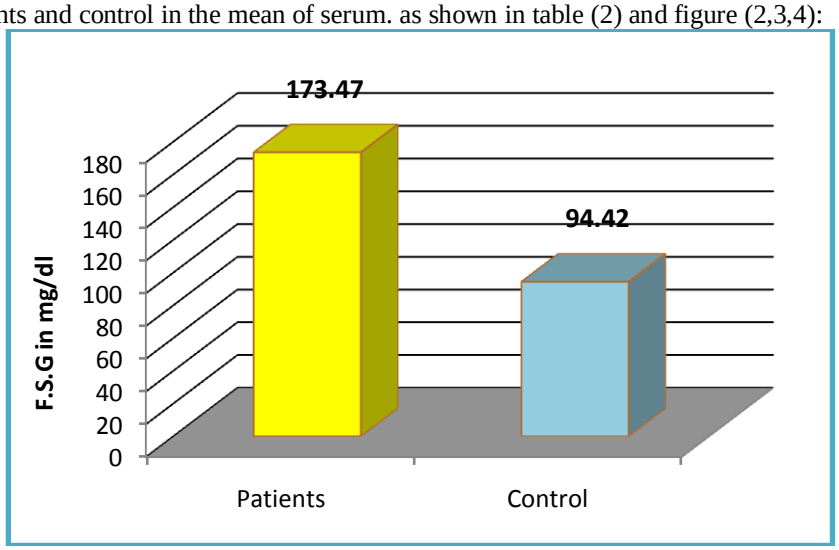


Figure(3-1): The mean of BMI for patients group and control group

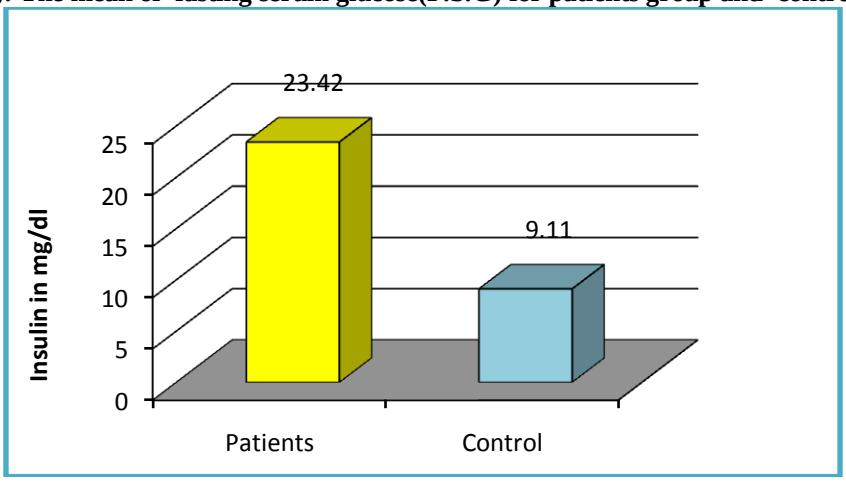
Table(2): the (mean ± SE) of (F.S.G , insulin, levels and HOMO(IR)) in patients and control group.

	F.S.G(mg/dl)	Insulin(mg/dl)	HOMO (IR)
Patient(n=60) mean ± SE	173.47 ± 8.60	23.42 ± 2.24	11.79 ± 1.69
Control(n=40) mean ± SE	23.42 ± 2.24	9.11 ± 0.54	2.13 ± 0.13
LSD value	11.79 ± 1.69	5.524 **	4.132 **
P-value	0.0001	0.0001	0.0001

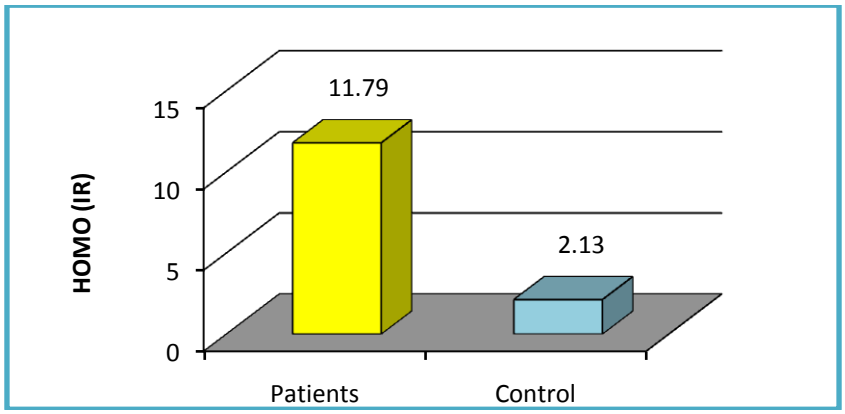
There was a significant difference (p=0.0001) in table (2) between (F.B.G ,insulin resistance and HOMO insulin) for patients and control in the mean of serum. as shown in table (2) and figure (2,3,4):



Figure(2): The mean of fasting serum glucose(F.S.G) for patients group and control group .



Figure(3): The mean of Serum Insulin for patients group and control group .



Figure(4): The mean of Insulin Resistance(HOMA IR) for patients group and control group .

Table(3):Comparer(mean ± SE) between patients and control in lipid profile

Parameters / Group	Cholesterol	Triglyceride	HDL	LDL	VLDL
Patient(n=60) mean ± SE	194.01 ± 6.41	154.07 ± 11.42	39.31 ± 2.57	123.89± 6.45	30.81 ± 2.11
Control(n=40) mean ± SE	160.04 ± 3.66	107.02 ± 6.57	37.35 ± 1.21	99.82 ± 5.29	22.87 ± 1.41
LSD value	1.984 NS	29.77 **	6.578 NS	16.58 **	5.63 **
P-value	0.851	0.0001	0.556	0.0019	0.0001
** (P<0.01), NS: Non-significant					

The results in table (3) show a significant in the LDL , TG, VLDL levels for patients comprising with control group but there was non-significantly (p=0.556) in the mean of HDL levels in patients than that healthy control group, also there was non-significantly(p= 0.851)in the mean of the cholesterol levels in patients comprising with control group, as shown in figure (5):

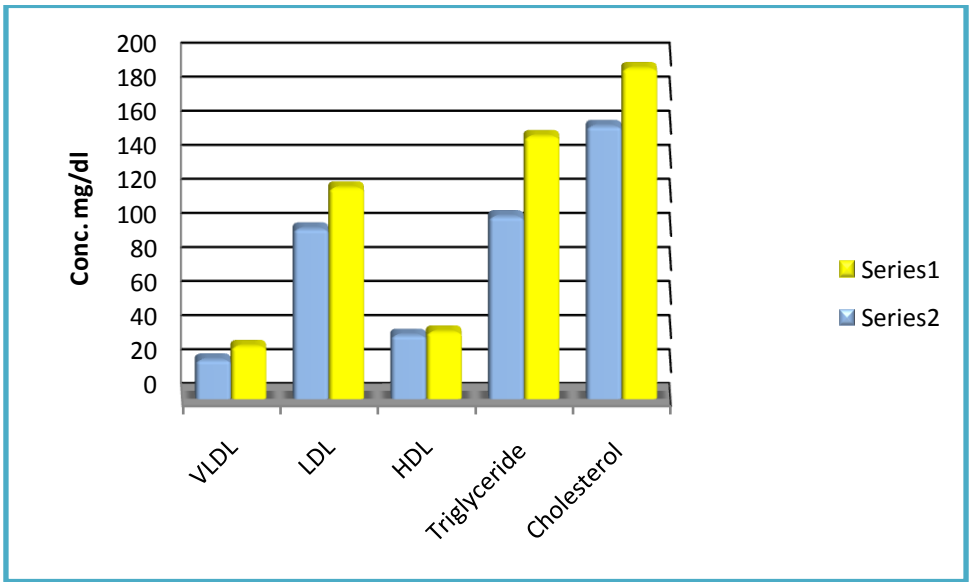


Figure (5): the means of lipid profile for patients group and control group .

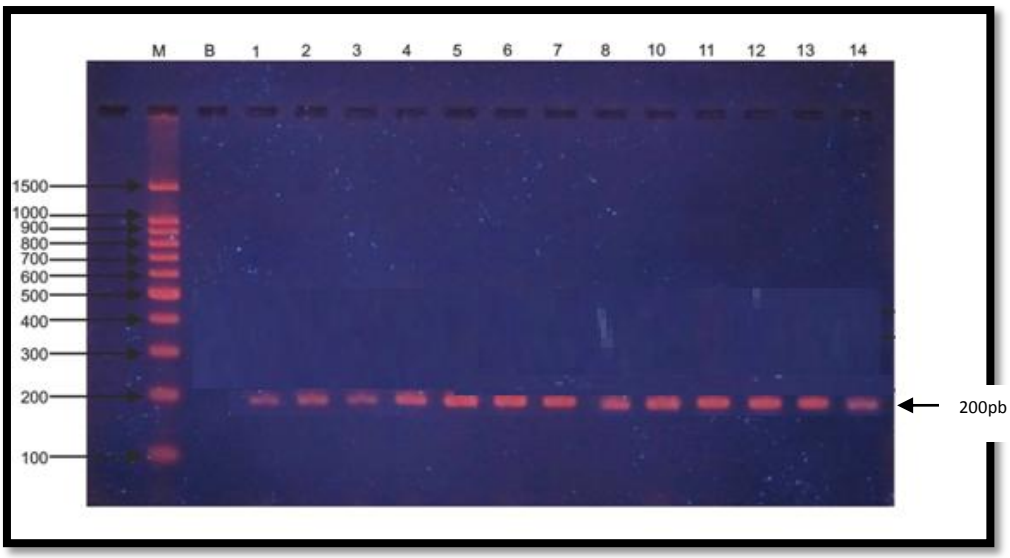
Table (4)A: Genotype distribution of OGG1 gene A/G polymorphism in healthy control and diabetic mellitus type 2 patients:

OGG1 Polymorphism	Patients (n= 60)	Control (n= 40)
Ser/Ser(GG)	16 (26.67%)	28 (70.00%)
Ser/Cys(GC)	42 (70.00%)	12 (30.00%)
Cys/Cys(CC)	2 (3.33%)	0 (0.00%)
Chi-square value (χ^2)	11.834 **	12.092 **
**** (P<0.01).**		

Table (4)B: Allele frequency of Gene in patients and control

Allele	Patients	Control
G	0.62	0.85
A	0.38	0.15
Total	1 (100%)	1 (100%)

The frequency distribution of *OGG1* gene polymorphism was represented in table (3). Frequency of *OGG1 ser/ser* (homozygous) genotype showed (26.67%) in patients and (70 %) in control .The differences were significant (P≤0.01). while ser/cys genotype showed higher significantly in diabetes mellitus Type2 (70%) compared to controls (30%). The differences were significant. *OGG1 cys/cys* genotype frequency showed significantly elevated in the diabetes mellitus type2(3.33%) comprising to controls (0.00%).



Figure(6): A representive multiplex PCR analysis of OGG1 polymorphism. OGG1 genes PCR product resolved by (2%) agarose gel electrophoresis (1hr/70v). Lane M, DNA molecular weight marker. Lane B, negative control. Lane (1-14) is samples. A 200 pb is present only in those individuals containing the OGG1 gene.

Table (5): Genotype distribution of OGG1 gene in healthy control and diabetic mellitus type 2 patients [F.S.G, Insulin and HOMO(IR)]:

Groups OGG1 Polymorphism	No.	Patients (n=60) mean ± SE			No.	Control (n=40) mean ± SE		
		F.S.G	Insulin	HOMO (IR)		F.S.G	Insulin	HOMO (IR)
GG	16	160.9± 14.17	21.55 ± 3.15	9.82 ± 2.47	28	96.04± 1.18	9.46± 0.65	2.24± 0.16
GC	42	179.2± 10.82	24.31 ± 2.93	12.64 ± 2.21	12	90.6± 1.98	8.26± 0.9	1.85± 0.21
CC	2	154± 61	19.80± 14.8	9.75 ± 8.60	0	0.00	0.00	0.00
P-value	---	0.601	0.831	0.753		N.S	N.S	N.S

Table (6): Genotype distribution of OGG1 gene for patients in lipid profile

Polymorphism	No.	Mean ± SE				
		Cholesterol	Triglyceride	HDL	LDL	VLDL
GG	16	149.81 ± 14.85	174.62 ± 19.84	37.99 ± 2.63	84.68 ± 9.69	35.18 ± 3.97
GC	42	183.64 ± 6.75	191.47 ± 13.83	40.45 ± 3.52	101.97 ± 6.50	36.38 ± 2.47
CC	2	176.50 ± 20.50	254.00 ± 114.00	26.00 ± 6.00	99.50 ± 8.50	51.00 ± 23.0
P-value	---	0.065	0.471	0.586	0.361	0.441
NS: Non-significant.						

Table (7): Genotype distribution of OGG1 gene for control in lipid profile

Polymorphism	No.	Mean ± SE				
		Cholesterol	Triglyceride	HDL	LDL	VLDL
GG	16	165.1±4.3	104.8± 7.6	34.28±1.13	124± 9.17	23.01± 1.7
GC	42	190.8± 3.24	112.17± 13.2	44.5± 1.81	123.8 ± 3.23	22.56 ± 2.68
CC	2	0.00	0.00	0.00	0.00	0.00
P-value	---	0.068	0.372 NS	0.42 NS	0.87 NS	0.57 NS
NS: Non-significant.						

The results showed the mean of lipid profile that of means were statistically not significant (p>0.05).

IV. DISCUSSION

In table (2) there was a significant increase (p= 0.0001) in the mean of fasting serum glucose levels of patients compared to control , this agrees with previous studies done by (*Walla 2015 , Baydaa et al 2013 , Al-Shamma et al 2013*)[9]. Vats et al.,(2013)[10],found that there were significant difference in mean of FBG in patients with T2DM as compared to control group in Indian population .

Chronic hyperglycemia is a key factor for the induction of vascular disease in type 2 diabetes patient. Increasing in intracellular glucose drives mitochondria, induced oxidative stress and the synthesis of advanced glycation end products with resultant alterations in the regulation of vascular wall homeostasis by endothelial cells(*Brown, 2008*)[11].

In table (3) there was significantly decrease (p= o.ooo1)in triglyceride levels and VLDL levels of diabetic group compared to healthy control . These results agree with other studies (*Abdulhussain et al 2012*)[12]. In diabetes mellitus, abnormally increased levels of lipids and lipid peroxides in plasma may be due to the abnormal lipid metabolism . Patients with type 2 diabetes frequently have an abnormal blood lipid profile consisting of moderately elevated LDL-C, moderately decreased HDL-C, and high TC and triglycerides . Thus, inadequate levels of HDL-C, in conjunction with more atherogenic forms of LDL-C may contribute to atherogenesis . The results of the present study showed approximately a two-fold increase in serum levels of all lipid fractions for diabetic group when compared with control group.

There was a non significant difference in the mean of cholesterol (p=0.851) and HDL(p=0.556) level were compared with control group, this result agree with previous studies done by (Guerra 2000)[13]. Bid *et al.*, (2010) [14]showed that there was no significant difference between patients and controls with lipid profile in Egyptian and Indian population, demonstrated the relation of elevation lipid profile and type 2 diabetes mellites in Egyptian and Indian population.

This variation in prevalence may be due to differences in BMI and possibly genetic variation and the results were in agreement with the findings of many similar study (Amin-ul-Haq. 2006 ,18)[15].

khan et al [16]reported that no significant differences were observed in the levels of serum TC, LDL, HDL. The most common abnormality found in diabetes is high triglycerides with Low HDL, and although if low density lipoprotein (LDL) might not be higher, its metabolism is abnormal .There is also an inverse relationship between serum levels of HDL-C and triglycerides in diabetic patients; with low serum HDL-C levels possible representing an independent risk factor for cardiovascular disease. Another studies, Nesto RW. (2005) showed that Insulin resistance, which is central to the metabolic syndrome and type 2 diabetes mellitus, leads to high levels of very low-density lipoprotein (VLDL),which contain a high concentration of triglycerides, resulting in high serum triglyceride level and low serum HDL-C levels .But Mumtaz A. S.(2010)described lipid abnormalities in diabetic patients with type 2 are as increased serum triglycerides, very low density lipoproteins, low density and lowering of high of high density lipoproteins. Several studies have reported an increased susceptibility to lipid peroxidation in patients with diabetes mellitus. The generation of free radicals may lead to lipid peroxidation and the formation of several types of damage in diabetes mellitus(Abdulhussain J. M. ,2012).[17]

The observed Ser/Ser , Ser/Cys and Cys/Cys genotype frequencies were 0.2667, 0.700 and 0.333, respectively (Table 4 -A).

The serine (wild-type) and cysteine (variant) allele frequencies were 0.62 and 0.38, respectively. The genotype and allele frequencies obtained from diabetic patients did not differ significantly from those found in control subjects with the OGG1 Ser326Cys polymorphism.

Genetic alterations in *OGG1* are thought to influence the development of oxidative stress and thus contribute to the pathophysiology of many diseases including cancer. While many sequence variants within the *OGG1* gene have been identified, the main focus has been on the Ser(326)-Cys variant, since several epidemiological studies have associated the Ser(326)Cys polymorphism with many types of cancer including kidney, colon and lung cancer .(Farook Thameem, 2010)[18].Carriers of Cys/Cys were found to have lower OGG1 activity and impaired ability to repair 8-OHdG than the carriers of Ser/Ser allele, thus contributing to the cancer risk.(Lee AJ,2005). [19]

Recently, the Ser(326)Cys variant was reported to be associated with decreased insulin sensitivity in subjects with normal glucose tolerance suggesting that genetic alterations in OGG1 may contribute to insulin resistance and potentially T2DM (Wang CL,2006).[20]

According to this results of diabetes Type2 ; the hyperglycemia stimulates reactive oxygen species production and increases oxidative stress. Excessive generation of ROS such as hydrogen peroxide(H_2O_2), superoxide(O_2^-), and hydroxyl radical (OH^-)along with reactive nitrogen species (RNS) like nitric oxide oxidize DNA, protein and other cellular components due to their damage and individuals with low antioxidant capacity are at increased risk of infection withT2DM (Ramprasath*et al*., 2011).[(21)]

v. CONCLUSION

From the present study the following points can be concluded :

1. The hyperglycemia, insulin resistance , abnormal change of lipoprotein , all these parameters are associated with T2DM.
2. The wild OGG1 Ser/Ser is more prevalence in control than patients.
3. The variant OGG1 Ser/Cys is more prevalence in patients than control.
4. The mutant genotype Cys/Cys may be conserved in patients and further study needs to elucidate that.
5. OGG1 genotypes do not have an effect on blood lipids given exposure to T2DM.

V. RECOMMENDATIONS

1-Further studies of T2DM , large cohort number of patients must be included .

2- Further studies to evaluation the role of gene and different kinds of cancer disease and Type1 DM.

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