

## “Glutathione S transferase P 1 (GSTP1) genetic polymorphism and Type 2 Diabetes Mellitus (T2DM) in Libyan subjects”

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**Abstract:** Oxidant stress is one of the major outcome of diabetes mellitus). Glutathione-S-transferase (GST) is one of the key enzymes involved in neutralizing hyperglycemia induced generation of reactive oxygen species (ROS). Therefore, present study was aimed to evaluate the role of GST along with oxidative stress markers and their correlation in patients with Type 2 diabetes mellitus with and without nephropathy

**Patients and methods:** In the present study, a total of 67 Type2 Diabetes Mellitus (T2DM) Libyan patients and 26 healthy controls were recruited from the Benghazi Center for Diagnosis and Treatment of Diabetes, Benghazi, Libya.

Blood samples taken from them were subjected to measurements of HbA1c, total cholesterol, triglycerides, and HDL-c using an auto analyzer with enzymatic techniques. The data were analyzed using SPSS version 18, P values < 0.05 were considered to be statistically significant. DNA is extracted from whole blood samples by using QIAamp DNA Blood Mini Kit(250), then amplified the desired gene with the specific primers using PCR thermal cycler TC 5000 and digested the PCR products with restriction enzyme ALW26I overnight. The GSTP1Ile105Val genotype was identified by separation of digested products on 2.5% agarose gel electrophoresis.

**Results:** In the present study, diabetic patients had significantly higher fasting blood glucose, HbA1c, total cholesterol, triglycerides, and LDL-c, and lower HDL-c compared to control group.

Our results demonstrate that the frequency of the GSTP 1 ILeu105val allele was lesser in diabetic patients compared to control subjects.

**Conclusion:** The present study suggests that GSTP1Ile105Val may not play a significant role in the development and control of hyperglycemia and diabetes mellitus in Libyan subjects .

**Key words:** Type 2 Diabetes Mellitus (T2DM), Libya, Glutathione S transferase isoenzymes

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### I. Introduction

Type 2 Diabetes Mellitus (T2DM) is one of the major non communicable diseases that afflicts a sizeable population in Libya. It is known that hyperglycemia induces greater generation of Reactive oxygen species (ROS) which may cause many of the complications associated with T2DM. <sup>1</sup>

Glutathione-S-transferase (GSTs) isoenzymes play a key role in the biotransformation of ROS.. A member of the glutathione-S-transferase (GST) family, GSTP1, which is located at 11q13, helps in the biotransformation of electrophilic compounds by promoting conjugation with glutathione (G.SH). It has been reported that two genetic polymorphisms of GSTP1 gene involves exon 5 and exon 6 of the gene. Exon 5 is the major translatable region that is linked to enzyme activity. The genetic polymorphism reported in exon 5 at the site -313, results in polymorphism at codon 105, where an adenosine-to-guanidine (A > G) transition causes an Isoleucine -to-Valine substitution. <sup>2-5</sup>

There are many studies carried out to find out the association between GSTP1 gene polymorphism and T2DM<sup>6-13</sup> In this preliminary study an attempt has been made to assess the association of genetic polymorphism in the GSTP1 gene leading to the development of T2DM in Libyan diabetic patients.

### II. Materials and Methods:

67 patients recruited from Benghazi Center for Diagnosis and Treatment of Diabetes and 26 healthy control subjects were taken for the present study. Libya. Informed consents were obtained from the subjects enrolled for the study. The Institute Research Ethics Committee approved the study protocol.

Venous blood samples were drawn from normal control and diabetic patients after at least 10 hours fasting, and analyzed for blood glucose, HbA1c, total cholesterol, triacylglycerol, and HDL-c using an auto analyzer with enzymatic techniques. LDL cholesterol was calculated according to Friedwald equation using the routine methods. DNA is extracted from whole blood samples by using QIAamp DNA Blood Mini Kit(250), then amplified the desired gene with the specific primers using PCR thermal cycler TC 5000 and digested the PCR products with restriction enzyme ALW26I overnight. To detect the GSTP1Ile105Val genotype the digested gene DNA products are subjected to 2.5% agarose gel electrophoresis and analysed.

**Genomic analyses:**

Genomic DNA was extracted from whole EDTA blood from peripheral mononuclear cells using Genejet™ column as described in the genomic DNA purification kit supplied by (using QIAamp DNA Blood Mini Kit (Qiagen, Germany): Integrity and purity of DNA extracted was tested by electrophoresis using 1% agar gel. The A313G GST PI was determined using PCR –RFLP according the method described by Harris etal.

In brief, 12 ul ( 50-150ng) of genomic DNA was mixed with 0.2 ml with 0.2 ul of each primer  
 Forward (5’ –ACCCCAGGGCTCTATGGGAA 3-‘)  
 Reverse ( 5’-TGAGGGCACAAGAAGCCCCT-3’)  
 in a sterile eppendorf tube with the primers concentration being 5pmol per reaction tube,  
 12.5 ul Dream Taq™ PCR Masler Mix ( 2 x) and completed to a final reaction volume of 25 ul using a nuclease free sterile water.

The conditions that was standardized for the thermo cycler was a 5 min initial denaturation phase 95°C, followed by 30 cycles of denaturation ( 94°C, 30 s), annealing ( 55°C, 30 s) and extension ( 72°C, 30 s) and a final elongation step of 5 min at 72°C.

The resulting 176 bp fragment generated by PCT was separated on a 2% agar gel and visualized using Ethidium Bromide staining to confirm its presence.

The PCR product obtained by using restriction endonuclease Alw 261 The RFLP obtained was digested in a 1.5 ml sterile eppendorf tube having 5ul of PCR product, 0.5 ul of the enzyme, 1 ul of enzyme buffer and a final volume of 15 ul made up with nuclease free sterile water.

The mixture was incubated at 37°C for 1 hr using a thermo mixer. The digested products were separated electrophoretically on a 2% agar gel. The three patterns obtained are :

A single undigested ban at 176 base pairs indicating the presence of a homozygote AA allele ( a wild type).The presence of restriction site resulting in 2 fragments ( 91 and 85 base pairs ) indicating the presence of GG homozygote mutant allele and lastly 3 bands ( 176, 91, 85 base pairs ) indicating the presence of A/G heterozygote mutant allele.

**Statistical analyses:**

The data were analyzed using SPSS version 18, P values < 0.05 were considered to be statistically significant .To compare observed frequencies of different GSTPi, genotypes among all subjects chi-square test was carried out .

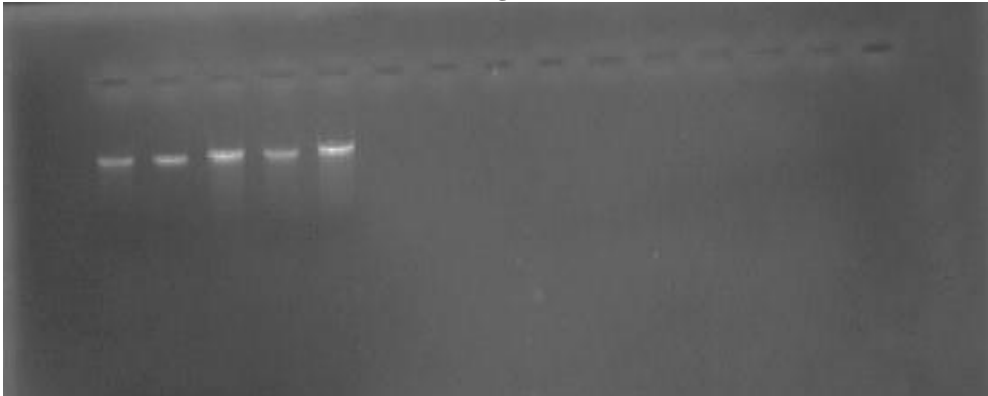
**III. Results**

**Table.1.** Biochemical Parameters Measured in the Libyan subjects

Parameter	Controls	Diabetes	P Value
BMI ( kg/m <sup>2</sup> )	24.40 ± 0.60	29.80±1.70	0.000
Blood Pressure:Systolic mm.Hg	113.45±5.50	117.20±9.20	0.010
Diastolic mm.Hg	76.05±2.70	82.45±5.50	0.010
Fasting Blood glucose(mg/dL)	89.70±6.80	163.50±64.50	0.000
Glycated Hemoglobin(%)	5.10±0.40	7.80±1.80	0.000
Total Cholesterol (mg/dL)	168.10±24.50	182.50±34.50	0.027
LDL-Cholesterol (mg/dL)	92.30±19.40	107.00±34.50	0.016
HDL Cholesterol (mg/dL)	67.15±10.42	46.35±9.20	0.000
Triglycerides(mg/dL)	119.75±43.10	144.00±69.20	0.040

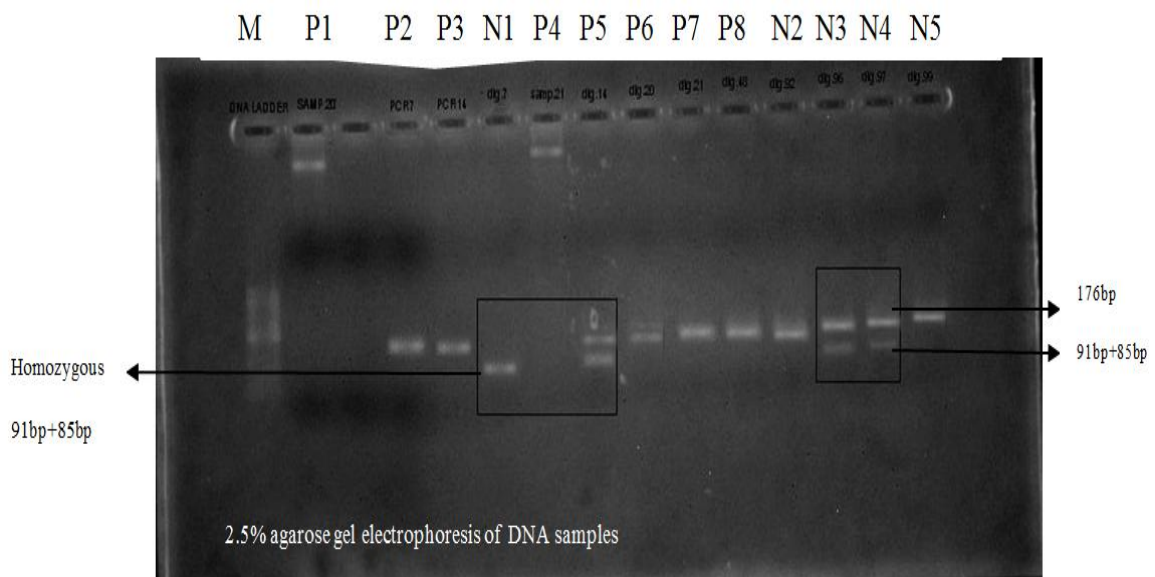
P<0.05 is considered as statistically significant

**Fig.1**



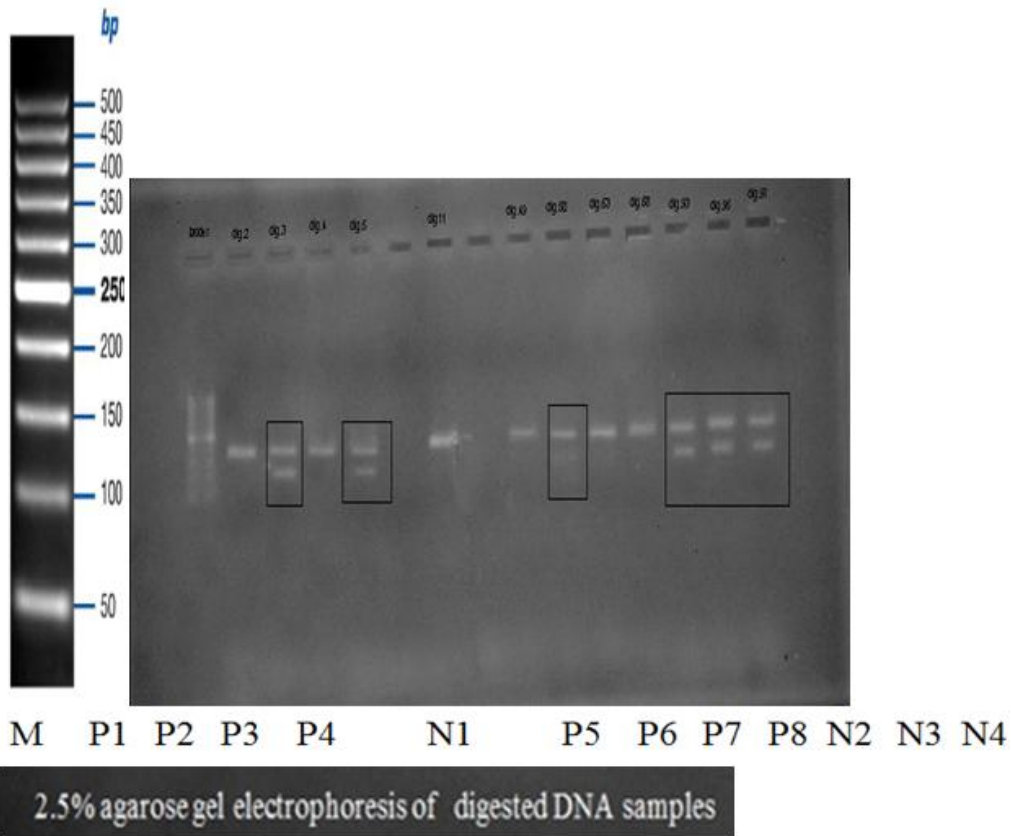
## Agarose gel electrophoresis of genomic DNA samples

**Fig.2**



P1 and P4 showing DNA sample after extraction, P2 and P3 showing PCR product of sample, P5, N3 and N4 showing PCR product of samples after digestion overnight with ALW261 and samples are heterozygous carrying A allele (176bp) and G allele which cuts by ALW261 into two bands one with 91 bp and other with 85bp, P6, P7, P8, N2 and N5 showing PCR products of samples after digestion overnight with ALW261 and samples are homozygous carrying two normal A alleles, while N1 showing PCR product of sample after digestion overnight with ALW261 and sample is homozygous carrying two polymorphic G allele.

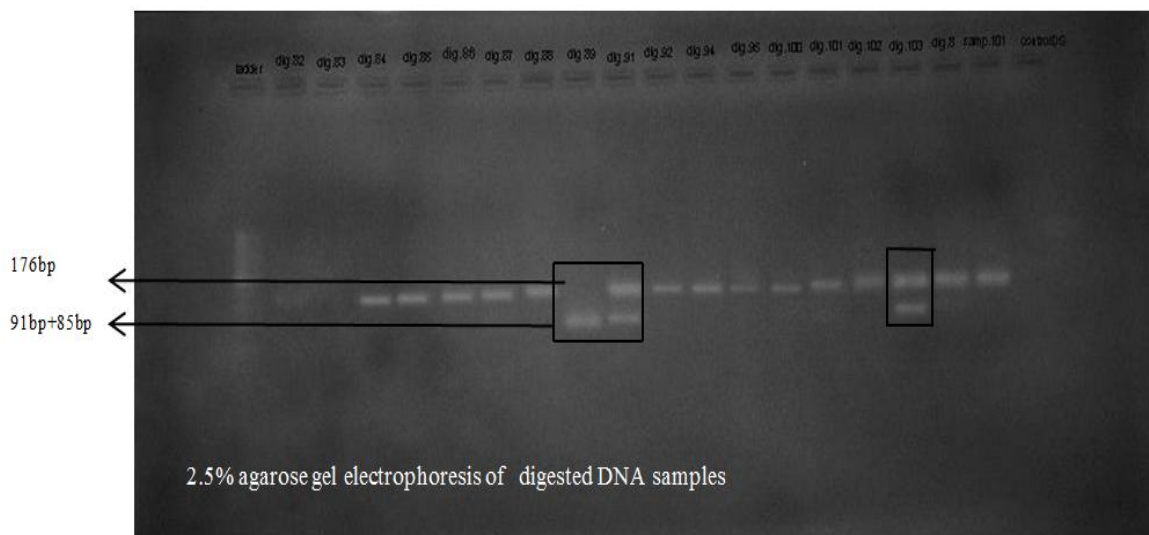
**Fig.3.**



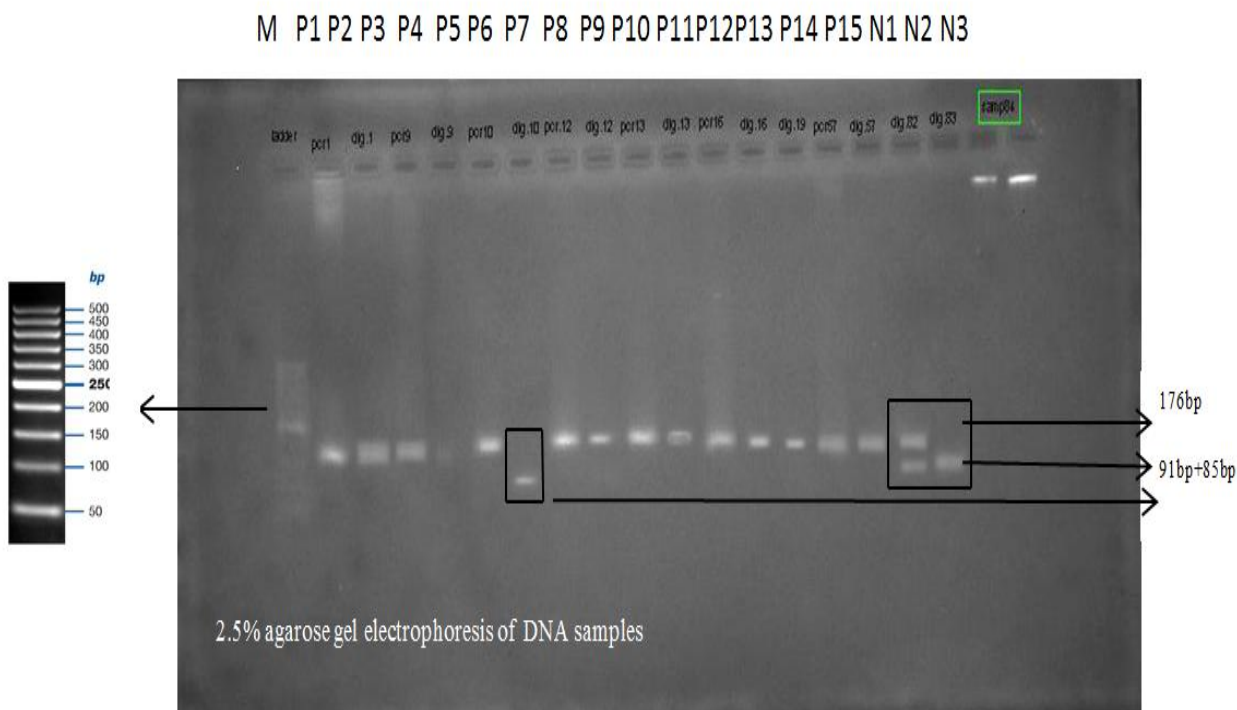
P2,P4,P6, N2,N3 and N4 showing PCR product of samples after digestion overnight with ALW261 and samples are heterozygous carrying A allele(176bp) and G allele which cuts by ALW261 into two bands one with 91 bp and other with 85bp. P1, P3, P5,P7, P8 and N1 showing PCR products of samples after digestion overnight with ALW261 and samples are homozygous carrying two normal A alleles.

**Fig.4.**

M N1 N2 N3 N4 N5 N6 N7 N8 N9 N10 N11N12 N13 N14 N15N16N17N18 C



N9 and N16 showing PCR product of samples after digestion overnight with ALW261 and samples are heterozygous carrying A allele(176bp) and G allele which cuts by ALW261 into two bands one with 91 bp and other with 85bp. N1,N2,N3,N4,N5,N6,N7,N10,N11,N12,N13,N14,N15,N17 and N18 showing PCR products of samples after digestion overnight with ALW261 and samples are homozygous carrying two normal A alleles, while N8 showing PCR product of sample after digestion overnight with ALW261 and sample is homozygous carrying two polymorphic G allele.



**Fig.5.** Electrophoretic pattern of DNA samples after digestion with

ALW261. (M = marker N = normal P = patient C = control). P1,P3,P5,P7,P9,P11 and P14 showing PCR product of samples and samples are homozygous carrying two normal A alleles. N1 showing PCR product of sample after digestion overnight with ALW261 and sample is heterozygous carrying A allele(176bp) and G allele which cuts by ALW261 into two bands one with 91 bp and other with 85bp. P6 and N2 showing PCR products of samples after digestion overnight with ALW261 and samples are homozygous carrying two normal A alleles, while N3 showing sample after extraction.

**Table.2.** Genetic Polymorphism seen in the Libyan Subjects studied

Genotype	patient	control
A allele, G allele	3 female and 1 male	2 female and 4 male
G allele, G allele	1male	2 female and 1 male
A allele, A allele	28 female and 34 male	6 female and 11 male

#### IV. Results and Discussion

- 1) Single undigested band at 176 base pairs indicating the presence of a homozygote AA allele (wild type),
- 2) The presence of a restriction site resulting in two fragments (91 and 85 base pairs) indicating the presence of a GG homozygote mutant allele,
- 3) And lastly three bands (176, 91 and 85 base pairs) indicating the presence of an A/G heterozygote mutant allele

G allele homozygosity(val/val) was detected in 3 samples of control subjects (including 2 female and 1 male), whereas 1 male patient was found to be homozygous for G allele(val/val)

6 of control subjects (including 2 female and 4 male) and 4 of patients(including 3 female and 1 male) were heterozygous for the G allele(Ile/val).

Our preliminary results are in agreement with the findings reported for Turkish and Japanese population, respectively suggesting that GSTP1 Ile105Val polymorphism may not play a significant role in the development of T2DM<sup>7-8</sup>this was in contrast to the studies conducted in certain Indian population which shows that GSTP1 Ile105Val polymorphism may play role in the development of diabetes mellitus.<sup>9-10</sup>

These contradictory results may be due to different ethnicity, geographical and environmental factors apart from differences in research methodologies adopted.<sup>11</sup>

This is the first preliminary study carried out to determine the association of T2DM with GSTP1Ile105Val gene polymorphism in the Libyan population. Our results showed that GSTP1 Ile/Val genotype may not play a significant role in the development of T2DM.

The findings presented here are preliminary because of the small number of subjects and that the study requires confirmation in a separate, larger cohort.

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