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DETERMINATION OF POLYMORPHISM OF GLUTATHIONE S TRANSFERASE (GST) IN THE IRAQI (DIABETIC AND NON-DIABETIC) ACROMEGALIC PATIENTS

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ABSTRACT : In Iraqi patients with acromegaly the present investigation included 40 control group and 70 patients with acromegaly divided 35 patients with Diabetic while another 35 patients without Diabetic, with ages between (29-72) years for the identification of GST activity polymorphisms by present and absent GSTM1, GSTT1, and PCR-RFLP, enzymatic digestions were carried out using BsmAI (Biolabs. England, UK) for GSTP1b and AciI (Biolabs, England, UK) for GSTP1c.the association GST Activity with GST genotype were investigated in a cohort of Iraq acromegaly patients comparing with the healthy control group. The results show a non-significant change in GSTP1b gene in both groups, while show high significantly in GSTP1c in diabetic and non-diabetic acromegaly groups.

Key words : GSTM1, GSTT1, GSTP1, acromegaly, diabetic, non- diabetic.

INTRODUCTION

Acromegaly is a disease of impaired somatic development and distorted ratios due to over-expression of growth hormone (GH) and growth factor I (IGF-I) like insulin (Evans *et al*, 1992). Pierre *et al* (2008) first identified the clinical features of acromegaly over 120 years ago, are caused by over-expression of GH by pituitary adenoma, whereas excessive GH and Insulin-like growth factor in adulthood contribute to acromegaly (Brzana *et al*, 2012). Diabetes mellitus is a subset from metabolic disorders known ashy per glycemia caused by the Secretion of pancreatic insulin, or insulin development, or both. GSTs are one of the defense systems against harmful oxidative stress effects. Glutathione S-transferases (GSTs) are the greatest group from phase II isoenzymes known to detoxify a variety from electrophilic compounds, inclusive carcinogens, environmental toxins and ROS intracellular damage (Yalin *et al*, 2007). Through combining them with glutathione, detoxification through GSTs is achieved. GSTs thus play a major role in antioxidant defense mechanisms and as cellular anti mutagens (Hayes *et al*, 2005; Almamoori *et al*, 2019). Collectively the 4% of all soluble proteins in the liver are human soluble GSTs, dimeric proteins of 50 KDa, both of which are subunits of the same GST class (Oakley, 2011). GST Five mu(ì) GST genes are GSTM1, GSTM2, GSTM3 GSTM4 and GSTM5 (SHERRATT *et al*, 1997), and the GSTP1 gene is present in three wild-type alleles (GSTP1 a) and two separate alleles (GSTP1b, GSTP1c). GSTP1 single nucleotide polymorphism (SNP) on exon 5 is caused by a guanine base replacing adenine at 105 positions leading to valine substitution with isoleucine amino acid such substitution due to the appearance from a new allele with alteration in specific substratum activity compared to wild-type allele (Pearson *et al*, 1993). GSTP1 gene polymorphism decreases the ability to conjugate glutathione electrophile material and thus sensitize cells to damage that caused by free radicals. The GSTP1 form was associated with the specific cancer risk, diabetic, renal failure (Zimniak *et al*, 1994) and heart disease (Rodríguez *et al*, 2014).

MATERIALS AND METHODS

In this study, the patient group consisted of selected 40 control and 70patients (35 diabetic and 35 non-diabetic groups) diagnosed with acromegaly in the National Diabetes Center, Mustansiriyah University during the period from May to December 2018 and the age range (29-72 years) some information has been taken from patients and healthy people such as age, sex, region, and infection renal failure, sugar, smoking or not smoking and

blood pressure.

DNA extraction

Genomic DNA was extracted from the whole blood cells by using Promega Wizard™ Genomic DNA Purification Kit.

The Primers

Primers sequences used to amplify the GSTM, GSTT, GSTP1b and GSTP1c (Rodríguez *et al*, 2014) as seen in Table 1.

PCR Working solution

The optimization of the polymerase chain reaction was accomplished after several trials. The PCR reaction mix for a DNA sample was prepared by mixing the following components as shown in Table 2.

PCR-RFLP of GSTP1b and GSTP1c genes

Enzymatic digestions were made using BsmAI (Biolabs. England, UK) for P1b (exon 5) and AciI (Biolabs, England, UK) for P1c (exon 6) for the identification of P1 polymorphisms. The polymorphism recognition was based on the existence of DNA fragments of different sizes. For exon 5, 176 bp, 91 bp and 85 bp fragments correspond to heterozygote P1b while fragments of 91 bp and 85 bp P1b correspond to homozygote and a fragment of 176 bp corresponds to wild P1a.As for exon 6 polymorphisms, the fragment of 332 bp corresponds to homozygote P1c; three fragments, 332bp, 174 bp and 158 bp correspond to heterozygote P1c and two fragments, 158 bp and 174 bp correspond to wild P1a.

Table 1: The Primers used in study.

Primers	Sequence	Amplicon
GSTM1	R-5'-GAACTCCCTGAAAAGCTAAAGC-3' F-5'-GTTGGGCTCAAATATACGGTGG-3'	215 bp
GSTT1	F-5'-TCACCGGATCATGGCCAGCA-3' R-5'-TTCCTTACTGGTCCTCACATCTC-3	480 bp
CYP1A1	F-5'-GAACTGCCACTTCAGCTGTCT-3' R-5'-CAGCTGCATTTGGAAGTGCTC-3'	312 bp
GSTP1b	F-5'-ACCCCAGGGCTCTATGGGAA-3' R-5'-TGAGGGCACAAGAAGCCCCT-3'	176 bp
GSTP1c	F-5-'TGGCAGCTGAAGTGGACAGGATT-3' R-5'-ATGGCTCACACCTGTGTCCATCT-3'	332 bp

Table 2 : The mixture of working solution.

PCR components	Amount
Go Taq Start Green Master Mix	12.5µL
Primer Forward	1µL
Primer Reverse	1µL
Distilled water (D.w)	8.5µL
DNA template	2µL
Final volume	25µL

RESULTS

GSTM1, GSTT1 and CYP1A1 genetic polymorphism

For each PCR reaction, two µl of genomic DNA have been used. The presence of GSTM1and GSTT1 genes was simultaneously analyzed using a traditional PCR protocol.

The product size bands of 215bp and 480bp respectively compared to the control gene CYP1A1 is shown in Fig. 1.

GSTP1b and GSTP1c polymorphism

GSTP1 b and GSTP1c polymorphisms were genotyped utilizing a PCR method through confrontation with primers as seen in Figs. 2, 3.

In the non-diabetic acromegalic patients' group show, a non-significant change was observed in the comparison between the patient and the control groups with Non-diabetic in GSTP 1b gene. The allele aa shows (P>0.492), while the allele ab shows (P>0.647) on the other hand, the allele bb shows a non-significant (p>0.467). To determine odds ratios and 95% confidence intervals for non-diabetic acromegalic group, various comparisons of genotypes as well as a vs. b alleles were analyzed as shown in Table 3. The odds ratio of non-diabetic acromegaly group of GSTP1b gene specific subpopulations for aa vs. ab and bb genotypes. The odds ratio for compared control with patients group for aa subjects was 0.7 (95% CI,0.28 to 1.72), ab subjects was1.28(95%CI, 0.52to3.14) and bb subjects was 3.52(95%CI, 0.14 to 85.67).

While GSTP1c gene the result shows high significantly in the genotype aa shows (P<0.0002), while the genotype ac shows (P<0.001) and the genotype cc (p<0.001). The odds ratio of non-diabetic acromegaly group of GSTP 1c gene specific subpopulations for aa vs. ac and cc genotypes. The odds ratio for compared control with patients group for aa subjects was 0.05 (95% CI, 0.012 to 0.248), ab subjects was 32.15 (95% CI, 6.77 to 152.74) and bb subjects was 0.24(95% CI, 0.11 to 0.51). Summarizes

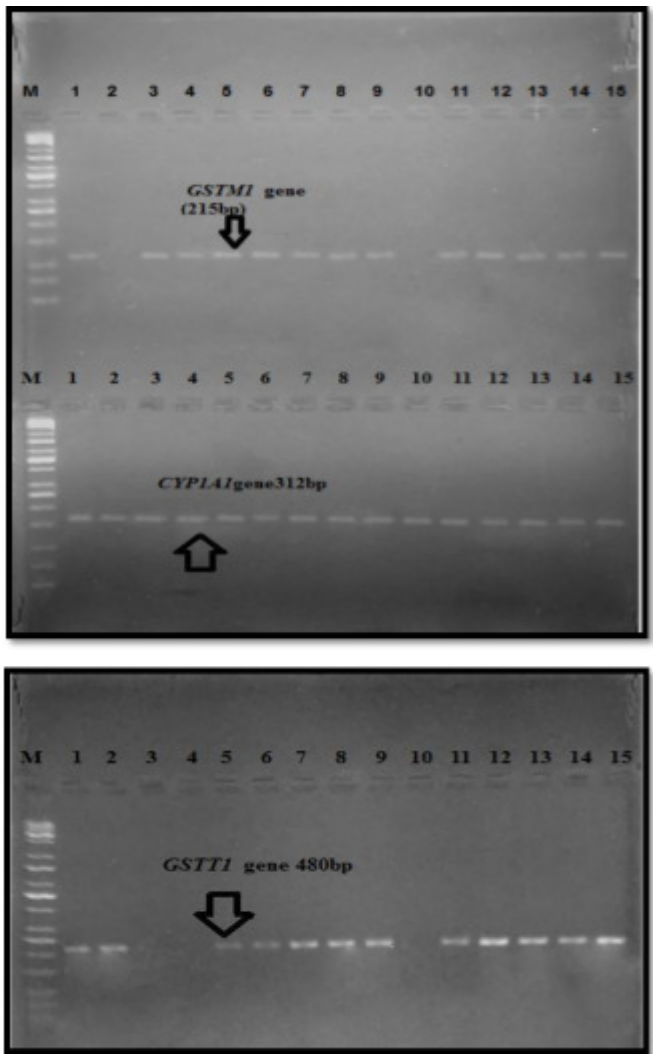

Fig. 1 : Agarose gel electrophoresis for partial *GSTM1,CYP1A1* and *GSTT1* genes fractionated by electrophoresis on a 2% agarosegel (60min., 1V/cm, 1X TBE) staining with EB and visualized under U.V. light Lane: 1 (M: 100bp ladder).

positive resultsof allele of RFLP in the sample study (patients and control). The result shows significant changes in GSTP1c when comparing observed and expected, this is a departure to the equation Hardy-Weinberg, while GSTP1b gene shows non-significant changes when comparing between observed and expected, this is not departure to the equation Hardy-Weinberg.

In diabetic group when comparison between the diabetic patient and the control groups GSTP 1b gene. The genotype aa shows (P>1.00), while the genotype ab shows (P>0.35) show anon-significant change, the genotype bb shows significant (p<0.04). To determine odds ratios and 95% confidence intervals for diabetic acromegaly group, various comparisons of genotypes as well as a vs. b alleles were analyzed as shown in Table 2. The odds ratio for compared control with patients group

Table 3 : Genotype distribution of *GSTP 1b & GSTP 1c* genes in healthy control and non-diabetic acromegaly patient.

Gene	Genotypes	Patients No. (35)		Control No. (40)		Observed	Percentage	Expecte d	Per centage	H-W	P value	O R	(95% CI)
		No.	%	No.	%								
GSTP1b	aa	17	48.57	23	57.5	17	48.57	18.57857	53.0816	0.177	0.492	0.7	0.28 - 1.72
	ab	17	48.57	17	42.5	17	48.57	13.84286	39.551		0.647	1.28	0.52 - 3.14
	bb	1	2.86	0	0	1	2.86	2.578571	7.36735		0.467	3.52	0.14 - 85.67
GSTP1c	aa	0	0.001	0	0	0	0	3.45714	9.87755	0.006	0.0002	0.05	0.012-0.248
	ac	22	62.86	38	95	22	62.86	15.0857	43.102		0.001	32.15	6.77 -152.74
	cc	13	37.14	2	5	13	37.14	16.4571	47.0204		0.001	0.24	0.11 - 0.51

Significant $p \leq 0.01, p \leq 0.05$, OR = Odd ratio, CI, confidence interval.

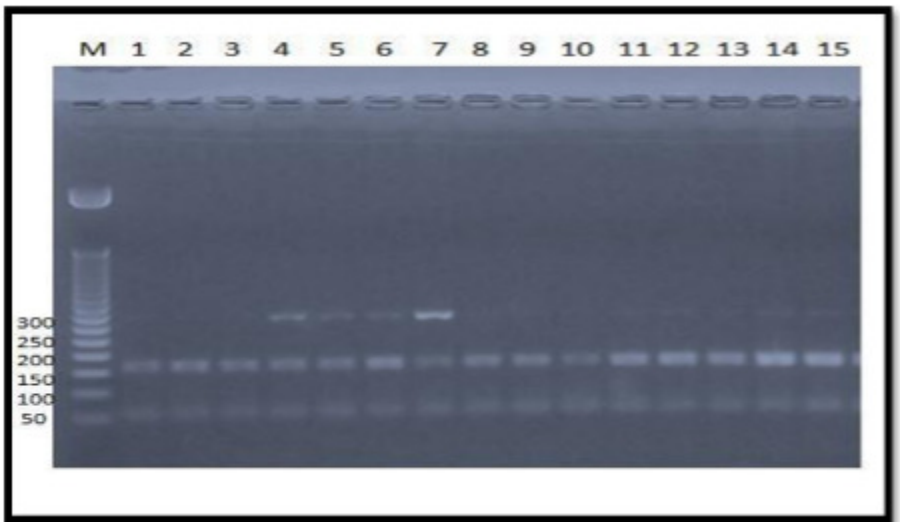

Fig. 2 : Agarose gel electrophoresis for lane 1-15 represented GSTP1c(332bp) PCR product digested with AciI restriction enzyme. Lane: 1 (M: 50bp ladder).

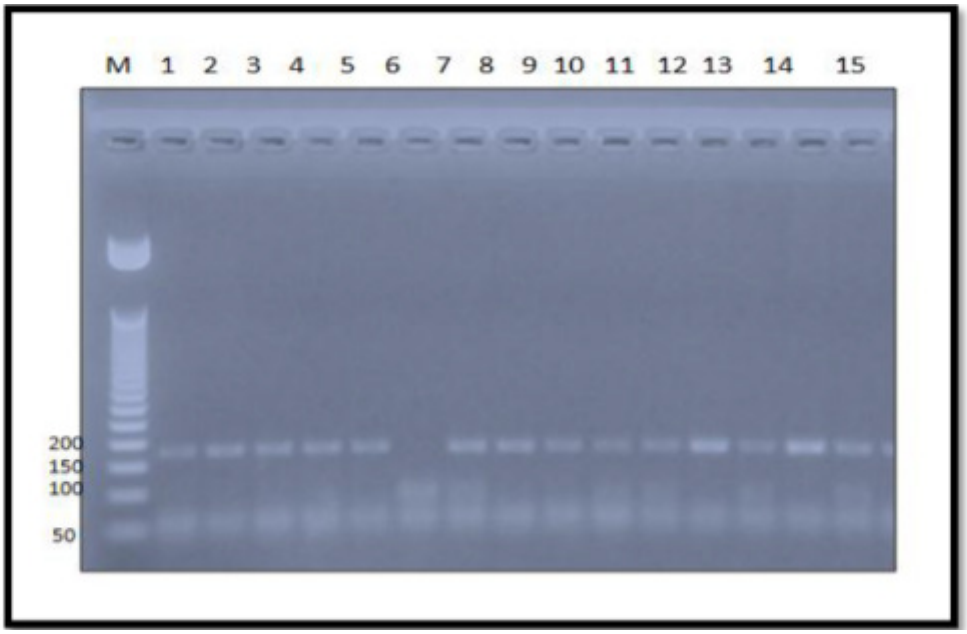

Fig. 3 : Agarose gel electrophoresis for lane 1-15 represented GSTP1b partial gene amplicon size 176bp PCR product digested with BsmAI (Biolabs, England, UK) restriction enzyme. Lane: 1 (M: 50bp ladder).

for aa subjects was 0.99 (95%CI, 0.40to2.44), ab subjects was 0.62 (95%CI, 0.24 to 1.58) and bb subjects was 11.57 (95% CI, 0.62 to 214.92). While, GSTP 1c gene the result shows a non-significant change in the allele aa shows (P>1.00), while the allele ac shows (P<0.001) and the allele cc (p<0.001) high significantly. The odds ratios of diabetic acromegaly group of GSTP 1c gene specific subpopulations for aa vs. ab and bb genotypes. The odds ratio for compared control with patients group for aa subjects was 0.37(95%CI, 0.02 to 9.00), ac subjects was 20.12 (95%CI, 4.28 to 94.65) and cc subjects was 0.05(95% CI, 0.01 to 0.23). Summarizes positive results of allele of RFLP in the sample study (patients and control). The result shows significant changes in GSTP 1c when comparing between observed and expected, this is a departure to the equation Hardy-Weinberg, while GSTP 1b gene shows a non-significant changes when comparing between observed and expected, this is not departure to the equation Hardy-Weinberg.

DISCUSSION

Acromegaly-diagnosed diabetes mellitus is associated with increased reactive oxygen species production and reduced Defenses against antioxidants. This leads to oxidative stress that in diabetes and its complications a specific pathogenic factor was considered. Studies have shown that people with diabetes (Palmer *et al*, 2003). Reduced antioxidant strength is associated with increased diabetes risk (Ha, 1995; Lehmann *et al*, 2000). GST family

Table 4 : Genotype distribution of *GSTP 1b & GSTP 1c* genes in healthy control and diabetic acromegaly patients.

Gene	Genotypes	Patients No. (35)		Control No. (40)		Observed	Percentage	Expecte d	Per centage	H-W	P value	O R	(95% CI)
		No.	%	No.	%								
GSTP 1b	aa	20	57.14	23	57.5	20	57.14	18.57857	53.0816	0.22	1.00	0.99	0.40 - 2.44
	ab	11	31.43	17	42.5	11	31.43	13.84286	39.551		0.35	0.62	0.24 - 1.58
	bb	4	11.43	0	0	4	11.43	2.578571	7.36735		0.04	11.57	0.62 - 214.92
GSTP 1c	aa	0	0.00	0	0	0	0	2.31429	6.61224	0.04	1.00	0.37	0.02 - 9.00
	ac	18	51.43	2	5	18	51.43	13.3714	38.2041		0.001	20.12	4.28 - 94.65
	cc	17	48.57	38	95	17	48.57	19.3143	55.1837		0.001	0.05	0.01 - 0.23

Significant $p \leq 0.01$, $p \leq 0.05$, OR = Odd ratio, CI : confidence interval.

members are to protecting cells against ROS as they can use a wide range from oxidative stress products as substrates (Gallou *et al*, 1993). The study by Zaki show there was a non-significant change in the frequencies from the GSTP1b gene polymorphisms among the patients and control groups in agreement with our study (Zaki *et al*, 2015). Significant differences of genotype frequency in acromegaly diabetic patients and controls (13.1% versus 5.1%, resp).The data indicate that the GSTP1 genotype plays a key role in individual susceptibility to DM but does not appear to influence the onset of DSPN in DM patients, this data is consistent with data published by Amer and Bid which showed that GSTP1c gene and its variant genotype may help DM (Baynes *et al*, 1999). Among patients harboring the GSTP1, Ramprasath also showed a significant risk of DM. Data published by other researchers do not support the role from GSTP1 polymorphism sin either Turkish or Iranian patients' appearance of DM (Giacco *et al*, 2010; Bid *et al*, 2010; Gönül *et al*, 2012). Nonetheless, with GSTP1 Ile105Val gene polymorphisms, which is different from our findings, no significant impact was observed (Sharma *et al*, 2012). A statistically significant association of GSTP1 Ile105Val heterozygous genotypes with T2DM was recorded in a recently published study on a North Indian population. There were some findings that established the relationship among GSTP1 gene polymorphism and DM disease growth in a study of Egypt (Yalin *et al*, 2007). The study found that in Turkish people and Japanese people respectively, polymorphism in GSTP1may not play an active role in the pathogenesis from the disease. These data can prevent differences in the selected study groups in ethnic groups (Bid *et al*, 2010). This is the first study of the relationship between GSTP1 gene and Acromegly in Iraq and very little work on the disease with the GSTP1 gene is taking place around the world. A comparable study is not available after comprehensive literature work to align the current study with others, but the effect from the presence of GSTM1 and GSTT1 has been examined to ascertain its association with diabetes in North India (Oniki *et al*, 2008). GSTM1 genotype interaction with T2DM has been observed so that GSTM1 gene polymorphism could be type 2 DM (Oniki *et al*, 2008) predictive marker in Iraq, the interaction between these genes and breast cancer was examined to determine the effect of GSTP1 gene polymorphism on breast cancer are trying to find out how GSTP1 to be a detoxifying agent that protects DNA from harmful exogenous and endogenous compounds of DNA. The prevalence of GSTP1 polymorphisim in the breast cancer population the control group is significantly higher and the odds ratio

is16.3, suggesting that the genotype of GSTP1 (Val/Val) is associated with increased breast cancer risk (Delles *et al*, 2008).

CONCLUSION

The allele (ac) in GSTP1c can be a predictor of Diabeties acromegaly disease in Iraqi society. The allele (cc) in GSTP1c is more visible in healthy patients and can be a protective factor for the development of diabetes.

REFERENCES

Almamoori A M J, Alwan W K, Al-Jassani M J, Khadairi M M, Salh S M and Mohammad N (2019) Evaluation of Dna Damage And Antioxidants Defense Systems In Type 2 Diabetes Mellitus Patients. *Ann. Trop. Med. Public Health* **22**(12), 141–150.

Baynes J W and Thorpe S R (1999) Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* **48**(1), 1-9.

Bid H K, Konwar R, Saxena M, Chaudhari P, Agrawal C G and Banerjee M (2010) Association of glutathione S-transferase (GSTM1, T1 and P1) gene polymorphisms with type 2 diabetes mellitus in north Indian population. *J. Postgraduate Medicine* **56**(3), 176.

Brzana J A, Yedinak C G, Delashaw J B, Gultelkin H S, Cook D and Fleseriu M (2012) Discordant growth hormone and IGF-1 levels post pituitary surgery in patients with acromegaly naïve to medical therapy and radiation: what to follow, GH or IGF-1 values?. *Pituitary* **15**(4), 562-570.

Delles C, Padmanabhan S, Lee W K, Miller W H, McBride M W, McClure J D and Brown M J (2008). Glutathione S-transferase variants and hypertension. *J. Hypertension* **26**(7), 1343-1352.

Evans H M and Long J A (1922) Characteristic effects upon growth, oestrus and ovulation induced by the intraperitoneal administration of fresh anterior hypophyseal substance. *Proc. Natl. Acad. Sci.USA* **8**(3), 38.

Gallou G, Ruelland A, Legras B, Maugendre D, Allannic H and Cloarec L (1993) Plasma malondialdehyde in type 1 and type 2 diabetic patients. *Clinica Chimica Acta* **214**(2), 227-234.

Giacco F and Brownlee M (2010) Oxidative stress and diabetic complications. *Circulation Res.* **107**(9), 1058-1070.

Gönül N, Kadioglu E, Kocabaº N A, Özkaya M, Karakaya A E and Karahalil B (2012) The role of GSTM1, GSTT1, GSTP1, and OGG1 polymorphisms in type 2 diabetes mellitus risk: a case–control study in a Turkish population. *Gene* **505**(1), 121-127.

Ha H (1995) Role of oxidative stress in the development of diabetic nephropathy. *Kidney Int*. **51**, S18-S21.

Lehmann R and Schleicher E D (2000) Molecular mechanism of diabetic nephropathy. *Clinica Chimica Acta* **297**(1-2), 135-144.

Oakley A (2011) Glutathione transferases: a structural perspective. *Drug Metabolism Reviews* **43**(2), 138-151.

Oniki K, Umemoto Y, Nagata R, Hori M, Mihara S, Marubayashi T and Nakagawa K (2008) Glutathione S-transferase A1 polymorphism as a risk factor for smoking-related type 2 diabetes among Japanese. *Toxicology Letters* **178**(3), 143-145.

Palmer C N, Young V, Ho M, Doney A and Belch J J (2003) Association of common variation in glutathione S transferase genes with premature development of cardiovascular disease in patients with systemic sclerosis. *Arthritis & Rheumatism* **48**(3), 854-855.

Pearson W R, Vorachek W R, Xu S J, Berger R, Hart I, Vannais D and Patterson D (1993) Identification of class-mu glutathione transferase genes GSTM1-GSTM5 on human chromosome 1p13. *Amer. J. Human Genetics* **53**(1), 220.

Pereira E C, Ferderbar S, Bertolami M C, Faludi A A, Monte O, Xavier H T and Abdalla D S P (2008) Biomarkers of oxidative stress and endothelial dysfunction in glucose intolerance and diabetes mellitus. *Clin. Biochem.* **41**(18), 1454-1460.

Rodríguez M, Mejia F, Lecourtois M, Domínguez V and Castillo J (2014) Influence of GSTT1, GSTM1 and GSTP1 Polymorphisms on the Development of Breast Cancer. *J. Cancer Therapy*.

Sharma A, Pandey A, Sardana S, Sehgal A and Sharma J K (2012) Genetic polymorphisms of GSTM1 and GSTT1 genes in Delhi and comparison with other Indian and global populations. *Asian Pac. J. Cancer Prev*. **13**(11), 5647-5652.

SHERRATT P J, Pulford D J, Harrison D J, Green T and Hayes J D (1997) Evidence that human class Theta glutathione S-transferase T1-1 can catalyse the activation of dichloromethane, a liver and lung carcinogen in the mouse: comparison of the tissue distribution of GST T1-1 with that of classes Alpha, Mu and Pi GST in human. *Biochem. J.* **326**(3), 837-846.

Yalin S, Hatungil R, Tamer L, Ates N A, Dogruer N, Yildirim H and Atik U (2007) Glutathione S transferase gene polymorphisms in Turkish patients with diabetes mellitus. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or Disease* **25**(5), 509-513.

Zaki M A, Moghazy T F, El-Deeb M M K, Mohamed A H and Mohamed N A A (2015) Glutathione S-transferase M1, T1 and P1 gene polymorphisms and the risk of developing type 2 diabetes mellitus in Egyptian diabetic patients with and without diabetic vascular complications. *Alexandria J. Med.* **51**(1), 73-82.

Zimniak P, Nanduri B, Piku³a S, Bandorowicz Piku³a J, Singhal S S, Srivastava S K and Awasthi Y C (1994) Naturally occurring human glutathione S transferase GSTP1 1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. *Europ. J. Biochem.* **224**(3), 893-899.