Investigate the Role of Glutathione S Transferase (GST) Polymorphism in Development of Hypertension in UAE Population

Kosar Hussain 1*, Neveen Salah 2, Sahar Hussain 3, Sara Hussain 4

1 Rashid Hospital, Dubai Health Authority, Dubai, United Arab Emirates
2 Department of Biochemistry, Dubai Medical College, Dubai, United Arab Emirates
3 UAE University, Faculty of Medicine & Health Sciences, Al Ain, United Arab Emirates
4 Dubai Medical College, Dubai, United Arab Emirates

* Corresponding author at: Kosar Hussain. Rashid Hospital, Dubai Health Authority, Dubai, United Arab Emirates. Tel/Fax: +971-553979119, E-mail: exquisite_k1807@hotmail.com

Received: 15 Apr 2012              Revised: 01 Jul 2012                    Accepted: 06 Jul 2012

Abstract

Background: GST is a family of enzymes that are important in protection of the body against oxidative stress.

Objectives: Investigate the association between GSTT1 and GSTM1 polymorphism and hypertension.

Materials and Methods: GSTT1 and GSTM1 genotypes were detected by PCR. The fragments were then analyzed by agarose gel electrophoresis.

Results: There is no significant association between GSTT1 & GSTM1 polymorphism and hypertension (OR = 2.4, P > 0.05 and OR = 1.6, P > 0.05)

Conclusions: GSTT1 & GSTM1 polymorphism can be considered a risk factor for hypertension.

Keywords: Hypertension; Glutathione S-Transferase T1; Polymorphism, Genetic

1. Background

Essential hypertension is a complex multi-factorial disorder with many genetic, environmental and demographic factors contributing to this disorder. Several experimental and clinical studies have highlighted the role of oxidative stress in development of hypertension (1-4). Our body possesses a number of protective antioxidant mechanisms to counteract the production of ROS. One such system is the family of Glutathione-S-Transferase (GST) that functions as detoxification enzymes (5). GST activity has been demonstrated in vascular tissue, and represents a main cellular defense mechanism against oxidative injury (6). The polymorphism in the GSTT1 and GSTM1 gene loci is caused by a gene deletion, causing GSTT1-0 (GSTT1 null) and GSTM1-0 (GSTT1 null) genotype respectively.

2. Objectives

The present study aimed to test the hypothesis that the loss of activity of the enzyme due to a deletion polymorphism in the GSTT1 and GSTM1 may affect the risk of developing hypertension. We have also studied other risk factors for hypertension.

3. Material and Methods

3.1. Subjects

The control group consisted of 33 non-hypertensive individuals with mean age 41.7 ± 13.6 years. The case group consisted of 30 hypertensive patients with mean age 40.1 ± 14 years. The study was conducted in the Biochemistry department in Dubai Medical College for girls. All subjects signed an informed consent to participate in the study. The hypertensive patients underwent a standardized evaluation consisting of a questionnaire, physical examination, and laboratory tests. Weight, height, waist and hip circumferences were measured. Body mass index (BMI) and Waist Hip Ratio (WHR) were calculated. Hypertension was defined as blood pressure ≥ 140/90 mmHg or current use of anti-hypertensive medication.

3.2. Anthropometric Measurements

Subjects with body mass index (BMI) ≥ 25 kg/m2 were considered positive for obesity as defined by World Health organization. Those with waist circumference...
(WC) > 88 cm for women and > 95 cm for men or with waist hip ratio (WHR) > 0.8 for women and > 0.95 for men were considered positive for abdominal obesity (7).

3.3. Laboratory Tests

Lipids and lipid fraction measurements were performed using routine enzymatic tests (DiaSys Kits) as previously described (8, 9).

3.4. DNA Extraction

DNA was extracted from white blood cells by a salting-out method (10).

3.5. Analysis of GSTT1 and GSTM1 Genotype

For genotype analysis, the GSTT1 and GSTM1 were amplified by using multiplex polymerase chain reaction (PCR) protocol (11-13). Polymerase chain reaction was done on 96 well Amp PCR System 9700 Thermocycler (Applied Biosystems). Primer sequences and PCR conditions were as follows (oligonucleotides were synthesized by Sigma Aldrich, Germany). For detection of GSTM1 polymorphism, the forward primer was 5'-GAACCTCCCAGAAAAAGCTA-AAGC-3' and reverse primer was 5'-GGTTGGCCTCAAATATAC-GTTGG-3'. For detection of GSTT1 polymorphism the forward primer was 5'-TTCTTACAGCTCATCATCCTC-3' and the reverse primer was 5'-TACAGGATCATGCCCAGCA-3'. In order to confirm that the PCR had worked in subjects homozygous for GSTM1 or GSTT1 gene deletion, one pair of primers was used as an internal control to amplify a 312 bp fragment of CYPIAI gene: the forward primer was 5'GAACCTCCACCTGACGTCT-3', and the reverse primer was 5'CAGCTGCAATTGGAAATGTCTC-3'. Multiplex PCR mixture was carried out in a 50-μL reaction volume containing 100 ng of genomic DNA, 0.2 μmol/L of each primer, 0.8 mmol/L dNTPs, 2.0 mmol/L MgCl2 in 10% PCR buffer and 1.5U of DNA polymerase (Promega, UK). PCR involved an initial 2-min denaturation at 95°C, 30 cycles of denaturation at 95°C for 1 min, annealing at 64°C for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for

| Genotype          | Cases (n = 30) | Control (n = 33) | Odds Ratio | 95% CI  | P-value | X²  |
|-------------------|---------------|-----------------|------------|---------|---------|-----|
| GSTM1 No. (%)     |               |                 |            |         |         |     |
| Null              | 18(60)        | 16(48.5)        | 1.6        | 0.6-4.3 | 0.360   | 0.8 |
| Non-null          | 12(40)        | 17(51.5)        |            |         |         |     |
| GSTT1 No. (%)     |               |                 |            |         |         |     |
| Null              | 9(30)         | 5(15.2)         | 2.4        | 0.7-8.2 | 0.157   | 2.0 |
| Non-null          | 21(70)        | 28(84.8)        |            |         |         |     |
| GSTT1 and GSTM1 No. (%) |   |                 |            |         |         |     |
| Null both         | 5(16.7)       | 2(6.1)          | 3.1        | 0.6-17.4| 0.181   | 1.8 |
| Others            | 25(83.3)      | 31(93.9)        |            |         |         |     |

| Parameter  | Control (n = 33) | Cases (n = 30) | OR  | 95% CI  | P-value | X²  |
|------------|-----------------|---------------|-----|---------|---------|-----|
| BMI, No. (%) |                 |               | 2.5 | 0.9-7.0 | 0.083   | 3   |
| normal     | 17 (51.5)       | 9 (30)        |     |         |         |     |
| overweight & obese | 16 (48.5) | 21 (70)       | 1.4 | 0.5-3.7 | 0.560   | 0.3 |
| Waist-hip ratio, No. (%) | 20 (60.6) | 16 (53.3) | 1.4 | 0.5-3.7 | 0.532   | 0.4 |
| normal     | 13 (34.9)       | 14 (46.7)     |     |         |         |     |
| abnormal   |                |               |     |         |         |     |
| TAG, No. (%) |                 |               | 1.8 | 0.7-4.9 | 0.250   | 1.3 |
| <150mg/dL  | 18 (54.5)       | 14 (46.7)     |     |         |         |     |
| ≥150mg/dL  | 15 (45.5)       | 16 (53.3)     |     |         |         |     |
| TC, No. (%) |                 |               |     |         |         |     |
| <200mg/dL  | 18 (54.5)       | 12 (40)       |     |         |         |     |
| ≥200mg/dL  | 15 (45.5)       | 18 (60)       |     |         |         |     |
| HDL, No. (%) |                 |               | 1.4 | 0.5-3.7 | 0.532   | 0.4 |
| ≥40mg/dL   | 18 (54.5)       | 14 (46.7)     |     |         |         |     |
| <40mg/dL   | 15 (45.5)       | 16 (53.3)     |     |         |         |     |
| LDL, No. (%) |                 |               |     |         |         |     |
| <160mg/dL  | 28 (84.8)       | 23 (76.7)     |     |         |         |     |
| ≥160mg/dL  | 5 (15.2)        | 7 (23.3)      |     |         |         |     |
5 min. Detection of different genotypes were done by 1.5% agarose gel electrophoresis, the presence of band 480 bp indicate the presence of GSTT1 while the presence of band of 215 bp indicate the presence of GSTM1.

3.6. Statistical Analysis

Statistical analysis was done with SPSS software version 11.0 (SPSS, Inc; Chicago IL). Difference in genotype prevalence and association between case and control group were assessed by the Chi-square test. Correlation coefficient, Odds Ratio (OR) and 95% CI were used to describe the strength of association.

4. Results

The distribution of genotypes of GSTM1 and GSTT1 in cases and control are shown in Table 1.

The results of anthropometric measurements & lipid profile among the cases and control are displayed in Table 2.

5. Discussion

Oxidative stress may contribute to the generation and/or maintenance of hypertension via a number of possible mechanisms (14-16). There is increasing interest in the role of GST polymorphism as a contributory factor to oxidative stress. Tang et al reported that subjects with GSTM1-0/GSTT1-0 had higher C-reactive protein and fibrinogen and lower total antioxidant status compared to patients with wild-type GSTM1/GSTT1 genes (17). A recent study by Rybka et al. has shown that the glutathione-related antioxidant defense system was enhanced in elderly hypertensive patients who were receiving anti-hypertensive treatment (18). On the other hand Marinho and colleagues demonstrated that although GST activity and total plasma glutathione levels were markedly decreased in hypertension but there is no correlation with the GSTM1 and GSTT1 deletion polymorphisms (19). In our study it was seen that null GSTM1 and GSTT1 were not a significant risk factor for hypertension. However in an Italian case-control study by Polimati et al. concluded that GSTT1 null individuals were significantly associated with increased risk of hypertension [P < 0.001; adjusted OR 2.24 (1.43-3.50)]. In the sub-analysis they have shown that the risk was significantly higher in female hypertensives [P < 0.001; adjusted OR 3.25 (1.78-5.95)] but not in male subjects (20). Also Capoluongo et al. have published a similar study that showed GSTM1 null-genotype is a risk factor for hypertension among elderly subjects (OR = 2.25, 95% CI = 1.36-3.72; P = 0.005), while null GSTT1 genotype was a minor risk factor for hypertension (OR = 1.24, 95% CI = 0.67-2.29, P = 0.52). However, they failed to demonstrate that a combined GSTM1 and GSTT1 null-genotype act synergistically to increase the risk of hypertension (21). Oniki et al. have also demonstrated that subjects who have combined GSTM1 and GSTT1 null genotypes have higher risk for hypertension (adjusted OR: 3.1; 95% CI: 1.0-9.5, respectively). (22).

Obese subjects are more prone to develop hypertension and various neurohormonal mechanisms are postulated to be involved (23, 24). Various clinical intervention trials have consistently found that weight loss effectively lowers blood pressure (25). We recommend further studies to assess the role of other genes involved in anti-oxidant pathway.

Acknowledgements

We are grateful to Mr. Khan and Mrs. Sahar for their technical support.

Financial Disclosure

None declared.

Funding/Support

None declared.

References

1. Weseler AR, Bart A. Oxidative stress and vascular function: implications for pharmacologic treatments. Curr Hypertens Rep. 2010;12(3):354-61.
2. Montezano AC, Touyz RM. Oxidative stress, Nox, and hypertension: Experimental evidence and clinical controversies. Ann Med. 2012;44 Suppl 1:S2-S16.
3. Schulz E, Gori T, Munzel T. Oxidative stress and endothelial dys-function in hypertension. Hypertens Res. 2011;34(6):665-73.
4. Schulz E, Jansen T, Wenzel P, Daiber A, Munzel T. Nitric oxide, tetrahydrobiopterin, oxidative stress, and endothelial dysfunction in hypertension. Antioxid Redox Signal. 2008;10(6):S15-26.
5. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology. 2009;61(3):35-46.
6. He NG, Awasthi S, Singhal SS, Trent MB, Boor PJ. The role of glutathione S-transferases as a defense against reactive electrophiles in the blood vessel wall. Toxicol Appl Pharmacol. 1998;152(1):83-9.
7. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:1-253.
8. Friedewald WT, Levy RJ, Fredrickson DS. Estimation of the contribution of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.
9. Rifai N, bachnorik PS, Albers JF. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Text book of clinical chemistry. 3RD ed. Philadelphi: W.B Saunders Company; 1999. pp. 809-61.
10. Josef S, David WR, Nina I, Kaaren AJ. MOLECULAR CLONING: Rapid Isolation Of Mammalian DNA. New York: Cold Spring Harbour Laboratory Press; 2002.
11. Abdel-Rahman SZ, Anwar WA, Abdel-Aal WM, Mostafa HM, Au WW. GSTM1 and GSTT1 genes are potential risk modifiers for bladder cancer. Cancer Detect Prev. 1998;22(2):329-38.
12. Joseph S, David WR. Gel Electrophoresis of DNA. Cold Spring Harbor Laboratory. 3rd ed.: Press New York; 2001.
13. Mittal RD, Srivastava DS, Mandhani A, Kumar A, Mittal B. Polymorphism of GSTM1 and GSTT1 genes in prostate cancer: a study from North India. Indian J Cancer. 2004;41(3):155-9.
14. Cracowski JL, Durand T, Bessard G. Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications. Trends Pharmacol Sci. 2002;23(3):160-6.
15. Schiffrin EL. Beyond blood pressure: the endothelium and atherosclerosis progression. Am J Hypertens. 2002;15(10 Pt 2):S5-S22.
16. Chen X, Touyz RM, Park JB, Schiffrin EL. Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. Hypertension. 2001;38(3 Pt 2):606-11.

17. Tang J, Wang MW, Jia EZ, Yan J, Wang QM, Zhu J, et al. The common variant in the GSTM1 and GSTT1 genes is related to markers of oxidative stress and inflammation in patients with coronary artery disease: a case-only study. Mol Biol Rep. 2010;37(1):405-10.

18. Rybka J, Kupczyk D, Kedziora-Kornatowska K, Motyl J, Czuczejko J, Szewczyk-Golec K, et al. Glutathione-related antioxidant defense system in elderly patients treated for hypertension. Cardiovasc Toxicol. 2011;11(1):1-9.

19. Marinho C, Alho I, Arduino D, Falcao LM, Bras-Nogueira J, Bischo M. GST M/T and MTHFR polymorphisms as risk factors for hypertension. Biochem Biophys Res Commun. 2007;353(2):344-50.

20. Polimanti R, Placentini S, Lazzarin N, Re MA, Manfelliotto D, Fucirelli M. Glutathione S-transferase variants as risk factor for essential hypertension in Italian patients. Mol Cell Biochem. 2011;357(1-2):227-33.

21. Capoluongo E, Onder G, Concolino P, Russo A, Santonocito C, Bernabei R, et al. GSTM1-null polymorphism as possible risk marker for hypertension: results from the aging and longevity study in the Sirente Geographic Area (ilSIRENTE study). Clin Chim Acta. 2009;399(1-2):92-6.

22. Oniki K, Hori M, Takata K, Yokoyama T, Mihara S, Maruhayashi T, et al. Association between glutathione S-transferase A1, M1 and T1 polymorphisms and hypertension. Pharmacogenet Genomics. 2008;18(3):275-7.

23. Kurukulasuriya LR, Stas S, Lastra G, Manrique C, Sowers JR. Hypertension in obesity. Med Clin North Am. 2011;95(5):903-17.

24. Rocchini AP. Obesity hypertension. Am J Hypertens. 2002;15(2 Pt 2):505-25.

25. Mulrow CD, Chiquette E, Angel L, Grimm R, Cornell J, Summerbell CD, et al. WITHDRAWN: Dieting to reduce body weight for controlling hypertension in adults. Cochrane Database Syst Rev. 2008(4):CD000484.