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CLINICAL & BASIC RESEARCH

Association of *Angiotensin-Converting Enzyme* and *Glutathione S-Transferase* Gene Polymorphisms with Body Mass Index among Hypertensive North Indians

Saliha Rizvi,[1] *Syed T. Raza,[1] Zeba Siddiqi,[2] Shania Abbas,[1] Farzana Mahdi[1]

ارتباط تعدد الأشكال الجينية لأنزيم تحويل الأنجيوتنسين والجلوتاثيون س–ترانسفيريز مع مؤشر كتلة الجسم بين الهنود الشماليين المصابين بارتفاع ضغط الدم

صليحة رضوي، سيد تسليم رضا، زيبا صديقي، شانيا عباس، فرزانة مهدي

abstract: ***Objectives:*** This study aimed to examine the association of *angiotensin-converting enzyme* (*ACE*) and *glutathione S-transferase* (*GST*) gene polymorphisms with body mass index (BMI) in hypertensive North Indians. ***Methods:*** This case-control study was carried out between May 2013 and November 2014 at the Era's Lucknow Medical College & Hospital, Lucknow, India, and included 378 subjects divided into three groups. One group constituted 253 hypertensive individuals (sustained diastolic blood pressure of >90 mmHg and systolic blood pressure of >140 mmHg) who were subcategorised according to normal (<25 kg/m^2) or high (≥25 kg/m^2) BMI. The third group consisted of 125 age-, gender- and ethnically-matched normotensive controls with a normal BMI. Gene polymorphisms were evaluated by polymerase chain reaction. The genotypic and allelic frequency distribution among both groups were analysed. ***Results:*** A significant difference was found between *GST theta 1*-null and *GST mu 1*-positive genotype frequencies among the hypertensive overweight/obese individuals and controls (P = 0.014 and 0.033, respectively). However, no difference was observed in the frequency of *ACE* polymorphisms. *ACE* insertion/insertion genotype (P = 0.006), insertion and deletion alleles (P = 0.007 each) and *GST theta 1*-null and *GST theta 1*-positive genotypes (P = 0.006 each) were found to differ significantly between hypertensive cases and controls, regardless of BMI. ***Conclusion:*** *ACE* and *GST* gene polymorphisms were not associated with BMI but were significantly associated with hypertension among the studied group of North Indians.

Keywords: Angiotensin Converting Enzyme; Glutathione Transferase; Genetic Polymorphisms; Hypertension; Body Mass Index; Obesity; India.

الملخص: الهدف: هدفت هذه الدراسة إلى دراسة ارتباط تعدد الأشكال الجيني لأنزيم تحويل الأنجيوتنسين (ACE) والجلوتاثيون س–ترانسفيريز (GST) مع مؤشر كتلة الجسم بين الهنود الشماليين ذوي مرض ضغط الدم المرتفع. **الطريقة:** أجريت هذه الدراسة المقارنة بين الحالات ومجموعة التحكم بين مايو 2013 ونوفمبر 2014 في كلية ايراس لكناو الطبية ومستشفى، لكناو، ولاية اوتار براديش، الهند، وشملت 378 شخص تم تقسيمهم إلى ثلاث مجموعات. تشكل المجموعة الأولى 253 من الأفراد المصابين بارتفاع ضغط الدم (ضغط الدم الانبساطي المستمر >90 مم زئبق وضغط الدم الانقباضي >140 مم زئبق) الذين تم تقسيمهم بعد ذلك حسب كتلة الجسم الى (<25 كجم/م^2) و(≥25 كجم/م^2) وتألفت المجموعة الثالثة من 125 متماثلي العمر والجنس والعرق و ضغط الدم و مؤشر كتلة جسم طبيعيين. تم تقييم الجينات متعددة الأشكال عن طريق تفاعل البلمرة المتسلسل. وقد تم تحليل توزيع التردد الوراثي وأليلية بين المجموعتين. **النتائج:** وجد فرق كبير بين وتيرة النمط الجيني ل GST ثيتا 1 المصفر و GST ميوالإيجابي بين الأفراد المصابين بارتفاع ضغط الدم و يعانون من زيادة الوزن/السمنة والضوابط الأصحاء (P = 0.014 و P = 0.033، على التوالي). ومع ذلك، لم يلاحظ أي اختلاف في تعدد أشكال ACE. بالنسبة للإدراج/الحذف الجيني ل ACE (P = 0.006)، وإدراج وحذف الأليلات (P = 0.007 لكل منهما) و GST ثيتا 1-ا المصفر و GST ثيتا 1 الإيجابي (P = 0.006 لكل منهما) وجد أن هناك اختلاف احصائي بين حالات ارتفاع ضغط الدم والمجموعة الضابطة بغض النظر عن مؤشر كتلة الجسم. **الخلاصة:** لم يرتبط تعدد الأشكال الجيني ل ACE و GST مع مؤشر كتلة الجسم لكن ارتبط إحصائيا مع ارتفاع ضغط الدم في مجموعة البحث من الهنود الشماليين.

مفتاح الكلمات: الإنزيم المحول للأنجيوتنسين؛ الجلوتاثيون ترانسفريز؛ تعدد الأشكال الجينية؛ ارتفاع ضغط الدم؛ مؤشر كتلة الجسم؛ السمنة؛ الهند.

Advances in Knowledge

- *To the best of the authors' knowledge, this is the first study to investigate the effect of single nucleotide polymorphisms in angiotensin-converting enzyme (ACE) and glutathione S-transferase (GST) genes on body mass index (BMI) in hypertensive North Indians.*

Departments of [1]Biochemistry and [2]Medicine, Era's Lucknow Medical College & Hospital, Lucknow, India
**Corresponding Author e-mail: tasleem24@gmail.com*

- *While ACE and GST gene polymorphisms were not found to be associated with BMI among this study population, they were significantly associated with hypertension.*

Application to Patient Care

- *The results of this study could be helpful in understanding the impact of genetic polymorphisms on BMI and on an individual's predisposition to hypertension.*
- *Additionally, these findings could contribute in the identification of novel factors leading to the development of a high BMI; this would assist in the prevention and cure of various related metabolic diseases.*

Hypertension and obesity are emerging global health concerns.[1] Many epidemiological studies have shown an association between these medical conditions.[2,3] Hypertension predisposes individuals to future weight gain as compared to their normotensive counterparts, which means that even hypertensive individuals at a normal weight are at significantly higher risk of becoming obese.[4] The body mass index (BMI) is used to classify individuals according to various weight categories. A high BMI is associated with an increased burden of diabetes, hypertension and cardiovascular diseases.[1] Aside from environmental and lifestyle factors, BMI is also influenced by genetic factors. Research has confirmed the involvement of different genetic polymorphisms in the metabolism and appetite of predisposed individuals, thus causing them to become overweight or obese.[1] More than 41 *loci* on the human genome have been linked with the development of obesity.[1]

The angiotensin-converting enzyme (ACE) is part of the renin-angiotensin system, which is responsible for regulating blood pressure and the balance of fluids and salts inside the body. Engeli *et al*. suggested that components of the renin-angiotensin system might be involved in the development of obesity and associated hypertension.[5] The insertion/deletion polymorphism occurs in intron 16 of the *ACE* gene due to the presence or absence of a 278 base pairs (bp) *Arthrobacter luteus* repetitive sequence.[6] This polymorphism has been found to drastically affect ACE activity where the deletion/deletion genotype increases ACE activity while the insertion/insertion genotype decreases activity.[7] A study conducted in Pakistan showed the association of the *ACE* gene polymorphism with obesity, suggesting a role for the deletion allele in adipogenesis and adipocyte metabolism.[8]

Hirata *et al*. confirmed the role of oxidative stress in linking obesity with hypertension.[9] Systemic oxidative stress levels increase obesity, which may lead to the development of obesity-related hypertension. Glutathione S-transferase (GST) plays a major role in combating reactive oxygen species, thus protecting the body against its harmful effects.[10] Evidence also indicates the involvement of oxidative stress with the pathogenesis of hypertension.[9] Among humans, the gene coding for *GST mu 1* (*GSTM1*) and *GST theta 1* (*GSTT1*) are located on chromosomes 1p13.3 and 22, respectively. Previous studies have demonstrated that *GST* polymorphisms were not associated with BMI in diabetic North Indians or with weight gain in schizophrenic Koreans.[11,12] Since there is a close link between hypertension and obesity, this study sought to investigate the association of *ACE* and *GST* gene polymorphisms with BMI among a selected group of hypertensive North Indians.

Methods

This case-control study was carried out between May 2013 and November 2014 at the Era's Lucknow Medical College & Hospital, Lucknow, India. A total of 253 subjects with either a recent diagnosis or history of hypertension were recruited from the Department of Medicine at Era's Lucknow Medical College & Hospital. These patients mostly resided in Lucknow and adjoining areas, including Hardoi and Barabanki. A diagnosis of hypertension was based on sustained diastolic blood pressure of >90 mmHg and systolic blood pressure of >140 mmHg. Hypertensive patients were subsequently subcategorised according to BMI into normal weight (BMI <25 kg/m^2) or overweight/obese (≥25 kg/m^2) groups. As a control group, 125 age-, gender- and ethnically-matched normotensive individuals of normal weight (BMI <25 kg/m^2) were recruited for inclusion in the study. Patients who visited the outpatient clinics for minor illnesses and with no history of major clinical diseases were recruited as controls. Subjects in either group with a history of smoking or evidence of cancer, organ dysfunction or metabolic disease were excluded. The sample size calculations were based on the proportion of *ACE* genotypes among the cases and controls using the following formula:

$$N = (Z\alpha + Z\beta)^2/\{\ln (1-e)\}^*[\{(1-p1)/p1\} + (1-p2)/p2\}]$$

Based on a 95% level of significance and 80% expected power (20% type 2 error), the minimum sample size was 103 in each group. Subject to the

availability of cases, this was then increased so that the power would increase correspondingly.

Clinical data were collected from each subject, including gender, age, alcohol consumption, waist-to-hip ratio (WHR), weight, height and family history. Quetelet's equation (weight in kg/height in m^2) was used to calculate the BMI of each participant. WHR was calculated by dividing the waist circumference at the narrowest point above the hip by the hip circumference at its greatest gluteal protuberance. Serum triglycerides were measured using the glycerol phosphate oxidase-peroxidase-amidopyrine method while serum cholesterol was measured by the cholesterol oxidase-peroxidase method. High-density lipoprotein cholesterol was measured by the immunoinhibition method using the Erba XL-300 fully automated chemistry analyser (Erba Diagnostics Mannheim GmbH, Mannheim, Germany). Very low-density lipoprotein levels were estimated by dividing measured triglycerides by 5 mg/dL units. Low-density lipoprotein cholesterol levels were estimated using the Friedewald formula.[13]

The DNA extraction was performed using 3 mL of venous blood collected from all participants in 0.5 M ethylenediaminetetraacetic acid tubes. A standard phenol-chloroform extraction method was used to isolate genomic DNA from the blood samples.[14] The DNA concentration was determined by a spectrophotometer and samples were stored at -20 °C. *ACE* insertion/deletion polymorphisms (rs464994) were analysed by genomic DNA amplification using polymerase chain reaction (PCR) technology.[15] PCR amplification was conducted in a total volume of 20 µL using: 10 pmol each of forward primer 5'-CTGGAGACCACTCCCATCCTTTCT-3' and reverse primer 5'-GATGTGGCCATCTTCGTCAGAT-3'; 200 ng of genomic DNA; 3 mM of magnesium chloride; 50 mM of potassium chloride; 10 mM of trisaminomethane hydrochloride at a pH of 8.4; 0.5 mM each of deoxyribonucleotide triphosphate; and 2 U of *Thermus aquaticus* (Taq) polymerase (Bioline Reagents Ltd., London, UK). The PCR amplification was performed using an initial denaturation at 94 °C for five minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 60 °C for one minute and 15 seconds, extension at 72 °C for two minutes and 30 seconds and final extension at 72 °C for five minutes. The amplified products were separated on a 2.0% agarose gel stained with 0.5 ug/mL of ethidium bromide and visualised using a bioimaging and gel documentation system (UVP LLC, Upland, California, USA).

The *GSTM1* and *GSTT1* genetic polymorphisms were analysed by genomic DNA amplification using the multiplex-PCR technique (MJ Mini™ Thermal Cycler, Bio Rad Laboratories Inc., Hercules, California, USA).[15] Primers for *GSTM1* were 5'-AACTCCCTGAAAAGCTAAAGC-3' and 5'-GTTGGGCTCAAATATACGGTGG-3', while primers for *GSTT1* were 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-TCACCGGACATGGCCAGCA-3'. To avoid false-negative readings, β-globin was used as an internal control. Primers for β-globin were 5'-CAACTTCATCCACGTTCACC-3' and 5'-GAAGAGCCAAGGACAGGTAC-3'. PCR amplification was conducted in a total volume of 25 µL using: 10 pmol of each primer; 100 ng of genomic DNA; 2.5 mmol/L of magnesium chloride; 0.2 mmol/L each of deoxyribonucleotide triphosphate; and 1 U of Taq polymerase (Bioline Reagents Ltd). The PCR amplification was performed using an initial denaturation at 94 °C for five minutes, followed by 30 cycles at 94 °C for one minute, 64 °C for one minute, 72 °C for one minute and a final extension of 72 °C for seven minutes. Amplified products were separated on a 1.5% agarose gel stained with 0.5 µg/mL of ethidium bromide and visualised using a bioimaging and gel documentation system (UVP LLC). Direct sequencing was carried out for approximately 10% of all samples for both polymorphisms studied and genotyping for 20% of the samples was further duplicated. The results were verified by two individual laboratory technicians to confirm genotyping results.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 12 (IBM Corp., Chicago, Illinois, USA). Variables were tested for normality using the Kolmogorov-Smirnov test. Variables with a normal distribution were expressed as means ± standard deviation while variables with a non-normal distribution were expressed as medians and interquartile ranges and were log transformed before statistical analysis. A Chi-squared test was used to compare genotyping data between cases and controls. A P value of ≤0.050 was considered significant. The odds ratio (OR) and 95% confidence interval (CI) was calculated to test the relative risk for association. Other variables were compared using the Student's t-test for normally-distributed variables.

Ethical approval for this study was granted by the Institutional Ethical Committee of the Department of Medicine at Era's Lucknow Medical College & Hospital (#ELMC/E-1/3942). Written informed consent was obtained from each subject prior to data collection.

Results

A total of 378 patients were included in this study,

Table 1: Characteristics of overweight/obese* hypertensive subjects and hypertensive subjects of a normal weight† in comparison to an age-, gender- and ethnically-matched control group† in North India (N = 378)

Characteristic	Mean ± SD		*P* value‡	Mean ± SD		*P* value‡
	HH group (n = 150)	Control group (n = 125)		HN group (n = 103)	Control group (n = 125)	
Male-to-female ratio	83:67	72:53	0.706	58:45	72:53	0.845
Age in years	43.42 ± 13.29	40.03 ± 10.28	0.017	42.02 ± 16.65	40.03 ± 10.28	0.290
BMI in kg/m^2	28.87 ± 3.78	21.92 ± 2.60	<0.001	21.68 ± 1.93	21.92 ± 2.60	0.424
SBP in mm Hg	147.63 ± 21.16	110.00 ± 10.61	<0.001	146.96 ± 17.87	110.00 ± 10.61	<0.001
DBP in mm Hg	97.85 ± 4.02	75.00 ± 9.20	<0.001	93.50 ± 5.67	75.00 ± 9.20	<0.001
SC in mg/dL	194.92 ± 43.27	166.73 ± 24.17	<0.001	181.11 ± 56.88	166.73 ± 24.17	0.017
Median TG in mmol/L^{-1} (IQR)	2.12 (1.50–2.62)	1.32 (0.95–2.10)	<0.001	1.94 (0.96–1.98)	1.32 (0.95–2.10)	<0.001
HDL in mg/dL	41.24 ± 8.53	55.23 ± 9.59	<0.001	45.77 ± 8.79	55.23 ± 9.59	<0.001
VLDL in mg/dL	34.41 ± 20.55	30.83 ± 11.64	0.070	33.53 ± 16.7	30.83 ± 11.64	0.166
LDL in mg/dL	102.82 ± 47.44	87.80 ± 19.83	<0.001	103.78 ± 31.93	87.80 ± 19.83	<0.001
WHR	0.93 ± 0.02	0.86 ± 0.08	<0.001	0.86 ± 0.06	0.86 ± 0.08	0.667

SD = standard deviation; HH = hypertensive overweight/obese group; HN =hypertensive normal weight group; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; SC = serum cholesterol; TG = triglycerides; IQR = interquartile range; HDL = high-density lipoproteins; VLDL = very low-density lipoproteins; LDL = low-density lipoproteins; WHR = waist-to-hip ratio.

**BMI ≥25 kg/m^2. †BMI <25 kg/m^2. ‡Calculated using the Student's t-test. A P value of ≤0.050 was considered significant.*

ranging in age from 26–60 years old. There were 103 subjects in the hypertensive normal weight group, 150 subjects in the hypertensive overweight/obese group and 125 subjects in the control group. Obesity-related parameters were higher in the hypertensive groups compared to the control group [Table 1]. For *ACE* polymorphisms, a single 490 bp band represented an insertion/insertion genotype, two bands of 490 bp and 190 bp represented an insertion/deletion genotype, whereas the deletion/deletion genotype was confirmed by a single band of 190 bp [Figure 1]. For *GST* genetic polymorphisms, the band sizes observed were 215 bp, 480 bp and 268 bp for *GSTM1*, *GSTT1* and β-globin, respectively [Figure 2]. Negligible discrepancy in genotyping was observed.

The genotype frequencies of *ACE* insertion/deletion, deletion/deletion and insertion/insertion genotypes among the hypertensive overweight/obese group and the hypertensive normal weight group were 53.34%, 19.33% and 27.33% and 54.37%, 16.50% and 29.13%, respectively (OR = 0.96, 1.22 and 0.91; 95% CI = 0.56–1.66, 0.59–2.49 and 0.50–1.67; *P* =

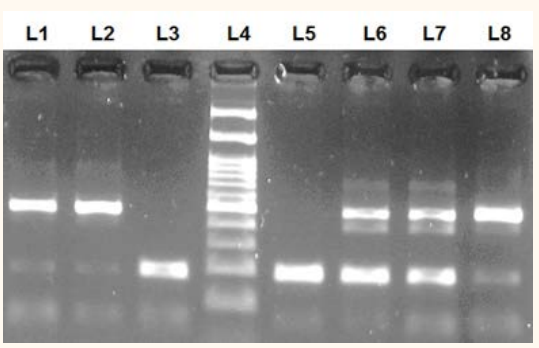

Figure 1: Agarose gel image showing polymerase chain reaction products for an *angiotensin-converting enzyme* polymorphism. Lanes 1, 2 and 8 show the insertion/insertion genotype at 490 base pairs (bp), lanes 6 and 7 show the insertion/deletion genotype at 490 bp and lanes 3 and 5 show the deletion/deletion genotype in a single band of 190 bp. Lane 4 is a 100 bp ladder.

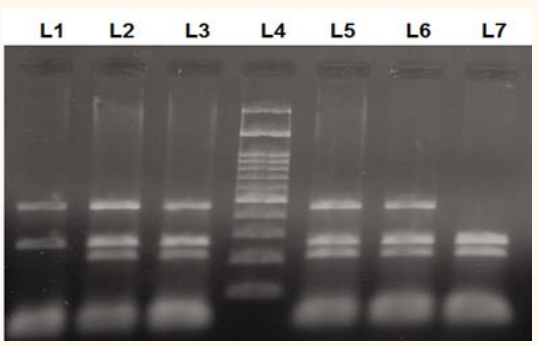

Figure 2: Agarose gel image showing polymerase chain reaction products of *glutathione S-transferase* (*GST*) polymorphisms. The presence or absence of *GST mu 1* (*GSTM1*) and *GST theta 1* (*GSTT1*) was detected by 480 base pairs (bp) and 210 bp bands, respectively. β-globin was considered an internal control at 260 bp. Lanes 1 and 7 show *GSTM1*- and *GSTT1*-null genotypes, respectively, and lanes 2–5 show *GSTM1* and *GSTT1* wild-type genotypes. Lane 4 is a 100 bp ladder.

Table 2: Frequencies of *angiotensin-converting enzyme* and *glutathione S-transferase* genetic polymorphisms among overweight/obese* hypertensive subjects and hypertensive subjects of a normal weight† in North India (N = 253)

Polymorphism			n (%)		OR	95% CI	χ^2	*P* value‡	Power
			HN group (n = 103)	HH group (n = 150)					
ACE	Genotype	I/D	56 (54.37)	80 (53.34)	0.96	0.56–1.66	0.02	0.889	0.610
		D/D	17 (16.50)	29 (19.33)	1.22	0.59–2.49	0.30	0.586	0.813
		I/I	30 (29.13)	41 (27.33)	0.91	0.50–1.67	0.10	0.757	0.622
	Allele	I	116 (56.31)	162 (54.00)	0.91	0.61–1.34	0.25	0.620	0.735
		D	90 (43.69)	138 (46.00)	1.10	0.75–1.63	0.25	0.620	0.735
GST	Genotype	*GSTT1*/*GSTM1*-positive	49 (47.57)	80 (53.34)	1.26	0.73–2.18	0.70	0.403	0.900
		GSTT1-null	16 (15.53)	20 (13.33)	0.86	0.39–1.86	0.16	0.693	0.654
		GSTM1-positive	65 (63.12)	100 (66.67)	1.19	0.67–2.11	0.34	0.559	0.822
	Allele	*GSTT1* -positive	87 (84.47)	130 (86.67)	1.17	0.54–2.54	0.16	0.693	0.753
		GSTM1-null	38 (36.89)	50 (33.33)	0.84	0.47–1.50	0.34	0.559	0.763

OR = odds ratio; CI = confidence interval; HN = hypertensive normal weight group; HH = hypertensive overweight/obese group; ACE = angiotensin-converting enzyme; I = insertion; D = deletion; GST = glutathione S-transferase; GSTT1 = GST theta 1; GSTM1 = GST mu 1.

**BMI ≥25 kg/m². †BMI <25 kg/m². ‡Calculated using the Student's t-test. A P value of ≤0.050 was considered significant.*

Table 3: Frequencies of *angiotensin-converting enzyme* and *glutathione S-transferase* genetic polymorphisms among overweight/obese* hypertensive subjects in comparison to an age-, gender- and ethnically-matched control group† in North India (N = 275)

Polymorphism			n (%)		OR	95% CI	χ^2	*P* value	Power
			Control group (n = 125)	HH group (n = 150)					
ACE	Genotype	I/D	74 (59.20)	80 (53.34)	0.86	0.49–1.48	0.31	0.575	0.812
		D/D	34 (27.20)	29 (19.33)	0.68	0.35–1.31	1.35	0.245	0.922
		I/I	17 (13.60)	41 (27.33)	2.37	1.16–4.83	5.86	0.015	0.908
	Allele	I	108 (43.20)	162 (54.00)	1.60	1.08–2.36	5.60	0.018	0.882
		D	142 (56.80)	138 (46.00)	0.63	0.42–0.92	5.60	0.018	0.882
GST	Genotype	*GSTT1*/*GSTM1*-positive	63 (50.40)	80 (53.34)	1.22	0.70–2.10	0.49	0.486	0.811
		GSTT1-null	5 (4.00)	20 (13.33)	3.89	1.23–12.27	6.09	0.014	0.900
		GSTM1-positive	68 (54.40)	100 (66.67)	1.84	1.05–3.24	4.54	0.033	0.900
	Allele	*GSTT1* -positive	120 (96.00)	130 (86.67)	0.46	0.18–1.20	2.60	0.107	0.885
		GSTM1-null	57 (45.60)	50 (33.33)	0.61	0.35–1.07	2.94	0.086	0.879

OR = odds ratio; CI = confidence interval; HH = hypertensive overweight/obese group; ACE = angiotensin-converting enzyme; I = insertion; D = deletion; GST = glutathione S-transferase; GSTT1 = GST theta 1; GSTM1 = GST mu 1.

**BMI ≥25 kg/m². †BMI <25 kg/m². ‡Calculated using the Student's t-test. A P value of ≤0.050 was considered significant.*

0.889, 0.586 and 0.757). In the control group, the same *ACE* genotype frequencies were 59.20%, 27.20% and 13.60%, respectively (OR = 0.86, 0.68 and 2.37; 95% CI = 0.49–1.48, 0.35–1.31 and 1.16–4.83; *P* = 0.575, 0.245 and 0.015). Frequencies of insertion and deletion alleles were 54.00% and 46.00% for the hypertensive overweight/obese group compared to 56.31% and 43.69% for the hypertensive normal weight group (OR = 0.91 and 1.10; 95% CI = 0.61–1.34 and 0.75–1.63; *P* = 0.620 each) and 43.20% and 56.80% for the control subjects, respectively (OR = 1.60 and 0.63; 95% CI = 1.08–2.36 and 0.42–0.92; *P* = 0.018 each) [Tables 2 and 3].

The *GSTM1*-null genotype frequency was 33.33% for the hypertensive overweight/obese group, while it was 36.89% and 45.60% for the hypertensive normal weight and control groups, respectively (OR = 0.84 and 0.61; 95% CI = 0.47–1.50 and 0.35–1.07; *P* = 0.559

Table 4: Frequencies of *angiotensin-converting enzyme* and *glutathione S-transferase* genetic polymorphisms among hypertensive subjects in comparison to an age-, gender- and ethnically-matched control group in North India (N = 378)

Polymorphism			n (%)		OR	95% CI	χ^2	*P* value*	Power
			Hypertensive subjects (n = 253)	Control group (n = 125)					
ACE	Genotype	I/D	137 (54.15)	74 (59.20)	0.81	0.50–1.32	0.71	0.398	0.913
		D/D	45 (17.18)	34 (27.20)	0.59	0.34–1.04	3.33	0.068	0.935
		I/I	71 (28.07)	17 (13.60)	2.42	1.27–4.57	7.50	0.006	0.969
	Allele	I	279 (55.14)	108 (43.20)	1.59	1.13–2.24	7.25	0.007	0.940
		D	227 (44.86)	142 (56.80)	0.63	0.45–0.88	7.25	0.007	0.940
GST	Genotype	*GSTT1*/*GSTM1*-positive	128 (50.59)	63 (50.40)	1.02	0.63–1.64	0.01	0.936	N/A
		GSTT1-null	37 (14.69)	5 (4.00)	4.09	1.40–11.96	7.61	0.006	0.969
		GSTM1-positive	165 (65.22)	68 (54.40)	1.59	0.97–2.58	3.47	0.062	0.880
	Allele	*GSTT1* -positive	216 (85.37)	120 (96.00)	0.24	0.08–0.71	7.61	0.006	0.984
		GSTM1-null	88 (34.78)	57 (45.60)	0.63	0.39–1.03	3.47	0.062	0.910

OR = odds ratio; CI = confidence interval; ACE = angiotensin-converting enzyme; I = insertion; D = deletion; GST = glutathione S-transferase; GSTT1 = GST theta 1; GSTM1 = GST mu 1.

**Calculated using the Student's t-test. A P value of ≤0.050 was considered significant.*

and 0.086). The frequency of the *GSTT1*-null genotype was 13.33% among the hypertensive overweight/obese group as compared to 15.53% and 4.00% among the hypertensive normal weight and control groups, respectively (OR = 0.86 and 3.89; 95% CI = 0.39–1.86 and 1.23–12.27; *P* = 0.693 and 0.014). The frequencies of *GSTM1*- and *GSTT1*-positive genotypes were 66.67% and 86.67% in the hypertensive overweight/obese group, while they were 63.12% and 84.47% for the hypertensive normal weight group (OR = 1.19 and 1.17; 95% CI = 0.67–2.11 and 0.54–2.54; *P* = 0.559 and 0.693) and 54.40% and 96.00% in the control group, respectively (OR = 1.84 and 0.46; 95% CI = 1.05–3.24 and 0.18–1.20; *P* = 0.033 and 0.107). The frequency of the *GSTT1*-/*GSTM1*-positive genotype was 53.34% in the hypertensive overweight/obese group, 47.57% for the hypertensive normal weight group and 50.40% in the control group (OR = 1.26 and 1.22; 95% CI = 0.73–2.18 and 0.70–2.10; *P* = 0.403 and 0.486) [Tables 2 and 3].

The *ACE* insertion/insertion genotype frequency among the combined overweight/obese and normal weight hypertensive groups was 28.07% compared to 13.60% in the control group (OR = 2.41; 95% CI = 1.27–4.57; *P* = 0.006). The deletion/deletion genotype frequency was 17.18% among hypertensives compared to 27.20% among healthy controls (OR = 0.59; 95% CI = 0.34–1.04; *P* = 0.068). Among the hypertensives, frequencies of the insertion and deletion alleles were 55.14% and 44.86%, respectively, compared with 43.20% and 56.80% in the control group, respectively (OR = 1.59 and 0.63; 95% CI = 1.13–2.24 and 0.45–0.88; *P* = 0.007 each). The frequencies of the *GSTT1*- and *GSTM1*-null genotypes in the hypertensive groups were 14.69% and 34.78%, respectively (OR = 4.09 and 0.63; 95% CI = 1.40–11.96 and 0.39–1.03; *P* = 0.006 and 0.062). A significant difference was found between the *GSTT1*-null (14.69% versus 4.00%) and -positive (85.37% versus 96.00%; OR = 0.24; 95% CI = 0.08–0.71; *P* = 0.006) genotypes among the hypertensive groups in comparison to the control group. However, there was a non-significant difference between frequencies of *GSTM1*-positive genotypes (65.22% versus 54.40%; OR = 1.59; 95% CI = 0.97–2.58; *P* = 0.062) [Table 4].

Discussion

Hypertension and obesity may have a mutual or reciprocal relationship, where the presence of one potentially leads to the other.[4] Studies have shown that the prevalence of hypertension is directly related to a higher BMI.[2,3] In the current study, lipid profile variables (including serum cholesterol, triglycerides, low- and very low-density lipoproteins and WHR) were higher among the hypertensive individuals than the healthy controls, regardless of BMI category. Various studies have demonstrated increased plasma renin activity and plasma angiotensinogen, angiotensin II and aldosterone levels in obese individuals.[16–18] Cooper *et al.* pointed out that the *ACE* insertion/deletion polymorphism was linked to changes in ACE levels, but was inconsistently associated with BMI.[19]

A study of a Saudi population noted a higher frequency of the mutant deletion allele in hypertensive cases with a BMI of ≥30 kg/m^2 in comparison to controls, although the difference was not significant.[20] However, this result was reversed in the current study, as the frequency of the deletion allele among the hypertensive overweight/obese group was significantly lower than the control group. A similar study performed on Korean women also confirmed this finding (P = 0.063).[21] Similarly, Passaro *et al.* showed that subjects with different polymorphisms in the *ACE* gene did not show significant variability in BMI.[22] The frequency of the *ACE* deletion/deletion genotype among hypertensive overweight/obese individuals in the current study (19.33%) was similar to that found among an overweight Pakistani population (18.00%).[8] The frequency of the *ACE* insertion/insertion genotype in the hypertensive overweight/obese group of the current study (27.33%) was similar to that found among obese Pakistani individuals (26.00%); however, it was lower than the frequency found among overweight Pakistanis (32.00%).[8]

Eisenmann *et al.* reported that children with the *ACE* deletion/deletion genotype had a higher BMI compared to insertion/insertion carriers;[23] this is consistent with the results obtained in the current study when comparing the hypertensive groups according to BMI, although this was not found to be significant. These results are contradictory to another report which demonstrated that the mutant deletion allele was associated with a low BMI.[24] On further comparison of the hypertensive overweight/obese and control groups in the current study, a significant difference was found between the frequency of the insertion/insertion genotype and between the allele frequencies of insertion and deletion alleles. While no significant association was found between the *ACE* insertion/deletion polymorphism and BMI in the hypertensives, a significant association was observed with hypertension. This suggests that the *ACE* insertion/deletion polymorphism affects hypertension as opposed to BMI, with carriers of the insertion/insertion genotype being more prone to hypertension. The insertion/insertion genotype frequency in the combined North Indian hypertensive population of the current study (28.07%) was similar to that found in hypertensive Pakistanis (29.30%) and another North Indian population (28.62%).[15,25] There was a significant association between the *ACE* insertion/insertion genotype and hypertension; this contrasts with the results of a study conducted among South Indians, although it is similar to that of the other study on North Indians.[15,26]

The current study found an association between the *ACE* deletion/deletion genotype and hypertension, although this was not significant. The frequency of this genotype among hypertensives in the current study (17.18%) was higher than that found for Malaysian and Bangladeshi individuals (10.76% and 11.40%, respectively); however, a Chinese population had a lower frequency.[27–29] The insertion and deletion alleles were significantly related to hypertension in the current study, which correlates with the findings of a similar North Indian study.[15]

A positive correlation has been reported between various obesity variables like BMI and WHR, including oxidative stress markers such as reduced erythrocyte glutathione.[30] In the current study, significant variation was found between the *GSTT1*-null genotype frequency among the hypertensive overweight/obese and control groups. Similarly, the frequency of the *GSTM1*-positive genotype was significantly higher in the hypertensive overweight/obese subjects. However, this difference was not significant when compared with the hypertensive normal weight group. The frequencies of the *GSTM1*- and *GSTT1*-null genotypes among hypertensive overweight/obese subjects in the present study (33.33% and 13.33%, respectively) were lower than those of non-Hispanics (45.30% and 81.60%, respectively), African Americans (72.20% and 76.20%, respectively) and Hispanics (46.70% and 81.70%, respectively) with higher BMIs.[31] In contrast, the frequencies of *GSTM1*- and *GSTT1*-positive genotypes were higher when compared to overweight/obese non-Hispanics (54.70% and 18.40%, respectively), African Americans (27.80% and 23.80%, respectively) and Hispanics (53.30% and 18.30%, respectively).[31]

The *GSTM1*- and *GSTT1*-positive genotype frequencies were somewhat higher among the overweight/obese hypertensives in the current study than for hypertensives of a normal weight, while the null genotype frequencies were lower among the former than the latter. However, these differences were not significant. The *GSTM1*- and *GSTT1*-positive genotype findings were similar across all three groups; nevertheless, there was a slightly higher although non-significant frequency in the hypertensive overweight/obese group than the hypertensive normal weight group. No association was found between *GST* polymorphisms and BMI in Indian diabetic patients with null and positive *GST* genotypes.[11,32] Similarly, in Korean schizophrenic patients, no association was observed between *GST* polymorphisms and drug-induced weight gain.[12] In view of these results, it can be concluded that *GST* polymorphisms are not associated with a higher BMI among hypertensive patients.

In the present study, a significant difference was found between the *GSTT1*-null and -positive genotypes among hypertensives in comparison to control subjects. However, frequencies of *GSTM1*-null and-positive genotypes were not significant. The frequencies of the *GSTT1*- and *GSTM1*-null genotypes among hypertensive patients in the current study (14.69% and 34.78%, respectively) were lower in comparison to findings from Arab (30.00% and 60.00%, respectively), Chinese (58.10% and 60.00%, respectively) and Korean (55.40% and 51.80%, respectively, and 61.20% and 62.40%, respectively) populations, but similar to findings from another North Indian study (11.59% and 34.05%, respectively).[15,33–36] In comparison, the *GSTM1*-positive genotype was found in 65.22% of the hypertensives in the current study; this was higher than the results from Arab (40.00%), Chinese (40.00%) and Korean (48.20% and 37.60%) populations, but again similar to results from a North Indian study.[15,33–36]

Conclusion

Among the studied North Indian population, *ACE* and *GST* genetic polymorphisms (particularly *ACE* insertion/insertion and *GSTT1*-null and -positive genotypes) were significantly associated with hypertension but not BMI. Although they were not significantly associated with a higher BMI, *ACE* and *GST* polymorphisms may nevertheless be related to the development of hypertension in overweight or obese individuals.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

References

1. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: Pathophysiology, evaluation, and effect of weight loss. Arterioscler Thromb Vasc Biol 2006; 26:968–76. doi: 10.1161/01.ATV.0000216787.85457.f3.
2. Narkiewicz K. Diagnosis and management of hypertension in obesity. Obes Rev 2006; 7:155–62. doi: 10.1111/j.1467-789X.2006.00226.x.
3. Shah S, Anand P, Maiya M, Mukherjee S, Munjal YP, Wander GS, et al. Indian hypertension guideline. In: Munjal YP, Ed. Postgraduate Medicine (Recent Advances in Medicine). New Delhi, India: Ajanta Offset & Packagings Ltd., 2007. Pp. 315–25.
4. Julius S, Valentini M, Palatini P. Overweight and hypertension: A 2-way street? Hypertension 2000; 35:807–13. doi: 10.1161/01.HYP.35.3.807.
5. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. Hypertension 2000; 35:1270–7. doi: 10.1161/01.HYP.35.6.1270.
6. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidylcarboxypeptidase 1). Nucleic Acids Res. 1992; 20:1433. doi: 10.1093/nar/20.6.1433-a.
7. Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, et al. Angiotensin-converting enzyme in the human heart: Effect of the deletion/insertion polymorphism. Circulation 1995; 92:1387–8. doi: 10.1161/01.CIR.92.6.1387.
8. Javaid A, Mansoor Q, Bilal N, Bilal A, Shaukat U, Ismail M. ACE gene DD genotype association with obesity in Pakistani population. Int J Bioautomation 2011; 15:49–56.
9. Hirata Y, Satonaka H. Hypertension and oxidative stress. Japan Med Assoc J 2001; 44:540–5.
10. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol 2005; 45:51–88. doi: 10.1146/annurev.pharmtox.45.120403.095857.
11. Bid HK, Konwar R, Saxena M, Chaudhari P, Agrawal CG, Banerjee M. Association of glutathione S-transferase (GSTM1, T1 and P1) gene polymorphisms with type 2 diabetes mellitus in north Indian population. J Postgrad Med 2010; 56:176–81. doi: 10.4103/0022-3859.68633.
12. Park YM, Lee HJ, Kang SG, Choi JE, Cho JH, Kim L. Lack of association between glutathione S-transferase-M1, -T1, and -P1 polymorphisms and olanzapine-induced weight gain in Korean schizophrenic patients. Psychiatry Investig 2010; 7:147–52. doi: 10.4306/pi.2010.7.2.147.
13. Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. Clin Chem 1990; 36:15–19.
14. Sambrook J, Frisch EF, Maniatis T, Eds. Molecular cloning: A laboratory manual, 2nd ed. New York, USA: Cold Sping Harbor Laboratory, 1989. Pp. 9.14–19.
15. Abbas S, Raza ST, Chandra A, Rizvi S, Ahmed F, Eba A, et al. Association of ACE, FABP2 and GST genes polymorphism with essential hypertension risk among a North Indian population. Ann Hum Biol 2015; 42:461–9. doi: 10.3109/03014460.2014.968206.
16. Ruano M, Silvestre V, Castro R, García-Lescún MC, Rodríguez A, Marco A, et al. Morbid obesity, hypertensive disease and the renin-angiotensin-aldosterone axis. Obes Surg 2005; 15:670–6. doi: 10.1381/0960892053923734.
17. Kidambi S, Kotchen JM, Grim CE, Raff H, Mao J, Singh R, et al. Association of adrenal steroids with hypertension and the metabolic syndrome in blacks. Hypertension 2007; 49:704–11. doi: 10.1161/01.HYP.0000253258.36141.c7.
18. Massiéra F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, et al. Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. FASEB J 2001; 15:2727–9. doi: 10.1096/fj.01-0457fje.
19. Cooper R, McFarlane-Anderson N, Bennett FI, Wilks R, Puras A, Tewksbury D, et al. Angiotensinogen and obesity: A potential pathway leading to hypertension. J Hum Hypertens 1997; 11:107–11. doi: 10.1038/sj.jhh.1000391.
20. Ali A, Alghasham A, Ismail H, Dowaidar M, Settin A. ACE I/D and eNOS E298D gene polymorphisms in Saudi subjects with hypertension. J Renin Angiotensin Aldosterone Syst 2012; 14:348–53. doi: 10.1177/1470320312459976.
21. Kim K. Association of angiotensin-converting enzyme insertion/deletion polymorphism with obesity, cardiovascular risk factors and exercise-mediated changes in Korean women. Eur J Appl Physiol 2009; 105:879–87. doi: 10.1007/s00421-008-0973-6.
22. Passaro A, Dalla Nora E, Marcello C, Di Vece F, Morieri ML, Sanz JM, et al. PPARγ Pro12Ala and ACE ID polymorphisms are associated with BMI and fat distribution, but not metabolic syndrome. Cardiovasc Diabetol 2011; 10:112. doi: 10.1186/1475-2840-10-112.

23. Eisenmann JC, Sarzynski MA, Glenn K, Rothschild M, Heelan KA. ACE I/D genotype, adiposity and blood pressure in children. Cardiovasc Diabetol 2009; 8:14. doi: 10.1186/1475-2840-8-14.

24. Ludwig E, Corneli PS, Anderson JL, Marshall HW, Lalouel JM, Ward RH. Angiotensin-converting enzyme gene polymorphism is associated with myocardial infarction but not with development of coronary stenosis. Circulation 1995; 91:2120–4. doi: 10.1161/01.CIR.91.8.2120.

25. Ismail M, Akhtar N, Nasir M, Firasat S, Ayub Q, Khaliq S. Association between the angiotensin-converting enzyme gene insertion/deletion polymorphism and essential hypertension in young Pakistani patients. J Biochem Mol Biol 2004; 37:552–5. doi: 10.5483/BMBRep.2004.37.5.552.

26. Choudhury I, Jothimalar R, Patra AK. Angiotensin converting enzyme gene polymorphism and its association with hypertension in South Indian population. Indian J Clin Biochem 2012; 27:265–9. doi: 10.1007/s12291-012-0217-8.

27. Qu H, Lu Y, Lin S. [Meta-analysis on the association of ACE/ID polymorphism and essential hypertension in Chinese population.] Zhonghua Yu Fang Yi Xue Za Zhi 2001; 35:408–11.

28. Ramachandran V, Ismail P, Stanslas J, Shamsudin N, Moin S, Mohd Jas R. Association of insertion/deletion polymorphism of angiotensin-converting enzyme gene with essential hypertension and type 2 diabetes mellitus in Malaysian subjects. J Renin Angiotensin Aldosterone Syst 2008; 9:208–14. doi: 10.1177/1470320308097499.

29. Morshed M, Khan H, Akhteruzzaman S. Association between angiotensin I-converting enzyme gene polymorphism and hypertension in selected individuals of the Bangladeshi population. J Biochem Mol Biol 2002; 35:251–4. doi: 10.5483/BMBRep.2002.35.3.251.

30. Eriksson JW. Metabolic stress in insulin's target cells leads to ROS accumulation: A hypothetical common pathway causing insulin resistance. FEBS Lett 2007; 581:3734–42. doi: 10.1016/j.febslet.2007.06.044.

31. Duggan C, Ballard-Barbash R, Baumgartner RN, Baumgartner KB, Bernstein L, McTiernan A. Associations between null mutations in GSTT1 and GSTM1, the GSTP1 Ile(105)Val polymorphism, and mortality in breast cancer survivors. Springerplus 2013; 2:450. doi: 10.1186/2193-1801-2-450.

32. Pinheiro DS, Rocha Filho CR, Mundim CA, Júnior Pde M, Ulhoa CJ, Reis AA, et al. Evaluation of glutathione S-transferase GSTM1 and GSTT1 deletion polymorphisms on type-2 diabetes mellitus risk. PLoS One 2013; 8:e76262. doi: 10.1371/journal.pone.0076262.

33. Wang R, Wang Y, Wang J, Yang K. Association of glutathione S-transferase T1 and M1 gene polymorphisms with ischemic stroke risk in the Chinese Han population. Neural Regen Res 2012; 7:1420–7. doi: 10.3969/j.issn.1673-5374.2012.18.009.

34. Kim SJ, Kim MG, Kim KS, Song JS, Yim SV, Chung JH. Impact of glutathione S-transferase M1 and T1 gene polymorphisms on the smoking-related coronary artery disease. J Korean Med Sci 2008; 23:365–72. doi: 10.3346/jkms.2008.23.3.365.

35. Hussain K, Salah N, Hussain S, Hussain S. Investigate the role of glutathione S transferase (GST) polymorphism in development of hypertension in UAE population. Iran Red Crescent Med J 2012; 14:479–82.

36. Lee BK, Lee SJ, Joo JS, Cho KS, Kim NS, Kim HJ. Association of glutathione S-transferase genes (GSTM1 and GSTT1) polymorphisms with hypertension in lead-exposed workers. Mol Cell Toxicol 2012; 8:203–8. doi: 10.1007/s13273-012-0025-5.