Association of Polymorphisms (rs 1799782, rs25489 and rs25487) in *XRCC1* and (rs 13181) *XPD* genes with Acute Coronary Artery Syndrome in Subjects from Multan, Pakistan

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Abstract: Acute coronary artery syndrome (ACS) is the major cause of mortality in Pakistan with genetic and environmental influence on the incidence of the disease. This case-control study was designed to find out if a correlation is existing between ACS and single nucleotide polymorphisms (SNPs) in DNA repair genes *XPD* [at codon 751, rs 13181 (Lys to Gln)] and *XRCC1* [at codon 399, rs25487 (Arg to Gln); 280, rs25489 (Arg to His) and 194, rs 1799782 (Arg to Trp)] either individually or in various combination with each other (haplotype analysis). The objective of this study was to find out the association of various studied risk factors and serum lipid profile of the subjects with the disease, if any. PCR-RFLP method was used to determine genotype at specific codon in 221 subjects (115 ACS patients and 106 healthy controls) from Southern Punjab population. Genotypic and allelic frequency distribution among the cases and controls revealed that all the studied SNPs were not individually associated with the ACS. Haplotype analysis revealed that subjects having wild type combination of all three *XRCC1* SNPs had greater susceptibility to ACS than any other studied genotypic combinations. Analysis of risk factors revealed that hypertension (P<0.001), age (P=0.05), education (P<0.001), gender (P<0.001), family history (P=0.005), smoking habit (P=0.002) and diabetes (P<0.001) were significantly associated with the incidence of ACS. Serum lipid profile analysis indicated that cholesterol level was significantly higher (P=0.048) in patients (161.5mg/dL) than controls (142.1mg/dL) while triglyceride remained unaffected (P=0.87) when compared between the two treatments.

Keywords: *XRCC1; XPD*; polymorphisms; acute coronary artery syndrome; haplotype analysis; risk factors.

INTRODUCTION

Acute coronary syndrome (ACS) is among the major cause of mortality around the globe (Braunwald *et al.,* 2002). One fourth of ACS patients suffer from ST segment elevation myocardial infarction (STEMI) while three fourth have unstable angina or non ST segment elevation myocardial infarction (NSTEMI) (Gaziano *et al*., 2007). There are number of risk factors that are reported to be associated with ACS. On the basis of whether we can alter these risk factors or not, they are classified as modifiable (high blood pressure, smoking, obesity, abnormal lipids and high blood sugar) and unmodifiable (age, gender and genetics) (Deviprasad *et al*., 2009).

DNA damage can be endogenous, i.e. oxidative deamination and replication errors or it can be exogenous i.e., teratogens can damage the primary structure of DNA. In order to repair these damages human cells are provided with more than 150 reported repair genes essential for maintaining genomic stability (Gurubhagavatula *et al*., 2004). *XRCC1* (X-ray repair cross-complementing-1) is responsible to generate a protein that facilitates the efficient repair of DNA single-strand breaks generated due to ionizing radiation and alkylating agent exposure (Deviprasad *et al.,* 2009). *XPD* (Xeroderma pigmentosum complementation group D) produces a helicase that participates in nucleotide excision repair (NER) and it is also part of the transcription factor IIH resulting in production of *XPD* proteins (Clarkson *et al.,* 2005). Association of various *XPD* alleles has been reported with chromosomal aberrations, reduced efficiency of DNA repair, frequency of sister chromatid exchanges and increased DNA strand breaks due to UV exposure (Vodicka *et al.,* 2004). Genetic changes in DNA repair genes results variable DNA repair capacity between individuals leading to variety of diseases (Qu, and Morimoto, 2005). Three of non-synonymous single nucleotide polymorphisms (SNPs) in *XRCC1*, at codon 399 (Arg to Gln), 280 (Arg to His) and 194 (Arg to Trp) and one SNP in *XPD* at codon 751 (Lys to Gln) are studied most frequently among the patients of various diseases especially in different types of cancer (Hu *et al.,* 2005) but little information is available regarding the association of these SNPs with ACS in various

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populations of the world. The aim of this case-control study was to find out if there is any correlation existing between ACS and hereditary genetic defect in DNA repair genes *XRCC1* [at codon 194 (Arg to Trp), 280 (Arg to His) and 399 (Arg to Gln)] and *XPD* gene [at codon 751 (Lys to Gln)] individually or in combination to one another (haplotype analysis) and also to find out the association of various studied risk factors and serum lipid profile of the subjects with the disease.

MATERIAL AND METHODS

Subjects and data collection

One hundred and fifteen patients suffering from acute coronary artery syndrome (ACS) visiting Chaudhry Pervaiz Elahi Institute of Cardiology Multan were enrolled in this study. Medical diagnosis revealed that ninty three patients were suffering from acute myocardial infarction, eighteen had unstable angina and the remaining four were patients of stable angina. At the time of blood sampling, all subjects provided a complete clinical history including risk factors like diabetes, hypertension, family history, alcohol consumption, body weight, smoking habit and dyslipidemias. Written informed consent was obtained from all. Age matched one hundred and six volunteer controls also participated in the study. The enrolled subject hales from different cities of Southern Punjab and had different ethnic origins, gender and age ranges. The enrolled controls were healthy individuals having normal electrocardiogram with no other reported physiological disorder. Controls had no family history of diabetes, hypertension and/or heart disease and they had normal ranges for serum triglyceride and cholesterol levels. Subjects not fulfilling the above mentioned inclusion criteria were not included in the study. All experimental protocols and subject handling procedure was approved by the ethical committee of Bahauddin Zakariya University Multan, Pakistan.

DNA extraction

Blood samples (3-5ml) from each subject was preserved by adding 400-500µl of EDTA and stored at -4°C. DNA extraction was carried out by inorganic method following Taqddus *et al*., 2013.

Genotyping of XRCC1 codon 194

In exon 6, an Arg to Trp (codon 194) substitution was amplified to generate a 491 bp following Smith *et al*. (2008) (table 1). Restriction endonuclease *MspI* was used over night to digest PCR product at 37°C and analyzed on 2.5% agarose gel.

Genotyping of XRCC1 codon 280

An Arg to His (codon 280) substitution in exon 9 was amplified to generate an undigested fragment of 176 bp following Smith *et al*., 2008 (table 1). PCR products were digested with *RsaI* at 37°C overnight and analyzed on 2% agarose gel.

Genotyping of XRCC1 codon 399

An Arg to Gln (codon 399) substitution in exon 10 was amplified to generate a 615 bp DNA fragment following Yen *et al*., 2008 (table 1). PCR products were digested overnight with *MspI* at 37°C and analyzed on 1.5% agarose gel.

Genotyping of XPD codon 751

A Lys to Gln (codon 751) change was amplified in exon 23 to to produce a`344 bp fragment following Millikan *et al*., 2006 (table 1). *Pst* I was used to digest PCR products at 37°C overnight and analyzed on 3% agarose gel.

Measurement of serological parameters

For the separation of serum from the blood cells, 800µl of blood samples were centrifuged at 13000 rpm for 10 minutes in 1.5ml Eppendorf tubes Serological determination of cholesterol and triglyceride was carried out following the standard procedure provided with the diagnostic kits (Merck, Germany).

STATISTICAL ANALYSIS

The data was analyzed statistically using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Genotypic and allelic frequencies were calculated by direct counting method. The odd ratios were calculated to know the association of genotypes or alleles with the disease, if any, at 95% confidence intervals (95% CI). Pair-wise linkage disequilibrium (LD) test was carried out using software Haploview 4.1. Correlation between acute coronary artery syndrome and all the risk factors (Gender, age, cast, education, marital status, family history, diabetes, hypertension, smoking, alcohol, drug consumption and weight) associated with the disease were drawn to demonstrate the effect of each parameter in control and patients by using binary logistic regression. Chi square test was conducted to calculate the frequency of each risk factor in control and Patients. 2-Sample t-test was carried out to compare the triglyceride and cholesterol levels between control and ACS patients.

RESULTS

Genotyping results of XRCC1 codon 194

Upon restriction, amplicon containing Arg/Arg produced 293 and 178 bp, Arg/Trp had 313, 293 and 178 bp fragments and Trp/Trp had 313 and 178 bp fragments (fig. 1).

Genotyping of XRCC1 codon 280

Upon restriction, amplicon containing Arg/Arg produced 148 and 28 bp, Arg/His had 176, 148 and 28 bp and His/His had 176 bp fragments (fig. 2).

Genotyping of XRCC1 codon 399

Upon restriction, amplicon containing Arg/Arg produced 263 and 352 bp, Arg/Gln had 263, 352 and 615 bp and Gln/Gln had 615 bp fragments (fig. 3).

Genotyping of XPD codon 751

*Pst*I digestion resulted in two fragments of 234 and 110 bp for the wild-type (Lys/Lys); three fragments of 171, 110 and 63 bp for the mutatnt (Gln/Gln); and four fragments at 234, 171, 110 and 63 bp for the heterozygotes (Lys/Gln) (fig. 4).

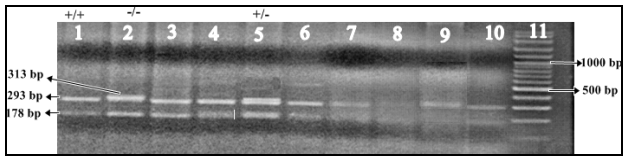

Fig. 1: Agarose gel image of PCR-RFLP assay for the detection of genotype at codon 194 of *XRCC1*. Lane 1, 3, 4, 6-10 homozygous wild genotype (Arg 194 Arg). Lane 5, heterozygous (Arg 280 Trp) genotype. Lane 2, mutant (Trp 194 Trp) genotype. Lane 11, 100 bp DNA ladder

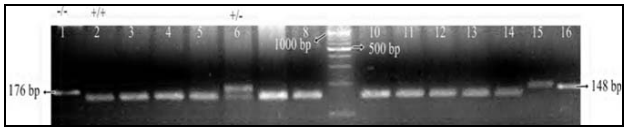

Fig 2. Agarose gel image of PCR-RFLP assay for the detection of genotype at codon 280 of *XRCC1*. Lane 1 and 15 uncut mutant PCR product (His 280 His). Lane 6, hetrozygous (Arg 280 His) genotype. Lane 9, 100 bp DNA ladder. Lane 2-5, 7-8, 10-14, 16, homozygous wild genotype (Arg 280 Arg).

Fig 3: Agarose gel image of PCR-RFLP assay for the detection of genotype at codon 399 of *XRCC1*. Lane 16, uncut PCR product. Lane 1, 2, 4, 7, 8, 11 and 12 heterozygous (Arg 399 Gln). Lane 3, 6, 9, 10 and 17 homozygous wild genotype (Arg 399 Arg). Lane 13 and 14 mutant (Gln 399 Gln). Lane 5, 15 and 18 are missing. Lane 19, 100 bp DNA ladder.

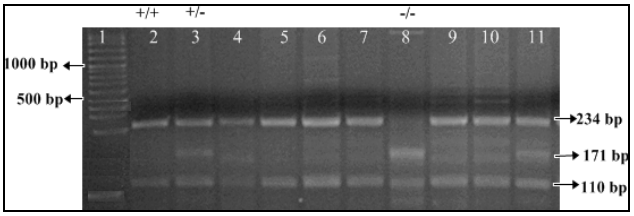

Fig. 4: Agarose gel image of PCR-RFLP assay for the detection of genotype at codon 751 of *XPD*. Lane 1, 100 bp DNA ladder. Lane 2, 4-7, 9 and 10 homozygous wild genotype (Lys 751 Lys). Lane 3 and 11 hetrozygous (Lys 751 Gln) genotype. Lane 8, mutant (Gln 751 Gln) genotype.

Correlation between risk factors and ACS

When various risk factors were compared between healthy controls and patients suffering from ACS, it was observed that family history (P=0.005), gender (P<0.001), education (P<0.001), age (P=0.05), hypertension (P<0.001), smoking habit (P=0.002) and diabetes (P<0.001) were the parameters that differed significantly among them and were associated with the incidence of cardiovascular diseases (table 2).

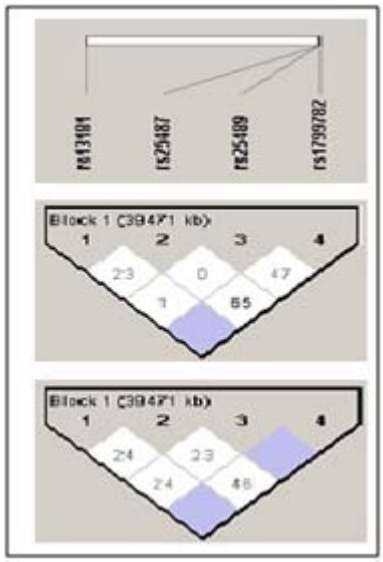

Fig. 5: (A) Schematic diagram of the four studied polymorphisms of *XRCC1* and *XPD* (B) LD pattern of gene polymorphisms in cases and (C) LD pattern of gene polymorphisms in controls from Southern Punjab population.

Association of genotypic and allelic frequency with ACS

Analysis of the distribution of genotypic and allelic frequency among the cases and controls revealed that all the studied SNPs are not individually associated with the ACS (table 3).

Combinational analysis of individual SNP

All the possible genotypic combination of *XRCC1* and XPD were analyzed through chi-square test to correlate a possible combination with ACS. Only one combination mentioned in table 4 was found significantly associated (P =0.041) with ACS indicating that subjects having wild genotype for three studied SNPs have more chances to develop heart disease (table 4).

Haplotype analysis

Analysis of distribution of various allelic combinations of all four studied SNPs in cases and controls revealed interesting results. All allelic combinations having significant P-values with higher frequency in control were protected combinations while those having higher frequency in cases were at higher risk to ACS (fig. 5, tables 5, 6) indicating that, in nature, there is variety of protective as well as alarming combinations for these four SNPs as far as incidence of ACS is concerned.

Table 1: Sequences of oligonucleotide primers used for genotyping of studied polymorphisms in *XPD* and *XRCC1*.

Gene	Polymorphism (rs number)	Primer Sequence	Amplified Product (bp)	References
XPD	Lys 751 Gln A/C (rs 13181)	F-5' TCAAACATCCTGTCCCTACT 3' R-5' CTGCGATTAAAGGCTGTGGA 3'	344	Millikan *et al*. (2006)
XRCC1	Arg 280 His G/A (rs25489)	F-5' GTCTTCTCCAGTGCCAGCTC3' R-5' AGCCACTCAGCACCACTACC3'	176	Smith *et al*. (2008)
	Arg 399 Glu G/A (rs25487)	F-5' TTGTGCTTTCTCTGTGTCCA3' R-5' TCCTCCAGCCTTTTCTGATA3'	615	Yen *et al*. (2008)
	Arg 194 Try C/T (rs 1799782)	F-5' GCCCCGTCCCAGGTA 3' R-5' CAAGACCCTTTCACT 3'	491	Smith *et al*. (2008)

Table 2: Analysis of the risk factors associated with acute coronary artery syndrome. P value indicates the results of chi-square test when each parameter was compared between control and patients.

Risk factors	Category	Control (N=106)	ACS patients (N=115)	P value
Age	25-35	16 (15%)	9 (78%)	0.05*
	35-45	27 (25%)	19 (17%)	
	45-55	26 (24%)	31 (27%)	
	55-65	34 (32%)	44 (38%)	
	65-75	3 (2.8%)	8 (7%)	
	75-85	0	4 (3.5%)	
Gender	Male	105 (99%)	83 (72%)	0.000***
	Female	1 (1%)	32 (28%)	
Marital status	Married	104 (98%)	112 (97%)	0.718^{ns}
	Unmarried	2 (2%)	3 (3%)	
Cast	Arain	6 (5%)	15 (13%)	0.254^{ns}
	Jutt	22 (21%)	18 (15%)	
	Rajpoot	10 (9%)	16 (14%)	
	Khan	12 (11%)	15 (13%)	
	Arabian	26 (24%)	17 (15%)	
	Malik	20 (19%)	22 (19%)	
	Others	10 (9%)	12 (10%)	
Education	Nil	23 (22%)	74 (64%)	0.000***
	Upto metric	53 (50%)	31 (27%)	
	upto graduation	21 (20%)	8 (7%)	
	Above graduation	9 (8%)	2 (2%)	
Family history	Yes	19 (18%)	40 (35%)	0.005**
	No	87 (82%)	75 (65%)	
Diabetes	Yes	9 (8%)	46 (40%)	0.000***
	No	97 (92%)	69 (60%)	
Hypertension	Yes	16 (15%)	60 (52%)	0.000***
	No	90 (85%)	55 (48%)	
Smoking	Yes	28 (26%)	54 (47%)	0.002**
	No	78 (74%)	61 (53%)	
Alcohol*	Yes	0 (0%)	1 (1%)	-
	No	106 (100%)	114 (99%)	
Drugs*	Yes	0 (0%)	1 (1%)	-
	No	106 (100%)	114 (99%)	
Weight	40-50	10 (9%)	7 (6%)	0.118^{ns}
	50-60	22 (21%)	32 (28%)	
	60-70	30 (28%)	42 (36%)	
	70-80	24 (22%)	24 (21%)	
	80 and above	20 (19%)	10 (8%)	

P<0.05 *= least significant P<0.01** = significant P<0.001*** = highly significant P>0.05 = non significant.

Table 3: Distribution of genotypic and allelic frequencies among cases and controls and their possible association with ACS.

Gene	Polymorphism (Amino acid change, Nucleotide Change, rs number and Position)	Genotype/allele	Control (N=106)	Cases (N=99)	χ^2- Value	*p*- Value	OR (95% CI)
XPD	Lys 751 Gln A/C rs 13181 4585919	AA AC CC A C	99(0.925) 5 (0.047) 2 (0.018) 203(0.948) 09(0.051)	92 (0.93) 5(0.05) 2(0.02) 189(0.954) 09(0.045)	0.224 0.086 0.141 0.360	0.636 0.770 0.707 0.549	1.0761 (0.3635-3.186) 0.9307 (0.2611-3.3175) 0.9327 (0.1289-6.7515) 1.0741 (0.4175-2.7633) 0.931 (0.3619-2.3952)
XRCC1	Arg 280 His G/A rs25489 44056412	GG GA AA G A	63(0.59) 19(0.17) 24(0.224) 145(0.677) 67(0.323)	59(0.59) 26(0.26) 14(0.14) 144(0.727) 54(0.272)	0.001 0.364 2.449 0.923	0.981 0.546 0.118 0.337	0.9933 (0.5685-1.7356) 0.6132 (0.3143-1.1964) 1.7857 (0.8569-3.7211) 0.8116 (0.53-1.2429) 1.2322 (0.8046-1.887)
	Arg 399 Glu G/A rs25487 44055726	GG GA AA G A	53(0.495) 30(0.28) 23(0.215) 136(0.635) 76(0.364)	40(0.40) 28(0.28) 31(0.31) 108(0.54) 90(0.46)	1.902 0.107 1.976 3.478	0.168 0.743 0.160 0.062	1.475 (0.8482-2.5649) 1.0009 (0.5448-1.8388) 0.6079 (0.3246-1.1385) 1.4912 (1.0033-2.2164) 0.6706 (0.4512-0.9967)
	Arg 194 Try C/T rs 1799782 44057574	CC CT TT C T	99(0.925) 04(0.37) 03(0.028) 202(0.9439) 13(0.0467)	94(0.949) 03(0.03) 02 (0.02) 191(0.964) 07(0.035)	0.018 0.012 0.005 0.022	0.895 0.912 0.945 0.882	0.7532 (0.2307-2.4528) 1.2549 (0.2737-5.7532) 1.4126 (0.231-8.6367) 0.5695 (0.2225-1.4578) 1.756 (0.686-4.4949)

Table 4: Combinational analysis of 3 genotypes of *XRCC1* to correlate their association with ACS. P-value indicate the result of Chi-Square test.

	XRCC1 280 (GG) / *XRCC1* 194 (CC)			χ^2- Value	*p*-Value
	XRCC1 399			6.366	0.041*
	AA	GA	GG		
Case	18	13	24		
Control	8	15	36		

P<0.05 = Least significant (*)

Table 5: Distribution of various allelic combinations of four studied polymorphisms among southern Punjab cases and controls and their association with ACS.

XPD	XRCC1			Haplotype	Case	Control	P-Value
Lys 751 Gln T/G rs 13181	Arg 399 Glu G/A rs25487	Arg 280 His G/A rs25489	Arg 194 Try C/T rs 1799782				
G	A			GA	0.521	0.621	0.0413*
G	G			GG	0.434	0.337	0.0431*
	A	A		AA	0.401	0.495	0.0549
	G	A		GA	0.329	0.213	0.0077**
	A		C	AC	0.533	0.625	0.0573
	G		C	GC	0.432	0.327	0.0286*
G	G	A		GGA	0.309	0.195	0.0074**
	G	A	C	GAC	0.306	0.185	0.0044**
G	G	A	C	GGAC	0.285	0.167	0.0041**

P>0.05 = Non significant; P<0.05 = Least significant (*); P<0.01=Significant (**)

Table 6: Distribution of associated alleles of four SNPs among southern Punjab cases and controls and their association with ACS.

SNP	Associated Allele	Case, Control Frequencies	P-Value
rs13181	T	0.045, 0.042	0.8994
rs25487	G	0.460, 0.358	0.0361*
rs25489	A	0.730, 0.708	0.6125
rs1799782	C	0.965, 0.953	0.5348

P>0.05 = Non significant; P<0.05 = Least significant (*)

Table 7: A comparison of serum biochemical parameters of control and patients of acute coronary syndrome. Data is expressed as Mean ± Standard Deviation (SD). P-value indicates the results of 2-sample t-test**.** N indicates the number of samples in each treatment.

Parameters	Control (N=100)	ACS Patients (N=100)	P-value
	Mean ± SD	Mean ±SD	
Cholesterol (mg/dL)	142.1±54	161.5±79	0.048*
Triglyceride (mg/dL)	123±48.6	124.2±54.6	0.87

P<0.05* = Least significant; P>0.05 = Non significant

Association of serum cholesterol and triglyceride levels with ACS

Analysis of result indicated high cholesterol level in serum among ACS patients (P=0.048) as compared to healthy subjects while triglyceride levels remained unaffected when compared between cases and controls (table, 7).

DISCUSSION

Acute Coronary artery syndrome (ACS) is a complex physiological abnormality caused by the interaction of multiple predisposing genes with the environmental risk factors (Guven *et al.,* 2007). In Pakistan, ACS is the major cause of mortality number of ACS suffering patients is very high (Taqddus *et al*., 2014). The lack of awareness makes it more difficult to lower the rate of annual deaths due to coronary vascular diseases. Delay in presentation to the hospital due to many factors such as lack of knowledge about mild chest pain, unavailability of urgent transport, poor public health care infrastructure, potential time lost by initial consultation with the patient's general practitioner resulting in delay to hospital presentation etc. leads towards increase in death rate due to ACS (Khan *et al.,* 2007). Therefore, it is very important to know the genetic background and the risk factors associated with ACS in Pakistani population to take prophylactic measures either to prevent the disease or delay its onset. Present study was designed to determine the association of four SNPs of DNA repair genes, *XRCC1* and *XPD*, with acute coronary artery syndrome.

Statistical Analysis of various genotypes of *XRCC1* and *XPD* studied codon revealed that phenotype of the subjects is not significantly influenced by the individual variation in genotypic pattern for these single nucleotide polymorphisms (table 3). Similar observation were made by Guven *et al*., 2007 as they had reported that *XPD* Lys751Gln and *XRCC1* Arg399Gln are not involved in causing ACS in Turkish population.

Various genotypic combinations of all four SNPs were combined and compared to calculate their effect on phenotype of subjects. Results revealed that *XRCC1* 280^{++}*/XRCC1* 194^{++} along with three genotypes of *XRCC1* 399 (++, +-and--) was the only combination that was significantly associated with the phenotype of subjects. It was further observed that subjects having wild type combination of all three *XRCC1* SNPs had greater incidence of ACS than any other genotypic combinations (table 4). Our results are in agreement with those of Al-Harithy *et al*. (2011) who had reported a significant association of various *XRCC1* genotypic combinations with colon cancer in Saudi population.

Haplotype analysis in the human genome is relatively new approach in population genetics studies (Stram and Seshan, 2012). This approach may lead us towards the development of more effective strategies to identify genetic variants with increased susceptibility to human diseases (Zhao *et al.,* 2008). Among the major objectives of haplotype analysis is to identify linkage disequilibrium (LD) patterns in different regions and different populations because the LD exists among markers it can play a role to infer population histories and to identify genetic variants underlying complex traits (Stram and Seshan, 2012). To our knowledge this is first haplotype analysis of the four DNA repair gene SNPs to report their association with ACS, so there are no reported literature to compare our findings but our study has revealed several genetic combinations which may result in ACS (tables 5, 6)

Analysis of studied risk factors that majority of the patients (38%) were within the age range of 55-65 years indicating a relationship (P=0.05) of ACS with age (table 2). Gender was highly significantly associated (P<0.001) with the disease as 72% of the diseased subjects were male (table 2). One of the justifications for this finding can be that males in our society are more exposed to environmental pollution, socio-economic tensions and smoking, which are the major risk factors for heart diseases. Our results are in agreement with Towfighi *et al*. (2009) and Morise *et al*. (1997) who had also reported the high prevalence of ACS among males with increase in age. Educational status had a highly significant correlation with ACS (P<0.001) and majority of the patients were uneducated. This can be linked to the lack of awareness regarding use of low cholesterol diet and regular exercise. Diabetes (P<0.001) and hypertension (P <0.001) were found significantly associated with ACS as majority of the ACS patients were suffering from these diseases (table 2). Our results are in agreement with those of Jayes *et al* 1992 and Abbas *et al*. (2003). A significant number of patients had family history of heart disease (P=0.05) (table 2) indicating the inheritance of the disease complementing the findings of Paise *et al*., 1996. Smoking has pronounced effect (P=0.02) on ACS incidence confirming Burazeri *et al*. (2007) who had also found a strong relationship between smoking and coronary artery syndrome and predicted 200% increase in chance of ACS in the smokers.

Serum parameters, cholesterol and triglyceride were determined in both patients and control, we found high cholesterol level among ACS patients (P=0.048) as compared to healthy subjects. Our results are in agreement with Kumar *et al*., 2011 who had also predicted higher cholesterol level in ACS patients (table 7). Triglyceride level was also studied among all the subjects but level of triglyceride was lower in patients as compared to control. In case of triglyceride our results are contradictory to Kumar *et al*. (2011) who predicted high triglyceride level in ACS patients.

CONCLUSION

We concluded that haplotypes are more powerful discriminators between cases and controls in this disease association study. Our results suggest that an analysis based on haplotypes can be advantageous over an analysis based on individual SNPs in the presence of multiple susceptibility alleles, particularly when linkage disequilibria between SNPs is weak. Our results indicated that studied *XRCC1* genotypes and several studied risk fators like age, gender, education, family history diabetes, hypertension and smoking has a strong influence on the incidence of cardio vascular diseases.

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