

INFLUENCE OF INTERLEUKIN-6 (-174 G/C) SINGLE NUCLEOTIDE POLYMORPHISM ON SERUM IL-6 LEVELS IN PREMATURE CORONARY ARTERY DISEASE

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Body

INTRODUCTION

Premature Coronary Artery Disease (PCAD) occurs as the foremost effect of coronary atherosclerosis. Premature CAD is defined as >70% stenosis in at least one vessel on coronary angiography in patients less than 45 years of age.¹ Timely detection and effective management are the key factors for the effective management of PCAD. One of the major events in the pathogenesis of atherosclerotic plaque formation and progression in CAD is inflammation. Multiple pro-inflammatory and anti-inflammatory cytokines play an active role in early atherosclerosis. Interleukin-6 (IL-6) is one of the key players in the modulation of immunological and inflammatory processes during various stages of premature coronary artery disease.²

IL-6 is an inflammatory cytokine which actively takes part in the immune-inflammatory process underlying the pathogenesis of CAD.³ The heritability of IL-6 has been estimated to be about >60%⁴ but still to date only a limited number of gene polymorphisms affecting IL-6 levels are known. The most widely studied of these is the -174 G/C variant in the IL-6 gene promoter region. While some studies have reported significant association of -174 G/C single nucleotide polymorphism with CAD,^{5,6} others have demonstrated no association between them.⁷ Keeping in view the role of IL-6 in the pathogenesis of CAD, it is being considered as a potential target for drug therapy because it has been seen that individuals with variants in IL-6 gene that hampers the IL-6 pathway were found to be at a reduced risk for coronary heart disease.⁸

There is still paucity of studies reporting the potential effect of the IL-6 gene promoter SNP -174 G/C (Rs. 1800795) on the serum IL-6 levels and the risk prediction of CAD in Premature coronary artery disease patients of Pakistan. Hence, we sought to assess the role of the IL-6 gene promoter SNP -174 G/C (Rs. 1800795) and IL-6 serum levels in the diagnosis and risk prediction of PCAD patients.

METHODS

This was a case-control study carried out at the Chemical Pathology Laboratory (CPL), Army Medical College, Rawalpindi, along with the Cardiovascular Genetics Institute, University College London, UK. Ethical approval was duly sought from the Institutional Review Committee of both the concerned institutions. Duration of the study was from July, 2014 to Jan, 2015.

Subjects: Three hundred and sixty four subjects aged less than 45 years reporting to the cardiac care centre with chest pain and scheduled to have angiography were recruited consecutively. Out of these 165 patients who had greater than 70% blockade in at least one coronary artery diagnosed angiographically, were selected as cases. Those who were angiographically free from the disease or had less than 10% stenosis in a single coronary artery

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were selected as controls (n=199). Cases who had previously been diagnosed with any blood disorder, carcinoma, renal, congenital heart disease, hepatitis, liver or thyroid disease or any acute infection were excluded. Demographic characteristics along with ethnicity were noted. Subjects were explained the process in detail and informed consent was taken in written form. The procedures adopted comply with the ethical standards of Helsinki Declaration of 1975.

Biochemical Analysis:

Blood samples were taken on the day which was scheduled for angiography of the respective patient preferably early in the morning. Ten millilitre blood sample was obtained by venipuncture. 7ml was transferred to a red top serum tube for serum analysis and 3 ml transferred to EDTA tube for DNA extraction.

Enzyme Linked Immunosorbent Assay (ELISA) technique was used for measuring the concentrations of serum IL-6 (Duo set kit-RandD systems) commercial kit using human monoclonal antibodies. The coefficient of variation (CV), intra assay, for IL-6 was 5.8% while the limit of detection was less than 2 ng/dl respectively. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN). Genotyping was carried out using Taqman Assay using the standard assay protocol at the Cardiovascular Genetics Institute, University College London, UK. For each Taqman genotyping assay there is a forward primer and a reverse primer which bind sequences either side of the SNP to be genotyped allowing amplification of the region during PCR. There are two allele specific probes labeled with a different fluorophore VIC or FAM at the 5' end. At the 3' end is a quencher which prevents fluorophore from fluorescing. During the process if only one fluorophore fluoresces the sample is homozygous for the corresponding allele.

If a signal from both fluorophores is present the sample is heterozygous. The components when using Taqman buffer are shown in Table 1. 2ul of the reaction mix was added per well. The plate was then centrifuged and the PCR performed using the for Taqman genotyping assays as below.

500C for 2 minutes

950C for 10 minutes

950C for 15 sec

600C for 1 minute

The intensity of each single nucleotide polymorphism (SNP) was based on the criteria of three clear clusters in two scales generated by Sequence Detection Systems (SDS) software version 2.3 (ABI).

Statistical analysis:-

Statistical analysis was done by SPSS-22 (SPSS Inc, Chicago) and MedCalc software version 9.6.4.0. Mean, SD, median was calculated for descriptive statistics. Mann-Whitney U test was applied for comparison of PCAD patients and controls. MedCalc software was used to construct Receiver operator characteristic curve (ROC) to estimate the diagnostic value and diagnostic odds ratio of IL-6. Genotype of -174G>C and allele frequencies were measured by the Chi-square and Fisher exact test, respectively. To evaluate possible relationship of -174G>C polymorphism and increased IL-6 levels with PCAD binary logistic regression analysis was carried out. A p-value of less than 0.05 qualified as significant.

RESULTS

Total 364 subjects consisting of 165 PCAD patients and 199 angio-negative controls participated in the study. Age of PCAD patients was 413.80 years while in controls it was 367.8 years. After applying the Kolmogorov-Smirnov test on data non-Gaussian distribution for cytokines was seen (Pless than 0.05). Demographic data of the subjects is demonstrated in table-II. Serum IL-6 levels were highly raised in PCAD patients (pless than 0.01). Area under

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curve (AUC) and 95% (CI) of IL-6 was 0.667 (0.592 - 0.737) [Fig-I]. A serum level of 3.7 ng/dL had the highest odds ratio of serum IL-6 for PCAD with the maximum sensitivity and specificity is shown in Table III.

In IL-6 -174 (G/C) polymorphism homozygous CC was significantly related to the occurrence of PCAD and serum IL-6 levels were highly raised in CC genotype subjects in contrast to subjects with GC and GG genotypes (pless than 0.05) (Fig 2). Genotypic and allelic distribution of IL-6-174G>C polymorphism is shown in Table IV. Results of binary logistic regression analysis are shown in Table V. Significant correlation was found among serum IL-6 levels, BMI, LDL, total cholesterol, cigarettes smoked per day and the extent of blockade in PCAD patients (Table VI).

DISCUSSION

We observed that the sensitivity and specificity of IL-6 was high for the diagnosis of PCAD. This is in agreement to studies done previously showing association of IL-6 with the degree of calcified coronary stenosis.⁹ This might be because IL-6 overexpression leads to plaque instability and progression.¹⁰

Ferroni et al., 2007 demonstrated that serum IL-6 had significant diagnostic potential in enzyme negative patients with chest pain.¹¹ The sensitivity and specificity of IL-6 has been shown to be 100% and 66% respectively for the prognosis of morbidity in acute coronary syndrome at a cut off value of 41pg/ml.¹²

TABLE I: REAGENT VOLUMES FOR IL-6 RS1800795 TAQMAN GENOTYPING ASSAY USING TAQMAN BUFFER

Reagent	Volume (ul)
Taqman master mix (Life Technologies)	410ul
Nuclease free water (Sigma)	389.5/399.9
40x/80x SNP Specific Assay (Life Technologies)	20.5/10.3

TABLE II: DEMOGRAPHIC CHARACTERISTICS OF PATIENTS AND CONTROLS

Variables	Cases (n=165)	Controls (n=199)	p-Value
Age (years) (Mean SD)	414.23	367.55	0.76
Gender(M/F)	155/ 10	194 / 5	0.63
Weight (Kg) (Mean SD)	73.111.7**	66.710.1	0.0009
Height (m) (Mean SD)	1.680.06	1.690.08	0.85
BMI (Kg/m ²) (Mean SD)	26.323.7**	23.63.5	less than 0.002
Positive Diabetes n (%)	61 (37)**	7(4)	less than 0.01
Positive Premature CAD Family History n (%)	73 (44)**	6 (3)	less than 0.01
Positive DM family history n (%)	61 (37)**	30 (15)	less than 0.01
Smokers n (%)	101 (61)*	78 (39)	less than 0.05
Total Cholesterol (mmol/l) (Mean SD)	4.93.77	4.51.38	0.56
Serum IL-6 (ng/dl) (Mean SD)	3.81.5	2.91.9	less than 0.01

TABLE III: DIAGNOSTIC PERFORMANCE OF IL-6 AT DIFFERENT CUTOFFS FOR DIAGNOSIS OF PREMATURE CORONARY ARTERY DISEASE

Biomarker	SN (%)	95%CI	SP (%)	95%CI	LR+	LR-	DOR
IL-6 (ng/dl)	70.27	58.5-80.3	82.65	73.7-89.6	4.05	0.36	11
> 3.2							
> 3.7*	77.03	65.8-86.0	80.61	71.4-87.9	3.97	0.28	14
> 3.4	78.38	67.3-87.1	78.57	69.1-86.2	3.66	0.28	13

TABLE IV: BINARY LOGISTIC REGRESSION ANALYSIS FOR PREDICTION OF PREMATURE CORONARY ARTERY DISEASE

Variables	B	Exp (B)	(95%CI)	Sig.
IL-6 (ng/dl)	0.020	1.030	(1.003-1.018)**	0.005
IL-6 -174 (G/C) Genotype: CC	0.320	1.072	(0.852-2.047)*	0.04

TABLE V: IL-6-174G>C GENOTYPE AND ALLELE FREQUENCIES OF STUDY POPULATION (N=300)

IL-6 -174 G/C Genotype (percentage)	PCAD patients (n=165)	Controls (n=199)	P-value
GG n (%)	110 (67)	147 (74)	
GC n (%)	45 (27)	50 (25)	0.04*a
CC n (%)	10 (6)	2 (1)	
IL-6-174G>C allele count			
G	265	344	0.027*b

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C

65

54

In the current study, IL-6 promoter region polymorphism -174 G/C was significantly related to PCAD, and it remained significant in multivariate analysis, even after accounting for the confounding variables. Minor allele C at -174G>C was associated with high serum IL-6 levels.¹³ To date, allele frequency information across Pakistan and even in South Asia is limited for the SNPs. Moreover the information that is available has conflicting results and there is marked variation in the risk allele frequencies of these risk SNPs in different populations. While some studies have marked C as the risk allele,¹⁴ others have demonstrated GG to be the genotype conferring the risk of CAD.¹² This is likely caused by the presence of population sub-structure.¹⁵

Another probable explanation may be that the functional SNP in the IL-6 is yet to be identified with -174 G/C being the tagging SNP in certain populations where significant association has not been observed. On performing logistic regression analysis after adjusting for the confounding variables an independent association was found between PCAD and raised serum IL-6 levels and CC genotype of -174G>C polymorphism. This is an important observation because a previous study demonstrates that IL-6 may have a limited role in predicting early mortality in CAD and its performance may be limited by age related factors.¹⁶

Whereas other studies have shown that interleukin-6 concentrations and IL-6 gene promoter SNP -174 G/C aid in the prognostic risk stratification of these CAD patients.¹⁷

We observed significant correlation of serum IL-6 levels with the extent of blockade in PCAD patients. This has been previously observed in a study where there was a positive association between the atherosclerotic burden and serum IL-6 level.¹⁸ This is probably because of the pro-atherogenic nature of IL-6 and its distinctive role in macrophage polarization and other stages of inflammation in atherosclerosis.¹⁹ Serum IL-6 levels also correlated significantly with the BMI in PCAD patients. This may be due to IL-6 altering the resistance to insulin in an individual. This correlation has been reported in a previous study in overweight, post-menopausal women.²⁰

Another study suggests that IL-6 may be secreted in relation to the increase in fat deposit resulting in a positive correlation with BMI and total cholesterol.²¹ As far as the correlation between smoking and IL-6 is concerned a moderate positive correlation between IL-6 and smoking load has been demonstrated earlier.²²

One of the probable explanations for this may be that the cigarette smoke activates alveolar macrophages and epithelial cells to release inflammatory mediators leading to increase in the oxidative stress through stimulatory effects on the acute phase response.²³

TABLE VI: CORRELATION OF SERUM INTERLEUKIN-6 LEVEL WITH DEGREE OF STENOSIS AND RISK FACTORS FOR PREMATURE CORONARY ARTERY DISEASE

Variables	IL-6 ng/dl) r	P-value
Body Mass Index (kg/m ²)	0.216**	pless than 0.01
Triglycerides (mmol/l)	0.189	p=0.32
Total Cholesterol (mmol/l)	0.349*	pless than 0.05
High Density Lipoprotein (mmol/l)	-0.258	p=0.15
Low Density Lipoprotein (mmol/l)	0.272**	pless than 0.01
Cigarettes per day	0.192*	pless than 0.05
Gensini score	0.160*	pless than 0.05

The strengths of our study are based on the factors that we have included angio-negative subjects as disease free controls for a better comparison. It is also the first kind of its study which has studied the diagnostic accuracy of IL-6 in PCAD patients who are young and whose number is increasing alarmingly in Pakistan and South Asia. Moreover, we have identified a cut off value for serum IL-6 level with the best possible sensitivity and specificity for the diagnosis and risk stratification of PCAD. The possible limitation of our study is its relatively limited sample size. Future research endeavors should also include acute coronary syndrome cases of PCAD for a better assessment of the diagnostic efficacy of IL-6 and the role of IL-6 -174 G/C SNP in PCAD patients.

CONCLUSION

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We have seen that IL-6 has good diagnostic performance for diagnosis of PCAD and may aid clinical assessment for better therapeutic protocol. We also identified a significant association of IL-6-174 G>C promoter region polymorphism with PCAD in Pakistani patients with the CC genotype contributing to raised serum levels IL-6 in patients.

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REFERENCES

1. Dhillon T, Niranjana S, Khanna A, Shetty S, Patel U, and Rupanagudi V. Premature Coronary Artery Disease [CAD] in the Asian Immigrant Population: Data from a New York City Hospital. *Chest J* 2004; 126(4): 790S.
2. Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. Recent Advances on the Role of Cytokines in Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2011; 31: 969-79.
3. Kishimoto T. IL-6: from its discovery to clinical applications *Int Immunol* 2010; 22(5): 347-52.
4. Shah T, Zabaneh D, Gaunt T, Swerdlow NI, Shah S, Talmud PJ, et al. Gene-Centric Analysis Identifies Variants Associated With Interleukin-6 Levels and Shared Pathways With Other Inflammation Markers. *Circ Cardiovasc Genet* 2013; 6: 163-70.
5. Sun GQ, Wu GD, Meng Y, Du B, Li YB. IL-6 gene promoter polymorphisms and risk of coronary artery disease in a Chinese population. *Genet Mol Res* 2014; 26; 13(3): 7718-24.
6. Maitra A, Shanker J, Dash D, John S, Sannappa PR, Rao VS, et al. Polymorphisms in the IL6 gene in Asian Indian families with premature coronary artery disease - the Indian Atherosclerosis Research Study. *Thromb Haemost* 2008; 99(5): 944-50.
7. Bhanushali AA, Das BR. Promoter variants in interleukin-6 and tumor necrosis factor alpha and risk of coronary artery disease in a population from Western India. *Indian J Hum Genet* 2013; 19(4): 430-36.
8. Hartman J, Frishman WH. Inflammation and atherosclerosis: A review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy. *Cardiol Rev* 2014; 22(3): 147-51.
9. Saremi A, Anderson RJ, Luo P, Moritz TE, Schwenke DC, Allison M, et al. Association between IL-6 and the extent of coronary atherosclerosis in the veterans affairs diabetes trial (VADT). *Atherosclerosis* 2009; 203(2): 610-14.
10. Koenig W, Khuseyinova N. Biomarkers of Atherosclerotic Plaque Instability and Rupture. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2007; 27: 15-26.
11. Ferroni P, Antonello R, Franco R, Palmirotta R, Guadagni F, Davi G, et al. Prognostic significance of interleukin-6 measurement in the diagnosis of acute myocardial infarction in emergency department. *Clinica Chimica Acta* 2007; 381(2): 151-56.
12. Mowafy A, El-Akabawy H, Hussein A, Abd El Hay A. Prognostic value of IL6 in young adults presenting with acute coronary syndrome. *Egyptian Heart J* 2015; 67(2): 151-8.
13. Niu W, Liu Y, Qi Y, Wu Z, Zhu D, Jin W. Association of interleukin-6 circulating levels with coronary artery disease: A meta-analysis implementing mendelian randomization approach. *Int J Cardiol* 2012; 157(2): 243-52.

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14. Satti HS, Hussain S, Javed Q. Association of Interleukin-6 Gene Promoter Polymorphism with Coronary Artery Disease in Pakistani Families. *Scientific World Journal*. 2013 Dec 2; 2013: 538365. doi: 10.1155/2013/538365.
15. Phulukdaree A, Khan S, Ramkaran P, Govender R, Moodley D, Chuturgoon AA. The Interleukin-6 -174 G/C Polymorphism Is Associated with Increased Risk of Coronary Artery Disease in Young South African Indian Men. *Metabolic syndrome and related disorders*. 2013; DOI: 10.1089/ met. 2012.0130
16. Reiner AP, Carlson CS, Ziv E, Iribarren C, Jaquish CE, Nickerson DA. Genetic ancestry, population sub-structure, and cardiovascular disease-related traits among African-American participants in the ***CARDIA Study***. *Hum Genet* 2007; 121(5): 565-75.
17. Kanikowska D, Pyda M, Korybalska K, Grajek T, Lesiak M, Breborowicz A, et al. Age-related limitations of interleukin-6 in predicting early mortality in acute ST-elevation myocardial infarction. *Immunity and Ageing* 2014; 11: 23.
18. Lai CL, Ji YR, Liu XH, Xing JP, Zhao JQ. Relationship between coronary atherosclerosis plaque characteristics and high sensitivity C-reactive proteins, interleukin-6. *Chin Med J (Engl)* 2011; 124(16): 2452-56.
19. Autieri MV. Proand Anti-Inflammatory Cytokine Networks in Atherosclerosis. Autieri MV. Pro-and anti-inflammatory cytokine networks in atherosclerosis. *ISRN Vascular Medicine* 2012 Nov 19; 2012.
20. Fenkci S, Rota S, Sabir N, Sermez Y, Guclu A, Akdag B. Relationship of serum interleukin-6 and tumor necrosis factor alpha levels with abdominal fat distribution evaluated by ultrasonography in overweight or obese postmenopausal Women. *J Investig Med* 2006; 54(8): 455-60.
21. Khaodhiar, LLing P, Blackburn GL, Bistrain BR. Serum Levels of Interleukin-6 and C-Reactive Protein Correlate With Body Mass Index Across the Broad Range of Obesity. *J Parenter Enteral Nutr* 2004; 28(6): 410-15.
22. Hacievliyagil SS, Mutlu LC, Temel I. Airway inflammatory markers in chronic obstructive pulmonary disease patients and healthy smokers. *Niger J Clin Pract* 2013; 16(1): 76-81.
23. Garcia-Rio F, Miravittles M, Soriano JB, Munoz L, Duran-Tauleria E, Sanchez G, et al. Systemic inflammation in chronic obstructive pulmonary disease: A population-based study. *Respir Res* 2010; 11: 63.

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