DOI: 10.7860/JCDR/2023/63067.18318

Original Article

Biochemistry Section

Association between Q192R Polymorphism of Paraoxonase-1 Gene and Risk of Coronary Artery Disease: A Case-control Study

HARLEEN KAUR[1], JASKIRAN KAUR[2], GURINDER MOHAN[3], NISHANT SHARMA[4], SAHIBA KUKREJA[5]

ABSTRACT

Introduction: Coronary Artery Disease (CAD) is a complex metabolic disorder in which lifestyle and genetic factors are known to play key roles in pathogenesis. Extensive studies have examined the role of Paraoxonase 1 (PON1) polymorphisms as genetic markers of CAD. However, the evidence regarding their role in the aetiology of CAD remains contradictory.

Aim: The aim of this study was to determine the genotypic/allelic frequency of the Q192R polymorphism of the PON1 gene and its association with the risk of CAD.

Materials and Methods: A case-control study was conducted at the Department of Biochemistry in collaboration with the Department of Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India, from November 2019 to December 2022. A total of 100 participants were divided into two groups: group I (CAD patients) and group II (healthy controls) to investigate the association between the Q192R polymorphism and CAD risk. The genotypic and allelic frequencies of the Q192R polymorphism of the PON1 gene and its association with CAD were determined using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP), followed by 2% agarose gel electrophoresis. The data were statistically analysed using the Chi-square test.

Results: There were a total of 50 participants in each group (29 males and 21 females), with a mean age of 47.02±8.79 years in group I and 37.70±7.29 years in group II (p-value <0.001). The present study confirmed that individuals with QR+RR genotype compared to QQ genotype (OR: 2.47; 95% CI: 1.10-5.55; p-value=0.03) of the Q192R polymorphism were associated with an increased risk of CAD. A higher frequency of the RR genotype of the Q192R polymorphism was detected in CAD patients, and it was associated with a 3-fold increased risk of developing CAD (p-value <0.05). However, the difference between QR versus QQ genotypes {Odds Ratio (OR): 2.13; 95% CI: 0.89-5.12, p-value=0.09} was found to be statistically insignificant. Furthermore, a significantly higher frequency of the R allele was observed in CAD patients compared to controls (OR 2.12, 95% CI: 1.18-3.84, p-value=0.01).

Conclusion: In conclusion, the Q192R polymorphism of the PON1 gene was associated with an increased risk of CAD in the Punjabi population. The results confirmed that the presence of the R allele was associated with an increased risk of CAD.

Keywords: Alleles, Cardiovascular disease, Genotypes, Metabolic disorders

INTRODUCTION

Cardiovascular Diseases (CVD), such as CAD and stroke, have emerged as primary health conditions related to high mortality and a pronounced socio-economic burden in developing countries [1]. The prevalence of CAD in India is 21.4% for diabetics and 11% for non-diabetics [1]. There are numerous risk factors for CAD. Several risk factors, such as high blood pressure, hypercholesterolemia, smoking, diabetes mellitus, obesity, sedentary lifestyle, poor eating habits, and stress, are modifiable. However, factors like age, sex, family history, and race cannot be changed and are considered non-modifiable risk factors [2]. The aetiology of CAD involves the interaction of multiple environmental and genetic factors that play an essential role in its pathogenesis [3].

According to genetic association studies, a number of genes involved in metabolism, the renin-angiotensin system, inflammation, and blood coagulation have been associated with the development of human diseases [4]. Dai X et al., have shown that genetic variables have an effect on the occurrence of CAD and myocardial infarction [5]. Paraoxonase 1 (PON1) has been extensively researched in relation to CVD, stroke, inflammation, and oxidative stress [6]. Lower serum PON1 activity is an independent risk factor for CAD because myocardial infarction patients had lower serum PON1 activity concentrations when compared to controls [4].

Paraoxonase 1 participates in the metabolism of lipoprotein phospholipids and is primarily expressed with High-density Lipoprotein (HDL) [7]. Human serum PON1 is one of the susceptibility genes that plays a major role in vascular pathology and is thus regarded as an emerging biomarker for CAD [8]. PON1 is a calcium-dependent enzyme with a molecular weight of 43 kDa, composed of 354 amino acids. It is synthesised from the PON1 gene, which is located between q21.3 and q22.1 on the long arm of human chromosome 7 (7q21-22) [6]. Human organs such as the liver, heart, kidney, lungs, brain, and small intestine have all been found to express PON1 Messenger Ribonucleic Acid (mRNA) [4]. PON1 is released into the blood by the liver, where it primarily associates with HDL and is regarded as a key factor for the antioxidative activity of HDL [8]. It has the ability to hydrolyse oxidised Low-density Lipoprotein (LDL)-cholesterol and lipid hydroperoxides, thus proving to be a potential atheroprotective molecule [9].

Human PON1 has several Single-nucleotide Polymorphisms (SNPs). The coding sequence (exons), non-coding sequence (introns), and other regulatory regions of the human PON1 gene contain nearly 160 polymorphisms [10]. The present study examined the Q192R SNP located in the coding region of the PON1 gene. There are three variants of this polymorphism: QQ, QR, and RR. The Q192R polymorphism is characterised by an exchange of the isoform glutamine (Q) to arginine (R), which disrupts the antioxidative potential of the PON1 enzyme and results in a greater risk of CAD [10].

Some studies have been conducted on Asian Indians, mainly focusing on other parts of India [8,11]. Therefore, the present case-control

study was designed to determine the genotypic frequency of the PON1 Q192R polymorphism and to explore its association with CAD risk in the Punjabi population in India.

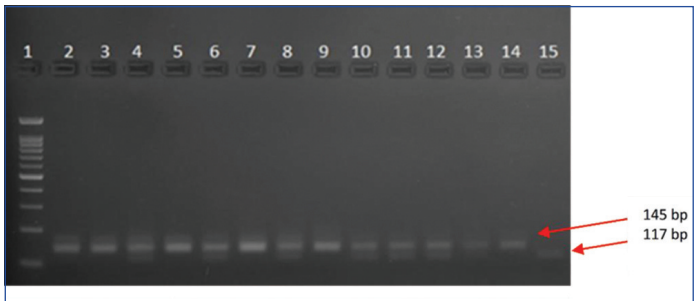
MATERIALS AND METHODS

A case-control study was conducted in the Department of Biochemistry, in collaboration with the Department of Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India, from November 2019 to December 2022. The study was carried out after obtaining approval from the Institutional Research and Ethical Committee (IEC No: Patho 70/2020 dated 12.02.2020). Written consent was obtained from every participant in the study.

Inclusion criteria: Patients with CAD were included. CAD was diagnosed by a cardiologist based on medical records showing medical indications, such as history, physical assessment, and ECG patterns compatible with CAD (e.g., presence of Q waves, ST elevation or depression, inverted T waves) [12]. Healthy individuals with no personal or family history of CAD were included as controls. Cases and controls were matched for sex and had an age <55 years (males) and <65 years (females).

Exclusion criteria: Participants with chronic kidney disease, hepatic disease, and autoimmune disease were excluded from the study.

A total of 100 participants presenting to the department during the study duration were enrolled in the study using purposive sampling. Out of the 100 participants, 50 were CAD patients (group I) and 50 were normal healthy controls (group II). After selecting participants based on the inclusion and exclusion criteria, weight, height, and Body Mass Index (BMI) were assessed in all participants and compared between both groups. A 2 mL blood sample was collected and mixed with an anticoagulant vacutainer containing Ethylenediaminetetraacetic Acid (EDTA) for use in molecular assays. Genomic DNA was extracted from nucleated blood cells using Qiagen DNeasy blood and tissue kits. The PON1 gene was amplified using ABI Veriti PCR to determine the Q192R polymorphism of the PON1 gene. The PCR product was visualised on 2% agarose gels with ethidium bromide staining to observe the 176-bp undigested amplicon. The MboI (↓GATC; New England Biolabs) restriction enzyme was used to digest the Q192R products. The presence of bands of 145 bp confirmed the presence of the Q allele, while a band of 117 bp confirmed the presence of the R allele at the Q192R locus, as shown in [Table/Fig-1].



[Table/Fig-1]: Gel documentation for genotyping the Q192R polymorphism of the PON1 gene.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) Version 21. Categorical variables were described using counts (%) and demographic continuous data using mean±Standard Deviation (SD). Demographic data were analysed using a t-test. A Chi-square test was used to analyse genotypic and allelic frequencies, and Odds Ratios (ORs) were calculated along with 95% Confidence Intervals (CIs). A p-value of ≤0.05 was defined as statistically significant.

RESULTS

Out of the 100 participants, 50 were enrolled in each of the two groups, I and II. In the present study, the mean age was 47.02±8.79 years in group I and 37.70±7.29 years in group II. Among the cases and controls, 29 (58%) were males and 21 (42%) were females. When comparing the mean weight, height, and BMI among both groups, it was found that weight and BMI were higher in CAD patients than in healthy controls [Table/Fig-2].

Characteristics		Group I (Mean±SD)	Group II (Mean±SD)	p-value
Gender, n (%)	Males	29 (58)	29 (58)	-
	Females	21 (42)	21 (42)	
Age (years)		47.02±8.79	37.70±7.29	**<0.001**
Weight (Kg)		69.62±12.31	64.98±11.51	**0.05**
Height (m)		1.65±0.074	1.67±0.09	0.145
BMI (Kg/m^2)		25.66±3.95	23.14±2.88	**<0.001**

[Table/Fig-2]: Distribution of gender and comparison of age, weight, height and BMI among both groups.
T-test used; The p-value in bold font was considered statistically significant

The present study compared the genotypic and allelic distribution of the Q192R polymorphism in CAD patients and normal healthy control participants to examine their association with CAD risk. The number of individuals carrying the QQ genotype and Q allele was higher in controls than in CAD patients, while the number of participants with the QR and RR genotypes and the R allele was higher in CAD patients compared to normal healthy controls. Q and R were identified as the major and minor alleles, respectively, in the healthy controls (73% vs 27%) as shown in [Table/Fig-3].

	Group I n (%)	Group II n (%)	χ^2	Odds ratio (95% CI)	p-value
Genotypes					
QQ	17 (34)	28 (56)	Reference range		
QR	22 (44)	17 (34)	2.92	2.13 (0.89-5.12)	0.09
RR	11 (22)	5 (10)	4.56	3.62 (1.07-12.24)	**0.03**
QR+RR	33 (66)	22 (44)	4.89	2.47 (1.10-5.55)	**0.03**
Alleles					
Q	0.56 (56)	0.73 (73)	Reference range		
R	0.44 (44)	0.27 (27)	6.31	2.12 (1.18-3.84)	**0.01**

[Table/Fig-3]: Distribution and association of genotypic/allelic frequency of Q192R polymorphism of PON1 gene in CAD patients and healthy controls.
Chi-square test; The p-value in bold font was considered statistically significant

DISCUSSION

Cardiovascular Diseases (CVD), particularly coronary artery disease and stroke, are the leading causes of death worldwide and major contributors to disability [13]. In the present study, CAD patients were found to be older compared to normal healthy controls. The significant higher mean age of CAD patients could be attributed to oxidative stress, which increases with age and leads to functional and electrical abnormalities, thereby contributing to CAD [14]. When comparing CAD patients to healthy controls, their BMI was significantly higher. This could be due to adipose cells, which are believed to be endocrine in nature and play a crucial role in maintaining body metabolism homeostasis. Adipose cells have the ability to produce proinflammatory cytokines such as Interleukin-6 (IL-6), C-reactive Protein (CRP), and tumour necrosis factor-alpha, as well as fat-related hormones like leptin and adiponectin, which contribute to the atherosclerotic process that can cause CAD [15].

High-density Lipoprotein-Cholesterol (HDL-C) can serve as an important independent marker for atherosclerosis and CAD [16].

Ethnicity	Place and year of the study	Sample size	Genotypic frequency						Allelic frequency			
			Control			CAD patients			Control		CAD patients	
			QQ	QR	RR	QQ	QR	RR	Q	R	Q	R
Present study	India, 2019-2022	100	56%	34%	10%	34%	44%	22%	73%	27%	56%	44%
Mohamed RH et al., [19]	Egypt, 2010	200	46%	34%	20%	12%	37.3%	50.6%	63%	37%	31%	69%
Sanghera DK et al., [20]	Singapore, 1997	347	47%	40%	13%	27%	59%	14%	67%	33%	57%	43%
Ferré N et al., [21]	Spain, 2002	215	49.3%	43.2%	7.5%	48.8%	40.4%	10.8%	75%	25%	69%	31%
Balcerzyk A et al., [22]	Poland, 2007	358	48%	39%	13%	60%	36%	4%	68%	32%	78%	22%

[Table/Fig-4]: Genotypic and Allelic Frequencies of Q192R polymorphism of PON1 gene among different populations [19-22].
Q (Glutamine); R (Arginine)

PON1, which resides in HDL-C particles, possesses antioxidant properties and has been extensively studied at the genotypic and phenotypic levels for its ability to counteract oxidative damage and reduce the risk of atherosclerosis [17]. PON1 has been associated with lipid metabolism as it can hydrolyse oxidised LDL-cholesterol and lipid hydroperoxides, establishing itself as a potential atheroprotective molecule [18].

The present study aimed to investigate the association between the Q192R polymorphism of the PON1 gene and the risk of CAD. Variations in the distribution of Q and R alleles, with reference to their major and minor allele states, have been reported in various ethnic populations [Table/Fig-4] [19-22]. Among the control participants in the present study, Q and R were identified as the major and minor alleles, respectively, at position 192 within the PON1 gene. Therefore, the distribution of the Q and R alleles of the PON1 gene in the Punjabi control population was similar to that reported in the Egyptian population [19]. However, it differed from that of the Hispanic population, where the R allele was predominant [11]. The present study also observed an increased frequency of QR and RR genotypes in CAD patients compared to healthy controls, indicating a significant association of the Q192R polymorphism with CAD risk in the studied population. This finding suggests that the PON1 gene may play an important role in CAD pathology. It is consistent with a previous study conducted in a northwest Punjabi population, where the QR and RR genotypes were associated with an increased risk of developing CAD [23]. Sanghera DK et al., also linked the Q192R polymorphism to an increased risk of CAD in Asian Indian communities [20]. However, negative results have been reported by Ferré N et al., in Spanish populations and Balcerzyk A et al., in Polish populations, which are contradictory to our findings [21,22].

In the present study, the Odds Ratios (ORs) were slightly higher, indicating an association of the RR genotype of the Q192R polymorphism with a three-fold increased risk of CAD in the Punjabi population. This suggests that the associations of the 192 R allele may be stronger in specific populations. These results are consistent with a previous study conducted by Gupta N et al., in the Indian population [23]. The R (Arg) allele (high-activity R isoform) is less effective than the Q (Gln) allele (low-activity Q isoform) in reducing the oxidative modification of LDL, which reduces lipid peroxide hydrolysis. This explains why the PON1-192 polymorphism is linked to an increased risk of CAD [16]. PON1 inhibits the oxidation of LDL-C and prevents the accumulation of oxidised lipids in blood vessels, thereby slowing down the atherosclerotic process and the progression of CAD [24].

Limitation(s)

The main limitation of the present study was that it was a time-bound study, where 100 samples were collected over a period of 1½ years. To validate the results, it is necessary to replicate the study with a larger sample size.

CONCLUSION(S)

The genotypic and allelic frequency of the Q192R polymorphism of the PON1 gene, specifically the R isoform (QR, RR, and R), was found to be higher in CAD patients compared to controls. The present study observed statistically significant associations between the QR+RR and RR genotypes and a 2-fold and 3-fold increased risk of developing CAD, respectively. The presence of the RR genotype and R allele can aid in early prediction, diagnosis, and management by providing valuable information about the disease risk, as they are more prevalent in CAD participants compared to normal healthy controls. This biomarker can be utilised for the diagnosis of CAD.

Acknowledgement

The authors express their gratitude to all the participants who were part of the present study.

REFERENCES

[1] Sreeniwas Kumar A, Sinha N. Cardiovascular disease in India: A 360 degree overview. Med J Armed Forces India. 2020;76(1):01-03. Doi: 10.1016/j.mjafi.2019.12.005. Epub 2020 Jan 13. PMID: 32020960; PMCID: PMC6994761.
[2] Hajar R. Risk Factors for coronary artery disease: Historical perspectives. Heart Views. 2017;18(3):109-14. Doi: 10.4103/HEARTVIEWS.HEARTVIEWS_106_17. PMID: 29184622; PMCID: PMC5686931.
[3] Wang H, Liu Z, Shao J, Jiang M, Lu X, Lin L, et al. Pathogenesis of premature coronary artery disease: Focus on risk factors and genetic variants. Genes & diseases. 2020;9(2):370-80. https://doi.org/10.1016/j.gendis.2020.11.003.
[4] Alharbi KK, Alnbaheen MS, Alharbi FK, Hasanato RM, Khan IA. Q192R polymorphism in the PON1 gene and familial hypercholesterolemia in a Saudi population. Annals of Saudi Medicine. 2017;37(6):425-32. Doi: 10.5144/0256-4947.2017.425. PMID: 29229890; PMCID: PMC6074118.
[5] Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial infarction. World J Cardiol. 2016;8(1):01-23. Doi: 10.4330/wjc.v8.i1.1. PMID: 26839654; PMCID: PMC4728103.
[6] Shunmoogam N, Naidoo P, Chilton R. Paraoxonase (PON)-1: A brief overview on genetics, structure, polymorphisms and clinical relevance. Vasc Health Risk Manag. 2018;14:137-43. Doi: 10.2147/VHRM.S165173. PMID: 29950852; PMCID: PMC6014389.
[7] Al-Hakeem MM, Abotalib Z, Alharbi KK, Khan IA. Relationship between the paraoxonase 1 gene glutamine 192 to arginine polymorphism and gestational diabetes mellitus in Saudi women. Clinical Biochemistry. 2014;47(15):122-25. Doi: 10.1016/j.clinbiochem.2014.07.005. PMID: 25036896.
[8] Kaur S, Bhatti GK, Vijayvergiya R, Singh P, Mastana SS, Tewari R, et al. Paraoxonase 1 gene polymorphisms (Q192R and L55M) are associated with coronary artery disease susceptibility in Asian Indians. International Journal of Diabetes and Metabolism. 2019;24(1-4):38-47. Doi: 10.1159/000494508.
[9] Moya C, Máñez S. Paraoxonases: Metabolic role and pharmacological projection. Naunyn Schmiedebergs Arch Pharmacol. 2018;391(4):349-59. Doi: 10.1007/s00210-018-1473-9. PMID: 29404699.
[10] Raza ST, Abbas S, Wani IA, Eba A, Mahdi F. Clinical implications of PON1 (rs662) and TNF-α (rs1799964) genes polymorphism in patients with coronary artery disease. Egypt J Med Hum Genet. 2022;23(1):01-07. https://doi.org/10.1186/s43042-022-00318-5.
[11] Rojas-García AE, Solís-Heredia MJ, Piña-Guzmán B, Vega L, López-Carrillo L, Quintanilla-Vega B. Genetic polymorphisms and activity of PON1 in a Mexican population. Toxicology and Applied Pharmacology. 2005;205(3):282-89. Doi: 10.1016/j.taap.2004.10.015. Epub 2004 Dec 8. PMID: 15922013.
[12] Fridman O, Gariglio L, Riviere S, Porcile R, Fuchs A, Potenzoni M. Paraoxonase 1 gene polymorphisms and enzyme activities in coronary artery disease and its relationship to serum lipids and glycemia. Archivos de Cardiologia de Mexico. 2016;86(4):350-57. Doi: 10.1016/j.acmx.2016.08.001. PMID: 27640339.
[13] Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: Update from the GBD 2019 study. J Am Coll Cardiol. 2020;76(25):2982-3021. Doi: 10.1016/j.jacc.2020.11.010. PMID: 33309175; PMCID: PMC7755038.
[14] Rodgers JL, Jones J, Bolleddu SI, Vanthenapalli S, Rodgers LE, Shah K, et al. Cardiovascular risks associated with gender and aging. J Cardiovasc Dev Dis. 2019;6(2):19. Doi: 10.3390/jcdd6020019. PMID: 31035613; PMCID: PMC6616540.

[15] Alkhawam H, Nguyen J, Sayanlar J, Sogomonian R, Desai R, Jolly J, et al. Coronary artery disease in patients with body mass index ≥30 kg/m(2): A retrospective chart analysis. J Community Hosp Intern Med Perspect. 2016;6(3):31483. Doi: 10.3402/jchimp.v6.31483. PMID: 27406452; PMCID: PMC4942517.
[16] Abd Elgwad ER, Behiry EG, Swailem FM, Ameen SG, Abdelhasib DM, Abd Elhamid RO. Association between Q192R polymorphism in the PON1 gene and statin responses in cardiac patients. Annals of Medicine and Surgery. 2018;31:01-05. Doi: 10.1016/j.amsu.2018.05.007. PMID: 29922459; PMCID: PMC6004736.
[17] Brites F, Martin M, Guillas I, Kontush A. Antioxidative activity of high-density lipoprotein (HDL): Mechanistic insights into potential clinical benefit. BBA Clin. 2017;8:66-77. Doi: 10.1016/j.bbacli.2017.07.002. PMID: 28936395; PMCID: PMC5597817.
[18] Meneses MJ, Silvestre R, Sousa-Lima I, Macedo MP. Paraoxonase-1 as a regulator of glucose and lipid homeostasis: Impact on the onset and progression of metabolic disorders. Int J Mol Sci. 2019;20(16):4049. Doi: 10.3390/ijms20164049. PMID: 31430977; PMCID: PMC6720961.
[19] Mohamed RH, Mohamed RH, Karam RA, Abd El-Aziz TA. The relationship between paraoxonase1-192 polymorphism and activity with coronary artery disease. Clin Biochem. 2010;43(6):553-58. Doi: 10.1016/j.clinbiochem.2009.12.015. PMID: 20026099.
[20] Sanghera DK, Saha N, Aston CE, Kamboh MI. Genetic polymorphism of paraoxonase and the risk of coronary heart disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 1997;17(6):1067-73. Doi: 10.1161/01.atv.17.6.1067. PMID: 9194756.
[21] Ferré N, Tous M, Paul A, Zamora A, Vendrell JJ, Bardají A, et al. Paraoxonase Gln-Arg(192) and Leu-Met(55) gene polymorphisms and enzyme activity in a population with a low rate of coronary heart disease. Clin Biochem. 2002;35(3):197-203. Doi: 10.1016/s0009-9120(02)00295-3. PMID: 12074827.
[22] Balcerzyk A, Zak I, Krauze J. Synergistic effects between Q192R polymorphism of paraoxonase 1 gene and some conventional risk factors in premature coronary artery disease. Arch Med Res. 2007;38(5):545-50. Doi: 10.1016/j.arcmed.2007.03.004. PMID: 17560461.
[23] Gupta N, Singh S, Maturu VN, Sharma YP, Gill KD. Paraoxonase 1 (PON1) polymorphisms, haplotypes and activity in predicting cad risk in North-West Indian Punjabis. PLoS One. 2011;6(5):e17805. Doi: 10.1371/journal.pone.0017805. PMID: 21629682; PMCID: PMC3101202.
[24] Otocka-Kmiecik A. Effect of carotenoids on Paraoxonase-1 activity and gene expression. Nutrients. 2022;14(14):2842. Doi: 10.3390/nu14142842. PMID: 35889799; PMCID: PMC9318174.

PARTICULARS OF CONTRIBUTORS:
1. PhD Student, Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
2. Professor, Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
3. Professor and Head, Department of Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
4. Statistician, Department of Community Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
5. Professor and Head, Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sahiba Kukreja,
Flat No. 202, Block-2, Cooperative Housing Complex,
Kapurthala-144601, Punjab, India.
E-mail: drsahibakukreja@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 08, 2023
- Manual Googling: Mar 16, 2023
- iThenticate Software: Apr 13, 2023 (24%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 25, 2023**
Date of Peer Review: **Mar 10, 2023**
Date of Acceptance: **Apr 24, 2023**
Date of Publishing: **Aug 01, 2023**