Original Article

DOI: 10.7860/JCDR/2023/59242.17653

Correlation of Homocysteine, Paraoxonase 1 and Malondialdehyde in Healthy Elderly Population: A Cross-sectional Study

Biochemistry Section

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ABSTRACT

Introduction: There is rapid increase in the life expectancy worldwide. Altered metabolism and biochemical interactions between homocysteine, Paraoxonase 1 (PON1) and Malondialdehyde (MDA) result in development and worsening of various diseases of elderly population.

Aim: To correlate the levels of homocysteine, PON1 and MDA in healthy elderly population with respect to age, vitamin B12 and lipid profile.

Materials and Methods: The present cross-sectional study was conducted in Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Pune, Maharashtra, India between July 2013 to July 2014. Study group consisted of 61 participants (30 elderly and 31 young healthy volunteers). Serum homocysteine and vitamin B12 were estimated by Chemiluminescence Microparticle Immunoassay (CMIA) while PON1 and MDA by spectrophotometry. Lipid profile was estimated by biochemistry autoanalyser. Statistical analysis was done by Pearson's correlation coefficient.

Results: Homocysteine and PON1 levels were found to be lower in elderly participants than in young participants (p-value <0.05, <0.01). The levels of vitamin B12 and MDA were higher in elderly participants than in young participants (p-value <0.01, <0.01). The levels of Total Cholesterol (TC) (p-value <0.01), High Density Lipoprotein (HDL) (p-value <0.05) and Low Density Lipoprotein (LDL) (p-value <0.05) were statistically significantly high in elderly participants as compared to young participants. Statistically significant negative correlation of homocysteine with vitamin B12 levels in both elderly participants (p-value <0.01) as well as young participants (p-value <0.01) were observed. There was no statistically significant correlation between homocysteine and PON1 well as MDA in both the groups. There was statistically significant negative correlation between the levels of homocysteine and TC (p-value <0.05), HDL (p-value <0.05) and LDL (p-value <0.01) in elderly participants.

Conclusion: Elderly population is not at the risk of developing diseases whose risk factor is homocysteine. Males are at higher risk of development of diseases because of homocysteine than females of any age group. Homocysteine is not pathogenic in old age even in the presence of other risk factors such as lipid peroxidation, decreased defense mechanisms to lipid peroxidation, raised levels of atherogenic lipids and overweight. Improvement in the vitamin B12 status can decrease the homocysteine levels.

Keywords: Atherogenic lipids, Chronic degenerative diseases, Vitamin B12

INTRODUCTION

According to the Government of India; elderly or old age consists of ages 60 years or above. There is a shift in the pattern of leading causes of disease and death. This is characterised by the waning of infectious and acute diseases and the emergence of chronic and degenerative diseases. These diseases are Cardiovascular Diseases (CVD), cerebrovascular accidents, diabetic complications, ocular diseases, fractures and Alzheimer's disease etc. Hyperhomocysteinemia is an independent risk factor and has causative role in CVDs like myocardial infarction [1], arterial and venous thrombosis [2], other coronary artery diseases [3,4], arrhythmias (like atrial fibrillation and ventricular arrhythmia) and heart failure [5]. Also, in diseases affecting central nervous system which are ischemic cerebral stroke [6], dementia and Alzheimer's disease [7], other neurocognitive and psychological impairments [8] and psychiatric disorders like bipolar disorder [9]. Homocysteine causes progression of diabetic retinopathy [10] and age related macular degeneration [11]. Also, involved in osteoporotic fractures [12]. Homocysteine is a sulphur containing amino acid formed from methionine. Hyperhomocysteinemia is involved in atherosclerosis, thromboembolism and vascular endothelial damage. It promotes formation of thrombin through activation of FactorV. Normally FactorV is associated with endothelial cells exclusively. But in atherosclerotic vessels it is associated with smooth muscle cells and macrophages [13]. Homocysteine increases thrombomodulin synthesis at Ribonucleic Acid (mRNA) level and inhibits protein C activation. Thrombus formed from such homocysteinylated fibrinogen has a higher resistance for lysis. Hyperhomocysteinemia inhibits Deoxyribonucleic Acid (DNA) synthesis in vascular endothelial cells leading to neutrophil migration across the endothelial surface which adds to damage and detachment of endothelial cells. It also produces connective tissue changes in the arteriosclerotic plaques like fibrosis, calcification, proteoglycan deposition [14].

PON1 a calcium dependent multifunctional enzyme. It is a 355 amino acid glycoprotein synthesised in the liver and transported in plasma by binding to High-density Lipoprotein (HDL). PON1 was identified as an organophosphate-hydrolysing enzyme in mammalian tissue. PON1 has also been shown to metabolise a number of drugs and prodrugs via its lactonase activity. It destroys covalent linkages between lipid peroxidation products and Low-density Lipoprotein (LDL). PON1 also degrades hydrogen peroxide, a major Reactive Oxygen Species (ROS). Thus, PON1 has antioxidant and antiatherogenic activity. Hepatic expression of the PON1 gene is down regulated by hyperhomocysteinemia [15]. MDA has been widely used for many years as a convenient biomarker for lipid peroxidation and oxidative stress. MDA is an end product of arachidonic acid and larger Poly-Unsaturated Fatty Acids (PUFAs). MDA is strongly reactive toward nucleophiles, such as basic amino acid residues (i.e., lysine, histidine and arginine) forming Advanced Lipid peroxidation End-Products (ALEs). ALEs promote intramolecular or intermolecular protein/DNA cross linking.

Under oxidative stress, MDA also undergoes oxidation by mitochondrial aldehyde dehydrogenase followed by decarboxylation to produce MDA Acetaldehyde (MAA) adducts. These MAA adducts are highly immunogenic [16].

It has been observed that biochemical interactions between homocysteine, PON1 and MDA result in development and acceleration of various diseases. Higher levels of homocysteine and MDA are correlated with lower levels of PON1. An increase in life expectancy should not only mean a decrease in mortality but it should also be in terms of maintaining a good 'quality of life'. Mortality and disability of old age are mostly due to CVDs, cerebrovascular accidents, diabetic complications, ocular diseases, fractures and Alzheimer's disease etc. Homocysteine is associated with these risk factors directly or indirectly [17,18]. So, reduction in homocysteine levels in middle aged and elderly adults will definitely help to increase life expectancy. So, this study was designed to understand the biochemical interactions between MDA, malodialdehyde and PON1 with respect to age, gender, vitamin status and Body Mass Index (BMI) etc.

MATERIALS AND METHODS

This was a cross-sectional study, conducted at Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Pune, Maharashtra, India, between July 2013 to July 2014. Institutional Ethical Committee (IEC) approval was obtained for the study. The study group was comprised of a total 61 participants. They were divided into two groups. Group I: The group comprised of 30 elderly healthy volunteers of age from 46 to 66 years of both genders. Group II: The group was comprised of 31 healthy young male and female volunteers of age from 15 to 45 years of both the genders.

The study was announced and willing individuals were asked for a specific history of any known condition as mentioned in exclusion criteria. Physical general and systemic examination was performed to measure the parameters required for the study and to confirm whether the history given was correct. Eligible individuals between the periods of two months were enrolled as a sample population and divided into two groups as stated.

Inclusion criteria for controls and cases: Healthy volunteers were included in the study

Exclusion criteria for controls and cases: Participants having conditions like women on hormone replacement therapy, pregnancy, history of CVDs, history of smoking and alcoholism, severe hepatic impairment, renal impairment, diabetes mellitus, hypothyroidism, malignancies. Participants on medications like vitamin B complex, Folate antagonists (Methotrexate, Phenytoin, Carbamazepine), vitamin B6 antagonists (Theophylline), Metformin, Diuretics, Nicotine were excluded from the study.

After obtaining written informed consent; participant's detailed physical and clinical examination was carried out. BMI was calculated and interpreted by National Centre for Health Statistics age and gender specific percentile curves [19].

One mL of fasting blood sample (after 12 hours of overnight fast) was collected from antecubital fossa without venous occlusion in a plain vacutainer. Blood was allowed to clot for one hour. Serum was separated after centrifugation at 2000 rpm for 10 minutes at room temperature. Following parameters were estimated in the serum which were free from haemolysis and turbidity [Table/Fig-1] [20-23].

STATISTICAL ANALYSIS

Data obtained was statistically analysed by using Statistical Package for the Social Sciences (SPSS) software version 17.0. Statistical tests used are: Unpaired t-test for finding the difference in the mean value in the groups; Pearson's correlation coefficient for finding correlation of homocysteine levels with PON1, MDA, vitamin B12 and lipid profile in both the groups. The p-value <0.01 and <0.05 was considered as significant.

S. No.	Parameter		Method
1	Homocysteine		Automated Chemiluminescence Microparticle Immunoassay (CMIA) system [20,21]
2	Vitamin B12		
3	Paraoxonase (PON1)		Eckerson HW et al., (1983) [22] Spectrophotometry
4	Malondialdehyde (MDA)		Wilbur KM et al., (1949) [23] Spectrophotometry
5	Serum Lipid profile		Automated Biochemistry analyser
	i	Total Cholesterol (TC)	Cholesterol Oxidase-peroxidase (CHOD-PAP)
	ii	Triglycerides (TG)	Glycerol phosphate oxidase
	iii	High Density Lipoprotein Cholesterol (HDL)	Direct HDL enzymatic
	iv	Low Density Lipoprotein Cholesterol (LDL)	Calculated by Friedewald's equation
	v	Very Low Density Lipoprotein Cholesterol (VLDL)	

[Table/Fig-1]: Estimation of biochemical parameters [20-23].

RESULTS

There was statistically significant difference in the levels of homocysteine, vitamin B12, PON1 and MDA between two groups. Homocysteine and PON1 levels were found to be lower in elderly participants than in young participants (p-value <0.05, <0.01). The levels of vitamin B12 and MDA were higher in elderly participants than in young participants (p-value <0.01, <0.01) [Table/Fig-2].

S. No.	Parameters (Unit)	Elderly participants Mean±SD	Young participants Mean±SD	p-value (t-test)
1	Homocysteine (µmol/L)	17.93±9.75	25.65±15.97	<0.05
2	Vitamin B12 (pg/mL)	272.97±196.34	166.42±59.27	<0.01
3	Paraoxonase (PON1) (IU/L)	46.70±37.58	69.90±37.20	<0.01
4	MDA (nmol/mL)	15.13±6.59	9.48±7.39	<0.01
5	Total cholesterol (mg/dL)	163.3±34.18	140.35±25.9	<0.01
6	Triglycerides (mg/dL)	131.77±75.85	111.23±68.2	0.27
7	High density lipoprotein (mg/dL)	44.7±10.66	39.61±7.22	<0.05
8	Very low density lipoprotein (mg/dL)	26.27±15.12	22.23±13.6	0.27
9	Low density lipoprotein (mg/dL)	93.27±26.81	78.52±23.31	<0.05
10	Weight (Kg)	69.40±8.25	60.23±12.14	<0.01
11	BMI (Kg/M^2)	26.69±2.87	23.53±5.63	<0.01

[Table/Fig-2]: Comparison of various parameters between Group-I and II.

There was statistically significant difference of weight and BMI between both the groups. The average weight was statistically significantly more in the elderly participants than young participants (p-value <0.01). According to BMI; elderly participants were overweight than young participants (p-value <0.01) [Table/Fig-2]. The levels of TC (p-value <0.01), HDL (p-value <0.05) and LDL (p-value <0.05) were statistically significantly high in elderly participants as compared to young participants. There was no statistically significant difference in the levels of triglyceride (p-value 0.27) and VLDL (p-value 0.27) between the two groups [Table/Fig-2].

Statistically significant difference between the levels of homocysteine in male population of elderly participants (p-value <0.01) as well as young participants (p-value <0.05) as compared to female populations of both the groups was observed [Table/Fig-3].

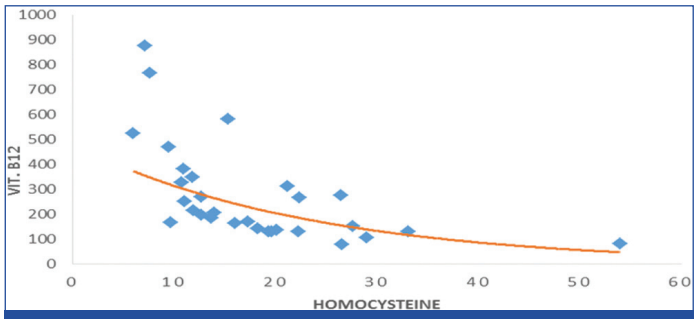
No.	Group	Gender	Homocysteine (Mean±SD)	p-value (t-test)
1	I (Elderly)	Male	26.27±12.48	<0.01
		Female	14.34±5.64	
2	II (Young)	Male	32.18±17.42	<0.05
		Female	16.44±7.32	

[Table/Fig-3]: Comparison of homocysteine levels with gender between Group-I and II.

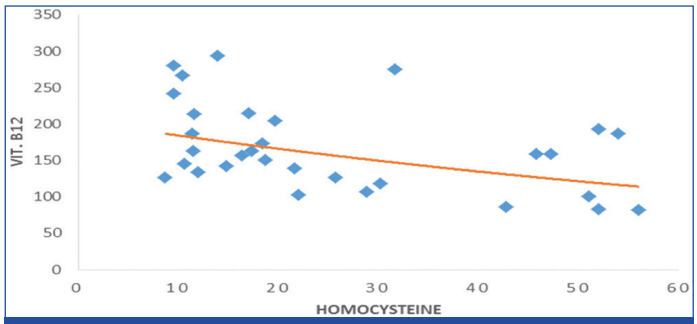
Statistically significant negative correlation of homocysteine with vitamin B12 levels in both elderly participants (p-value <0.01) as well as young participants (p-value <0.01) was found. There was no statistically significant correlation between homocysteine and PON1 well as MDA in both the groups. There was statistically significant negative correlation between the levels of homocysteine and total cholesterol (p-value <0.05), HDL (p-value <0.05) and LDL (p-value <0.01) in elderly participants [Table/Fig-4-6(a-c)].

S. No.	Parameter	Elderly participants		Young participants	
		Pearson's correlation	p-value	Pearson's correlation	p-value
1	Vitamin B12	-0.56	**<0.01**	-0.42	**<0.01**
2	Paraoxonase (PON) 1	-0.07	0.68	-0.01	0.92
3	Malondialdehyde (MDA)	0.003	0.98	0.22	0.23
4	Cholesterol	-0.36	**<0.05**	0.06	0.73
5	Triacylglycerol	0.27	0.13	-0.09	0.6
6	High Density Lipoprotein (HDL)	-0.39	**<0.05**	-0.06	0.72
7	Low Density Lipoprotein (LDL)	-0.49	**<0.01**	0.14	0.42
8	Very Low Density Lipoprotein (VLDL)	0.27	0.14	0.59	0.09

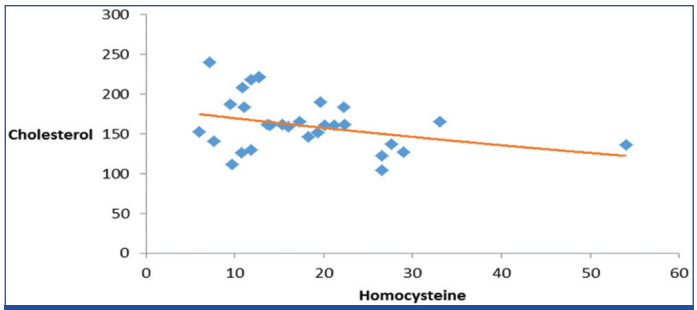
[Table/Fig-4]: Correlation of levels of homocysteine with various parameters between Group-I and II.



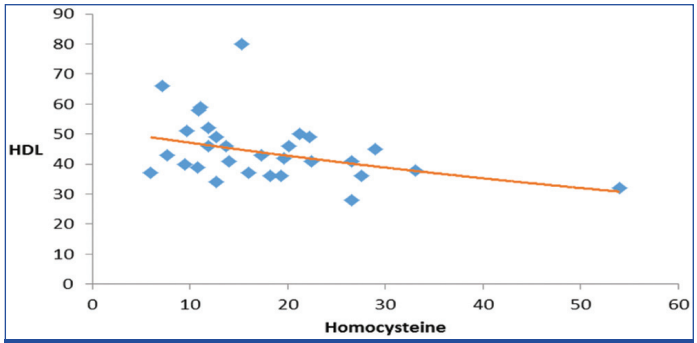
[Table/Fig-5a]: Correlation of levels of homocysteine with vitamin B12 in Group I.



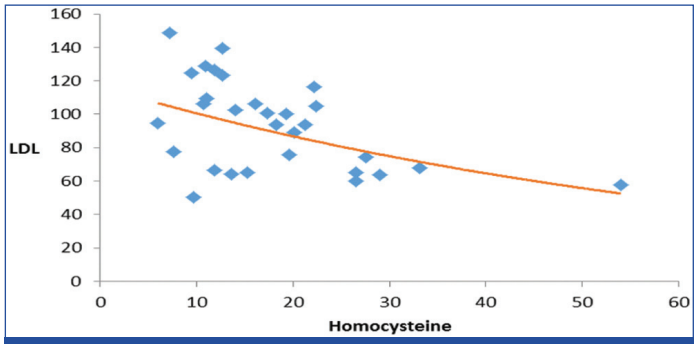
[Table/Fig-5b]: Correlation of levels of homocysteine with vitamin B12 in Group II.



[Table/Fig-6a]: Correlation of homocysteine levels with cholesterol in Group I.



[Table/Fig-6b]: Correlation of homocysteine levels with HDL in Group I.



[Table/Fig-6c]: Correlation of homocysteine levels with LDL in Group I.

DISCUSSION

This study was designed to correlate the levels of homocysteine, PON1 and MDA in healthy elderly population as compared to healthy young population. Furthermore comparison of levels of lipid profile, weight and BMI between elderly and young participants was also done. In present study, Homocysteine levels were statistically significantly lower in elderly participants than in young. Morris MS et al., found that the women of age group 17-54 years had low homocysteine levels and suggested that oestrogen plays an important role in maintenance of low levels of homocysteine [24]. In a similar study involving a population of 40-67 years of age; it was found that the homocysteine levels were low even though the vitamin B12 and folic acid status of that population was adequate. The study also states that; generally, homocysteine levels increase with age. Reasons for the higher homocysteine in elderly may be because of changes in renal function. Homocysteine levels may depend on muscle mass, hormone and vitamin status [25]. On the other hand; Gharaibeh MY et al., found that levels of hyperhomocysteinemia; low folate and vitamin B12 deficiency is more prevalent in healthy elderly population [26]. It has been observed that generally old age is associated with hyperhomocysteinemia and vitamin B12 deficiency; according to many studies. Present study had contrasting findings about homocysteine levels in healthy elderly population. The available data showing similar findings to present study was scarce.

In present study, statistically significantly lower levels of PON1 in elderly participants than in young was found. Cakatay U et al., studied that PON1 is low in the middle aged and elderly population [27]. The study suggested that decreased PON1 activity contributes to increased plasma oxidative protein damage in the ageing population. But in some studies it was found significantly higher serum concentrations PON1 in healthy elderly population [28,29]. In present study, vitamin B12 levels were statistically significantly higher in elderly participants than young. According to some recent studies conducted in urban South India as well as Gujarat; significantly high vitamin B12 levels were found in elderly population [30,31]. The correlation of vitamin B12 and homocysteine levels was found to be inverse. More recently, Zhang W et al., studied nutritional status of the elderly population in Rural North China. In contrast to present study findings; they found that severe deficiencies in folate and vitamin B12 levels exist along with hyperhomocysteinemia [32]. Folate and vitamin B12 supplementation is necessary to prevent hyperhomocysteinemia.

In present study, statistically significantly higher levels of MDA in elderly participants than in young was found. Akila VP et al., found an increase in MDA levels in the healthy as well as diabetic and

hypertensive elderly population [33]. Gil P et al., and Inal ME et al., study suggested that old age is associated with an increase in systemic oxidative stress in the form of increased levels of MDA [34,35]. Present study found that cholesterol, HDL and LDL levels were statistically significantly high in elderly participants as compared to young. There was no significant difference in triglycerides and VLDL levels. Some studies from Korea [36] and Sudan [37] found high prevalence of diabetes, hypertension and abnormal concentrations of serum triacylglycerol and total cholesterol, LDL and HDL were in elderly population.

In present study, it was found that the average weight of elderly participants was statistically significantly more than the young. So, also the BMI was statistically significantly more in elderly participants than young. Han TS et al., suggested that obesity is a common and increasing problem because of decreased physical activity and energy expenditure [38]. This leads to type II diabetes mellitus, arthritis and depression etc. Obesity in the elderly is potentially preventable and should be tackled carefully. According to BMI, the elderly population was overweight than the young population. In parallel to present study finding; Ganji V and Kafai MR, found that sex, age, BMI, serum folate and serum vitamin B12 are significant predictors of homocysteine concentration [39].

In this study, it was found that the levels of homocysteine in male population of both the groups were statistically significantly higher than the female population. In a study in China as well as two more studies by Lussier-Cocan S et al., and Leowattana W et al., found higher levels of homocysteine in men than women [40-42]. Present study found that increase in the vitamin B12 levels is associated with decrease in the homocysteine levels in both the groups. Wolters M et al., and another studies suggested that the vitamin B12 and folic acid deficiencies are associated with higher homocysteine levels in elderly population [17]. The hyperhomocysteinemia is because of lower concentrations of vitamin B12, vitamin B6 and folate [41]. This hyperhomocysteinemia can be lowered with the help of supplementation of vitamin B12 and folic acid [43]. Brattstrom L et al., stated that age, gender, folate, vitamin B12 and multivitamin usage are all important determinants of the plasma homocysteine concentration [44]. Gharaibeh MY et al., suggested that vitamin B12 deficiency in the elderly group along with hyperhomocysteinemia may be due to the high incidence of atrophic gastritis leading to inadequate nutritional intake [26].

Present study did not find any significant correlation of homocysteine with PON1 and MDA in any age group. Cavalca V et al., observed no significant correlation between homocysteine and MDA levels in the patients of coronary artery disease [45]. In contrast; a study in the patients of age related macular degeneration found increased homocysteine and MDA and decreased PON1 levels. In this study, it was found that the levels of homocysteine were inversely correlated to levels of TC, HDL and LDL in elderly participants. Present study did not find any correlation of homocysteine with triglycerides and VLDL in any of the age groups. In a study done to correlate the homocysteine levels and lipid profile in elderly patients admitted to Intensive Care Unit (ICU) of the Cardiology Department, it was found that a negative statistical correlation exists between serum homocysteine levels and the concentration of HDL but, not in homocysteine and LDL-C as well as TG [46]. Rosolová H et al., found a negative correlation of homocysteine with triglyceride and HDL levels [46]. El Oudi M et al., found hyperhomocysteinemia is associated significantly with elevated total cholesterol, LDL cholesterol and lower vitamin B12 levels [47].

It is quite clear that changing dietary habits has been inviting all kinds of serious illnesses to human beings. Cells of younger individuals could lead with normal metabolic pathways though being in compromising scenarios like low vitamin B12 and high homocysteine status. But as age advances, the antioxidant status goes down and cannot stand the damaging mechanisms like lipid peroxidation. The non communicable diseases are slow killers and one is caught at a younger age; the suffering is horrifying. So, younger generations should be made conscious about the preference towards nutritious food which is the only way out.

Limitation(s)

The sample size was too small to establish the exact mechanisms of pathological actions of homocysteine in healthy elderly population. This study was cross-sectional in design. So, development and progression of the diseases in the healthy elderly population; related to homocysteine cannot be observed. Effect of supplementation of vitamin B12 was not done. So, further risk reduction cannot be evaluated. This study should be conducted on a large sample size. Comparison of PON1 and MDA between gender populations was considered as a scope for further research.

CONCLUSION(S)

Elderly population is not at the risk of developing diseases whose risk factor is homocysteine. Males are at higher risk of development of diseases because of homocysteine than the females of any age group. Homocysteine is not pathogenic in old age even in the presence of other risk factors such as lipid peroxidation, decreased defence mechanisms to lipid peroxidation, raised levels of atherogenic lipids and overweightness. Improvement in the vitamin B12 status can decrease the homocysteine levels.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 26, 2022
- Manual Googling: Nov 02, 2022
- iThenticate Software: Dec 08, 2022 (11%)

ETYMOLOGY: Author Origin

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: **Jul 22, 2022**
Date of Peer Review: **Aug 27, 2022**
Date of Acceptance: **Dec 24, 2022**
Date of Publishing: **Mar 01, 2023**