DOI: 10.7860/JCDR/2022/59268.17371

Original Article

Biochemistry Section

Evaluation of Serum Vitamin B12 and Vitamin D Levels in Infertile Males with Suboptimal Semen Parameters- A Pilot Study from Eastern India

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ABSTRACT

Introduction: According to World Health Organization (WHO), infertility is a disease defined by the inability to conceive a child after one year or more of unprotected and regular sexual intercourse. Not only female partner is responsible but male partner related factors play a crucial role in infertility. A key role of vitamin D in male reproductive organs has been suggested. Role of vitamin B12 in spermatogenesis has also been emphasised.

Aim: To delineate if there is any significant association between serum vitamin B12 and vitamin D levels with semen parameters among infertile males belonging to the Eastern India.

Materials and Methods: This cross-sectional study was conducted in Department of Biochemistry at Institute of Post Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital, Kolkata, West Bengal, India, from May 2020 to July 2021. Fifty two infertile males of 25 to 40 years of age, with suboptimal semen parameters (semen volume, sperm count, sperm motility and sperm morphology were considered) were selected. Fasting (12 hours) blood samples were collected for estimation of serum vitamin B12 and vitamin D levels. Significance of association between each parameter with serum vitamin B12 and vitamin D levels was determined using Chi-square test and Fisher's-exact test. Results were analysed using Statistical Package for Social Sciences (SPSS) version 25.0. A p-value less than 0.05 was considered as statistically significant.

Results: Low serum vitamin D levels (<20 ng/mL) were detected in 10 (83.33%) subjects with low semen volume and in 15 (65.21%) With low sperm count. No significant statistical association was found for vitamin D levels with semen volume and sperm count. Among subjects with abnormal sperm motility and morphology, low vitamin D levels were found in 37 (75.51%) and 16 (69.56%), respectively. A significant statistical association was found between vitamin D level and sperm motility (p-value=0.005) but not with sperm morphology. Amongst subjects with low semen volume and low sperm count, low vitamin B12 levels (<200 pg/mL) were seen in 5 (41.6%) and 15 (65.21%), respectively. A significant statistical association was found between vitamin B12 level and sperm count (p-value=0.003). Among subjects with abnormal sperm motility and morphology, low vitamin B12 levels were present in 19 (38.77%) and 13 (56.52%) and there was a significant association between the variables p-value=0.037 and p-value=0.049, respectively.

Conclusion: It can be concluded that vitamin B12 and vitamin D levels in infertile male subjects disturb normal physiological mechanisms required for being fertile. Hence, vitamin B12 and vitamin D supplementation may be suggested for improvement of semen quality.

Keywords: Association, Conception, Infertility, Spermatozoa

INTRODUCTION

Infertility is a major barrier which hampers the right of individual and couples to have offspring [1]. Infertility is a disease defined by the inability to conceive a child after one year or more of unprotected and regular sexual intercourse [2]. According to a report by the World Health Organization (WHO), one in every four couples in developing countries is affected by infertility [3]. Global rates of male infertility ranges from 2.5% to 12% [4]. In North America, the estimated male infertility rate is between 4.5-6% while it is 9% in Australia. But the rate is as high as 8-12% in Eastern Europe [4,5] and male factor infertility prevalence is 42.2% in Nigeria [6]. In a study from Mongolia the infertility prevalence due to male factors was reported as 25.6% [7].

According to the WHO report, the prevalence of primary infertility in India was 3.9% (age-standardized to 25-49 years) and 16.8% (age-standardized to 15-49 years) [8]. Moreover, the prevalence of primary infertility has also been shown to vary across different states, for example 37.39%, from Bihar [9], 22.4% from Kashmir [10] and also the tribes and castes within the same region in India. Male infertility can be divided into two categories. The first type of abnormality is of secretory origin which corresponds to an alteration of the production of the spermatozoa by the testes. Second type of abnormality is excretory in origin which is defined as a defect of sperm delivery in the genital tract. However, in 30% of cases male infertility disorder remains unexplained [11].

One main cause of non obstructive male infertility is deficiency in the semen volume and its quality [12,13]. Previous research has shown meaningful positive relationships between semen quality and biochemical parameters like serum vitamin B12, vitamin D levels specially in early 1960s [14]. Researchers have emphasized a role of vitamin B12 in spermatogenesis, and hence in semen quality [14,15]. According to published literature plasma vitamin B12 concentrations in infertile men were found to be lower than in fertile men [16]. Other studies have also shown the positive effects of vitamin B12 on different sperm parameters [15,17]. Similarly, a number of previous animal and human studies have shown that vitamin D is necessary for optimal function of male reproduction system [9,18]. Some studies found a positive correlation between serum vitamin D and sperm motility [19-22]. But, there are no studies to delineate the relation with male factor infertility with serum levels of vitamin B12 as well as vitamin D from the eastern part of India.

Therefore, the present study was carried out in a tertiary care hospital in the eastern zone of India, with the primary objective to measure serum vitamin B12 and vitamin D levels in cases of male infertility with suboptimal semen parameters.

MATERIALS AND METHODS

This was a hospital-based cross-sectional study conducted at Department of Biochemistry, Institute of Post Graduate Medical Education an Research and Seth Sukhlal Karnani Memorial Hospital (IPGME&R and SSKM Hospital), Kolkata, West Bengal, India, from May 2020 to July 2021. The study was conducted after approval from the Institutional Ethics Committee (IPGME&R/IEC/2020/300). Subjects were properly informed about the study and consents were taken before enrolment.

Infertile males with suboptimal semen parameters attending in Infertility Clinic under the Department of Gynaecology and Obstetrics, Institute of Post Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital, were recruited. As it was a pilot study, using the sample size rules of thumb [23], 52 male infertile patients with suboptimal semen parameters who attended the hospital and gave consent were studied.

Inclusion criteria: Male subjects of 25 to 40 years of age, with history of infertility after unprotected intercourse for more than one year, non obstructive type infertility, and semen tests showing suboptimal level of one or more semen parameters were included in the study. The WHO reference values for semen analysis were utilised to assess the semen reports.

Lower reference limits, the fifth centiles (with 95% confidence intervals), were as follows: semen volume, 1.5 mL (1.4-1.7); sperm count, 15 million/mL (12-16); progressive motility, 32% (31-34); and morphologically normal forms, 4% (3.0-4.0) [13].

Exclusion criteria: Subjects with any kind of acute illness, chronic diseases like hypertension, chronic liver disease, chronic kidney disease, malignancy etc., any endocrinal disorder like diabetes mellitus, thyroid disorder etc., taking vitamin B12, vitamin D supplementation, drugs altering the biochemical parameters under the study and subjects with history of smoking, alcoholism were excluded from the study.

Study Procedure

After the relevant history and clinical examination, semen samples were collected after three days of sexual abstinence and then were analysed in the Department of Pathology, IPGME&R and SSKM Hospital. Semen analysis [24] was done and patients with suboptimal level of one or more semen parameters were selected for this study.

After 12 hours of fasting, 10 mL sample of venous blood was collected in a red capped vial by aseptic measures for serum vitamin B12 (reference value 206-678 pg/mL) [25] and vitamin D levels (optimal value 25-80 ng/mL) [26] estimation in the Department of Biochemistry, IPGME&R and SSKM Hospital. Complete clot formation was ensured prior to centrifugation and then samples were separated by centrifuging at 3000 rpm for five minutes and were analysed within 24 hours.

Vitamin B12 levels in serum were estimated by chemiluminescence method in ADVIA Centaur CP (SIEMENS) by competitive immunoassay method using direct chemiluminescent technology in which vitamin B12 from the patient samples competed with vitamin B12 labelled with acridinium ester in the Lite Reagent, for a limited amount of purified intrinsic factor, which was covalently coupled to paramagnetic particles in the solid phase and analysed accordingly [27]. Serum 25 OH vitamin D level was estimated by chemiluminescence method in ADVIA, Centaur (SIEMENS) which is competitive immunoassay using direct chemiluminescent technology. Here, the amount of vitamin D present in the patient's sample and the relative light units detected by the system shows an inverse relation [28].

STATISTICAL ANALYSIS

Data was entered into a Microsoft excel spreadsheet and then analysed using Statistical Package for Social Sciences (SPSS) version 25.0. Results had been summarised descriptively using mean and standard deviation for numerical variables and count and percentages for categorical variables. All variables were tested for normality using Kolmogorov-Smirnov test. The comparison between the parameters of semen analysis and different biochemical parameters (vitamin B12 and vitamin D) were done by Chi-square test and Fisher's-exact test. A p-value <0.05 was considered as statistically significant.

RESULTS

Fifty two male infertile subjects between 25 to 40 years of age (mean 30.65±3.98 years) were studied. Their mean serum vitamin B12 level was 230.40±96.67 pg/mL and serum vitamin D level showed a mean value of 22.89±16.68 ng/mL. Kolmogorov-Smirnov test showed in case of vitamin B12 the value of K-S statistic (D) was 0.16 and p-value was 0.12 and for vitamin D, K-S statistic (D) and p-value was 0.17 and 0.08, respectively. Hence, the data was normally distributed. Result analysis revealed that majority of the males with low semen volume had a normal serum Vitamin B12 but Vitamin D were low. There was no significant association between the vitamins and semen volume [Table/Fig-1].

Semen volume	Vitamin B12		Vitamin D	
	Low (<200 pg/mL)	Normal	Low (<20 ng/mL)	Normal
Low (<1.5 mL per ejaculate) (n=12)	5 (41.6%)	7 (58.33%)	10 (83.33%)	2 (16.66%)
Normal (n=40)	17 (42.5%)	23 (57.5%)	27 (67.5%)	13 (32.5%)
p-value	0.959		0.288	

[Table/Fig-1]: Association between semen volume and serum vitamin B12 and vitamin D level.
*Calculated by Fisher's-exact test

In subjects with low sperm counts majority have low serum levels of vitamin B12 and vitamin D but significant association of sperm count was found only with vitamin B12 levels [Table/Fig-2].

Sperm count	Vitamin B12		Vitamin D	
	Low (<200 pg/mL)	Normal	Low (<20 ng/mL)	Normal
Low (<15 million/mL) (n=23)	15 (65.21%)	8 (34.78%)	15 (65.21%)	8 (34.78%)
Normal (n=29)	7 (29.13%)	22 (75.86%)	22 (75.86%)	7 (29.13%)
p-value	0.003*		0.4	

[Table/Fig-2]: Association between sperm count in semen and serum vitamin B12 and vitamin D level.
Calculated by Chi-square test, p-value <0.05 was considered as statistically significant

Majority of the subjects with abnormal sperm motility had normal serum vitamin B12 levels but low levels of vitamin D. Levels of vitamin D were significantly associated with sperm motility but not levels of vitamin B12 [Table/Fig-3].

Sperm motility	Vitamin B12		Vitamin D	
	Low (<200 pg/mL)	Normal	Low (<20 ng/mL)	Normal
Abnormal (n=49) (<32%)	19 (38.77%	30 (61.22%)	37 (75.51%)	12 (24.48%)
Normal (n=3)	3 (100%)	0	0	3 (100%)
p-value	0.069		0.005*	

[Table/Fig-3]: Association between sperm motility in semen and serum vitamin B12 and vitamin D level.
Calculated by Fisher's-exact test, p-value <0.05 was considered as statistically significant

Among the subjects with abnormal sperm morphology, majority had low serum levels of vitamin B12 and vitamin D but significant statistical association with sperm morphology was found only in case of vitamin B12 [Table/Fig-4].

Parameters		Vitamin B12		Vitamin D	
		Low (<200 pg/mL)	Normal	Low (<20 ng/mL)	Normal
Sperm morphology (Frequency (%)	Abnormal (n=23) (<4%)	13 (56.52%)	10 (43.47%)	16 (69.56%)	7 (30.43%)
	Normal (n=29)	9 (31.03%)	20 (68.96%)	21 (72.41%)	8 (27.58%)
	p-value	0.049*		0.822	

[Table/Fig-4]: Association between sperm morphology in semen and serum vitamin B12 and vitamin D level.
Calculated by Chi-square test

DISCUSSION

The present study aimed to find the association between different semen parameters with serum levels of vitamin B12 and vitamin D in cases of non obstructive male infertility.

Significant associations were found between vitamin B12 and vitamin D levels with some of the semen parameters. A significant association was found between vitamin B12 level and sperm count (p-value=0.003), and sperm morphology (p-value=0.049) but the results were insignificant in case of semen volume (p-value=0.959) and sperm motility (p-value=0.069). Stuhlinger MC et al., suggested that vitamin B12 deficiency might reduce sperm function through hyperhomocysteinemia-induced nitric oxide depletion by inhibiting nitric oxide synthase pathways, which reduced the amount of nitric oxide produced [29], while a study by Miraglia E et al., showed nitric oxide is crucial for adequate sperm motion [30]. Evidence suggested a possible homocysteine toxicity to sperm because low levels of vitamin B12 in the body reduced the catalytic activity of methionine synthase, so less synthesis of methionine from homocysteine [31].

It has been found that in human being vitamin B12 is transferred from the blood to the male reproductive organs which suggests that vitamin B12 may have a role in spermatogenesis [14,15]. In 2006, Chatterjee S et al., found that vitamin B12 had positive effects on sperm parameters, particularly sperm count [32]. Study by Dhillon VS et al., revealed plasma vitamin B12 concentrations are lower in infertile men compared to fertile [16]. In 2013, Abad C et al., found that an oral antioxidant treatment including vitamin B12 improved sperm motility, and DNA integrity [33].

In the present study, a significant association was found between serum vitamin D level and sperm motility (p-value=0.005) but insignificant to semen volume (p-value=0.288), sperm count (p-value=0.4), and sperm morphology (p-value=0.822). In the study by Zhu et al., serum vitamin D level in Chinese infertile men was significantly lower in oligospermia, asthenospermia, oligoasthenospermia, and azoospermia than those in fertile men [34]. Another cross-sectional study by Abbasihormzi Sh et al., showed no association between daily dietary intake of vitamin D and calcium with sperm parameters, whereas, a positive association was found between sperm motility with vitamin D categorised in oligoasthenoteratozoospermic men (p-value=0.028) [35]. In the cross-sectional study by Jensen MB et al., a positive association between serum vitamin D inactivating enzyme and sperm motility (p-value<0.004) was shown among Danish men [20]. The key role of vitamin D in male reproductive system has been suggested, with vitamin D receptor and vitamin D metabolising enzymes in the testis and spermatozoa [36].

One study by Ramlau-Hansen CH et al., indicated that low vitamin D was not a risk factor for poor semen quality although a high vitamin D level was associated with lower crude median total sperm count and percentage of normal sperm morphology but the association was non significant [37]. On the contrary, studies by Tirabassi G et al., and Hammoud AO et al., showed that vitamin D deficiency might damage sperm motility, suggesting that circulating 25-hydroxy-vitamin D3 had a key role in modulating sperm motility [22,38].

Hence, to summarise, in the present study, there was significant association between serum vitamin B12 and vitamin D levels with different semen parameters which corroborates with findings of most of the earlier studies performed in different parts of the world.

Limitation(s)

Inspite of every sincere effort, the present study had some lacunae. The sample size was small as it was a pilot study but it should be followed by another study with adequate sample size. The study was done in a single center, in a tertiary care hospital, so selection bias could not be excluded. As the study was carried out in a tertiary care hospital, so hospital bias could not be ruled out.

CONCLUSION(S)

In view of the results obtained from the study done, it can thus be concluded that reduced vitamin B12 and vitamin D levels in infertile male subjects disturb normal physiological mechanisms required for being fertile. So estimation of vitamin B12 and vitamin D levels should be included during routine investigations for male infertility. It may also be suggested that corrective measures with vitamin B12 and vitamin D supplementation in males could result in successful conception.

Acknowledgement

The authors acknowledge the work of authors, editor and publisher of all articles, journals and books whose work were used as reference. The authors are also thankful to the patients, all the faculties and technological stuffs of the Department of Biochemistry, Department of Pathology and Department of Gynecology and Obstetrics, Institute of Post Graduate Medical Education and Research, Kolkata for their help, support and encouragement. The authors appreciate specially to Meghna Mukherjee, Statistician, Institute of Post Graduate Medical Education and Research, Kolkata, for her help during compiling data.

REFERENCES

[1] Zegers-Hochschild F, Dickens BM, Dughman-Manzur S. Human rights to in vitro fertilization. International Journal of Gynecology & Obstetrics 2013;123(1):86-89.
[2] Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril. 2009; 92:1520-24.
[3] Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. PLoS Med. 2012;9(12):e1001356.
[4] Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13:37.
[5] Population Council. New Delhi: Population Council; 2004. Infertility. Looking Back, Looking Forward: A Profile of Sexual and Reproductive Health in India; pp. 67-72.
[6] Ikechebelu JI, Adinma JI, Orie EF, Ikegwuonu SO. High prevalence of male infertility in southeastern Nigeria. J Obstet Gynaecol. 2003;23(6):657-59.
[7] Bayasgalan G, Naranbat D, Tsedmaa B, Tsogmaa B, Sukhee D, Amarjargal O. et al., Clinical patterns and major causes of infertility in Mongolia. J Obstet Gynaecol Res. 2004;30(5):386-93.
[8] Infecundity, infertility, and childlessness in developing countries. DHS Comparative Reports No 9. Calverton, Maryland, USA: ORC Macro and the World Health Organization; 2004. World Health Organization.
[9] Singh K, Kumari R, Ranjan A, Bharti G. Analysis of causes and clinical pattern of infertility in couples coming to a tertiary care centre in Bihar, India. Int J Reprod Contracept Obstet Gynecol. 2017; 6(6):2279-83.
[10] Zargar AH, Wani AI, Masoodi SR, Laway BA, Salahuddin M. Epidemiologic and etiologic aspects of primary infertility in the Kashmir region of India. Fertil Steril. 1997;68(4):637-43.
[11] Cavallini G. Male idiopathic oligoasthenoteratozoospermia. Asian J Androl. 2006; 8(2):143-57. Doi: 10.1111/j.1745-7262.
[12] Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. Fertil Steril. 2002; 77(5):873-82.
[13] Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231-45.
[14] Watson A.A. Seminal vitamin B12 and sterility. Lancet. 1962;2:644.

[15] Boxmeer JC, Smit M, Weber RF, Lindemans J, Romijn JC, Eijkemans MJ, et al. Seminal plasma cobalamin significantly correlates with sperm concentration in men undergoing IVF or ICSI procedures. J Androl. 2007;28(4):521-27.
[16] Dhillon VS, Shahid M, Husain SA. Associations of MTHFR DNMT3b 4977 bp deletion in mtDNA and GSTM1 deletion, and aberrant CPG island hypermethylation of GSTM1 in non-obstructive infertility in indian men. Mol. Hum. Reprod. 2007;13(4):213-22.
[17] Banihani AS. Vitamin B12 and Semen Quality. Biomolecules. 2017;7(2):42.
[18] Tak YJ, Lee JG, Kim YJ, Park NC, Kim SS, Lee S, et al. Serum 25-hydroxyvitamin D levels and testosterone deficiency in middle-aged Korean men: A cross-sectional study. Asian J Androl. 2015; 17(2):324-28.
[19] Blomberg Jensen M, Bjerrum PJ, Jessen TE, Nielsen JE, Joensen UN, Olesen IA, et al. Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. Hum Reprod. 2011;26(6):1307-17.
[20] Blomberg Jensen M, Jorgensen A, Nielsen JE, Bjerrum PJ, Skalkam M, Petersen JH, et al. Expression of the vitamin D metabolizing enzyme CYP24A1 at the annulus of human spermatozoa may serve as a novel marker of semen quality. Int J Androl. 2012;35(4):499-10.
[21] Yang B, Sun H, Wan Y, Wang H, Qin W, Yang L, et al. Associations between testosterone, bone mineral density, vitamin D and semen quality in fertile and infertile Chinese men. Int J Androl. 2012;35(6):783-92.
[22] Tirabassi G, Cutini M, Muscogiuri G, Delli Muti N, Corona G, Galdiero M, et al., Association between vitamin D and sperm parameters: Clinical evidence. Endocrine. 2017;58(1):194-98.
[23] Browne RH. On the use of a pilot sample for sample size determination. Stat Med 1995;14(17):1933-40.
[24] Godkar PB, Godkar DP. Textbook of Medical Laboratory Technology, 3rd ed. Mumbai, India. Bhalani Publishing House, 2014.
[25] Burtis AC, Ashwood ER, Bruns DE. Reference information. In: Tietz (6 eds). Fundamentals of clinical chemistry. Missouri:Saunders. 2008:863.
[26] Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clin Proc. 2010;85(8):752-57; quiz 757-8.
[27] Chen X, Ren F, Xu J, Yu Z, Lin X, Bai Z, et al. A Rapid Quantitative Chemiluminescence Immunoassay for Vitamin B12 in Human Serum. Clin Lab. 2020;66(3):10.7754/Clin.Lab.2019.190604.
[28] Rollins G. Vitamin D Testing-what's the right answer? Labs grapple with confusing analytics, evidence. Clin Lab News. 2009;35(7):01-07.
[29] Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP. Homocysteine impairs the nitric oxide synthase pathway: Role of asymmetric dimethylarginine. Circulation. 2001;104(21):2569-75.
[30] Miraglia E, De Angelis F, Gazzano E, Hassanpour H, Bertagna A, Aldieri E, et al. Nitric oxide stimulates human sperm motility via activation of the cyclic GMP/protein kinase G signaling pathway. Reproduction. 2011;141(1):47-54.
[31] Banerjee RV, Matthews RG. Cobalamin-dependent methionine synthase. FASEB J. 1990;4(5):1450-59.
[32] Chatterjee S, Chowdhury RG, Khan B. Medical management of male infertility. J Indian Med Assoc. 2006;104(4):74, 76-77.
[33] Abad C, Amengual MJ, Gosalvez J, Coward K, Hannaoui N, Benet J, et al. Effects of oral antioxidant treatment upon the dynamics of human sperm DNA fragmentation and subpopulations of sperm with highly degraded DNA. Andrologia. 2013;45(3):211-16.
[34] Zhu CL, Xu QF, Li SX, Wei YC, Zhu GC, Yang C, et al., Investigation of serum vitamin D levels in Chinese infertile men. Andrologia. 2016;48(10):1261-66.
[35] Abbasihormozi Sh, Kouhkan A, Alizadeh AR, Shahverdi AH, Nasr-Esfahani MH, Sadighi Gilani MA, et al. Association of vitamin D status with semen quality and reproductive hormones in Iranian subfertile men. Andrology. 2017;5(1):113-18.
[36] Cito G, Cocci A, Micelli E, Gabutti A, Russo GI, Coccia ME, et al. Vitamin D and Male Fertility: An Updated Review. World J Mens Health. 2020;38(2):164-77.
[37] Ramlau-Hansen CH, Moeller UK, Bonde JP, Olsen J, Thulstrup AM. Are serum levels of vitamin D associated with semen quality? Results from a cross-sectional study in young healthy men. Fertil Steril. 2011;95(3):1000-1004.
[38] Hammoud AO, Meikle AW, Peterson CM, Stanford J, Gibson M, Carrell DT. Association of 25-hydroxy-vitamin D levels with semen and hormonal parameters. Asian J Androl. 2012;14(6):855-59.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 24, 2022
- Manual Googling: Oct 31, 2022
- iThenticate Software: Nov 21, 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. No

Date of Submission: **Jul 23, 2022**
Date of Peer Review: **Aug 06, 2022**
Date of Acceptance: **Nov 22, 2022**
Date of Publishing: **Dec 01, 2022**