DOI: 10.7860/NJLM/2022/52107.2587

Original Article

Pathology Section

Seroprevalence of Transfusion Transmitted Infections among Multiple Transfused Patients in Indian Scenario: Step for Safe Transfusion Practices

SWATI PATEL[1], VISHWADEEP KESARWANI[2], HIMANSHU SONI[3], NEEHARIKA SAH[4], MANJU PUROHIT[5]

ABSTRACT

Introduction: Transfusion Transmitted Infections (TTIs) is a worldwide problem both in past and in the present days. The risk of TTIs is increased due to the constant need to receive blood transfusion in patients requiring multiple transfusions. The problem of TTIs runs parallel to the prevalence of the infections in the blood donor group and to the number of units transfused.

Aim: To estimate the current prevalence of blood TTIs in patients requiring multiple units of blood transfusions and to determine their association with age, gender, various disease category and number of units transfused.

Materials and Methods: This cross-sectional retrospective study was conducted in the Department of Pathology, RD Gardi Medical College, Ujjain, Madhya Pradesh, India from January 2017 to December 2020. Patients of all the age groups and both gender from Outpatient and Inpatient Department, requiring regular transfusions and having a history of 10 or more units of blood transfusion were enrolled. Serological detection of TTIs was done by Enzyme-Linked Immune Sorbent Assay (ELISA) method. Statistically the results were analysed using Statistical Package for Social Sciences (SPSS) version 19.0.

Results: In this study, 480 patients were included of which 293 (61.1%) were males and 187 (38.9%) females. Maximum 195 (40.6%) were children in age group of <1-10 years. A total of 63 (13.12%) patients with Hepatitis C virus, 9 (1.87%) patients with Hepatitis B virus and 2 (0.42%) patients with Human Immunodeficiency Virus (HIV) infection were found at the end of study. Highest seropositivity of TTI was seen in patient who had undergone >400 number of transfusions , thus association was established but no association detected with age and sex of the patients. Majority of TTIs were positive in thalassemia patients 164 (34.17%) followed by patient undergoing haemodialysis 151 (31.45%).

Conclusion: The results of this study raise an alarm to the existence of a significant risk of TTIs in our society. There is requirement of advanced and vigilant screening of donors with proper strategies to reduce the breach in safe blood transfusion practices.

Keywords: Haemodialysis, Hepatitis, Human immunodeficiency virus, Prevalence, Thalassaemia

INTRODUCTION

The Transfusion Transmitted Infections (TTIs) is a challenge to the services of transfusion all over the world. The problems of TTI parallels to the prevalence of the infections in the blood donor community [1]. Various viruses, parasites and bacteria are important causes of concern on patient undergoing multiple transfusions. Risk of infections transmitted by transfusion creates a major problem in patients receiving long term transfusions [2-4].

Multiple transfusions of blood are required predominantly in patients suffering from haemoglobinopathies, bleeding disorders, bone marrow failures, patient dependant on haemodialysis, leukaemias and acute blood loss etc. Prevalence of HIV and post transfusion hepatitis among blood donors varies in various parts of country. This might be due to low viral load and mutant strains which are not able to detect in early phases of infection. Inspite of proper vigilance and quality control, measures are needed to prevent this problems. Proper use of sensitive laboratory tests may help Indian blood transfusion services to decrease incidence of TTIs [5].

In India, as per the Drug and Cosmetics (1st amendment) Rules 1992 [3] Act, screening of each unit of donated blood for the presence of markers of TTIs is mandatory [6,7]. Professional blood donation has been banned in India since 1997 [8]. Units of blood and its product are screened with assays of steadily increasing sensitivity for Hepatitis B surface Antigen (HBsAg) since 1971, against HIV since 1989 and against Hepatitis C virus since 2001 [9,10].

According to World Health Organisation (WHO), every year upto sixteen million hepatitis B, five million Hepatitis C and 1.60 lac of HIV new cases were seen due to lack of good quality screening of blood donors [11]. Though the blood transfusion is life saving intervention but it is never devoid of risk and carries the potential threat for TTIs [12]. Patients with multiple transfusions are at higher risk of acquiring TTIs due to the blood transfusion from donors infected by various blood borne infectious agents [13]. Despite the healthy status of donors, donated blood may carry various pathogens in the blood [14]. With every unit of blood, there is 1% chance of acquiring transfusion associated problems including TTIs [11].

Limited studies report the burden of blood-borne infections following multiple transfusion of blood from central India. The need to explore the burden of TTIs in these patients cannot be overemphasised. The current study was done to determine the prevalence of Hepatitis B, Hepatitis C, HIV infection in multitransfused individuals of Ujjain and surrounding area, further to estimate the prevalence of blood TTIs and to determine their association with age, gender, various disease category and number of units transfused in these patients.

MATERIALS AND METHODS

This cross-sectional retrospective study was conducted in the Department of Pathology at RD Gardi Medical College, Ujjain, Madhya Pradesh, India, over a period of four years i.e., January 2017 to December 2020. The protocol was designed in accordance with the ethical standards of the committee for the protection of the

human subjects of the institute (Ethical clearance number 77/2017). Total 480 patients were included in the study after obtaining written consent before transfusion from each patient or from their guardians in case of children. The first recorded case was from Mehidpur, Mehidpur district, Ujjain, nine year old male with complaints of tiredness, easy fatiguiability on 2nd January 2017.

Inclusion criteria: Patients of all age group and both gender from Outpatient and Inpatient Department, requiring regular transfusions and having a history of 10 or more units of blood transfusion, with atleast one unit monthly and last transfusions four weeks prior to sampling were included in the study.

Exclusion criteria: Patients who required less than 10 units of blood and not given consent for study as a part of their management were excluded at this study.

Demographic data, like age, sex, place of residence, clinical history, date of first transfusion, frequency of transfusion was taken or recorded from blood bank record.

Methodology

4 mL venous blood samples were collected from each multiple transfused patient in the study with due consent in clot activator vacutainer. The blood was left to clot and centrifuged for 10 minutes. At 1000 rpm to separate serum, which was stored at -20°C until analysis of, HBsAg, anti-HCV and HIV antibodies was done. Serological detection of HIV, HBV, and HCV was done by ELISA method. HBsAg, anti-HCV was performed using a third-generation and HIV by fourth-generation ELISA based on chemiluminescent microparticle immunoassay kit. All ELISA kits were supplied from Meril Diagnostics Pvt., Ltd., Gujarat, India. Interpretation of the results was performed according to manufacturer's instructions as: a cut-off value of less than 0.210 OD for HCV, 0.110 OD for HBV and 0.210 optical densities (OD) for HIV was considered negative [15].

STATISTICAL ANALYSIS

Statistically the results were analysed using Statistical Package for Social Sciences (SPSS) version 19.0, interpreted as percentage by applying chi-square test and the tables were prepared in Microsoft Excel.

RESULTS

In the present study, 480 multiple transfused patients were enrolled out of which 61.1% were males and 38.9% were females, male: female ratio of 1.57:1 and 15.42% of patients were seropositive for TTIs. The TTIs were more prevalent in females and males [Table/Fig-1]. It also represents the distribution of the different TTIs in male and in female. The highest prevalence among all TTIs in multiple transfused patients was of HCV, 63 (13.12%).

Gender distribution	N (%)	HBV Reactive n (%)	HCV Reactive n (%)	HIV Reactive n (%)	Overall TTIs Prevalence (%)
Female	187 (38.9%)	3 (1.6%)	26 (13.9%)	2 (1.07%)	31 (16.58)
Male	293 (61.1%)	6 (2.04%)	37 (12.63%)	0 (0.0%)	43 (14.68)
Total	480	9 (1.87%)	63 (13.12%)	2 (0.42%)	74 (15.42%)

[Table/Fig-1]: Distribution of multiple transfused patients and their prevalence of TTIs. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus

[Table/Fig-2] shows maximum prevalence of TTIs, 44 (22.56%) was found in age group of <1-10 years followed by 11-20 years age group, 16 (20.77%). The prevalence of HCV (1.54%) which was maximum in <1-10 year of age, whereas majority of HBV (10.81%) and HIV (5.4%) were seen in 21-30 years of age group.

[Table/Fig-3] shows the prevelance of TTIs according to disease category where majority of thalassaemia patients 52 (31.71%) showed seropositivity followed by haemodialysis patients 16 (10.59%). Multiple transfused category of haematoncology and other's including

Age wise distribution (in years)	Total patients N (%) (N=480)	HBV Reactive n (%)	HCV Reactive n (%)	HIV Reactive n (%)	Overall TTIs Prevalence (%)
<1-10	195 (40.6)	2 (1.02)	42 (21.54)	0 (0.0)	44 (22.56)
11-20	77 (16.04)	0 (0.0)	16 (20.77)	0 (0.0)	16 (20.77)
21-30	37 (7.71)	4 (10.81)	0 (0.0)	2 (5.4)	6 (16.21)
31-40	29 (6.04)	0 (0.0)	1 (3.45)	0 (0.0)	1 (3.45)
41-50	40 (8.33)	0 (0.0)	2 (5.0)	0 (0.0)	2 (5.0)
> 50	102 (21.25)	3 (2.94)	2 (1.96)	0 (0.0)	5 (4.90)

[Table/Fig-2]: Distribution of prevalence of various TTI detected in various age groups.

anaemia of chronic disease, aplastic anaemia, chronic liver disease, chronic renal failure, refractory megaloblastic anaemia showed no seropositivity for any of the TTI markers investigated. Maximum prevalence of seropositive was of HCV (30.49%) in multi-transfused thalassaemia patients, whereas HBV (2.83%) and HIV (1.89%) were most commonly found in patient who received blood due to acute blood loss.

Disease category	Total patients N (%)	HBV Reactive n (%)	HCV Reactive n (%)	HIV Reactive n (%)	Overall TTIs Prevalence
Thalassaemia	164 (34.17)	2 (1.22)	50 (30.49)	0 (0.0)	52 (31.71)
Haemodialysis	151 (31.45)	4 (2.65)	12 (7.95)	0 (0.0)	16 (10.59)
Sickle cell disease	9 (1.87)	0 (0.0)	1 (11.11)	0 (0.0)	1 (11.11)
Haemato-oncolgy	16 (3.33)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Acute blood loss	106 (22.1)	3 (2.83)	0 (0.0)	2 (1.89)	5 (4.72)
Others	34 (7.08)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[Table/Fig-3]: Distribution of prevalence of TTIs according to disease category.

[Table/Fig-4] shows highest number of multi-transfused patients {136 (28.3%)} received number of transfusions between 10-100. Overall highest seropositive of TTI was seen in patient of >400 transfusion group [Table/Fig-4]. This represents exponentially increasing seropositivity of HCV and HBV with increase in number of units transfused.

No. of transfusion	Total patients N (%)	HBV Reactive n (%)	HCV Reactive n (%)	HIV Reactive n (%)	TTI positive N (%)
10-100	136 (28.3)	3 (2.2)	3 (2.2)	1 (0.73)	5 (5.15)
101-200	107 (22.2)	3 (2.8)	4 (3.74)	1 (0.93)	8 (7.48)
201-300	75 (15.6)	0 (0.0)	15 (20)	0 (0.0)	15 (20)
301-400	75 (15.6)	0 (0.0)	16 (21.33)	0 (0.0)	16 (21.33)
> 400	87 (18.3)	3 (3.44)	25 (28.7)	0 (0.0)	28 (32.2)

[Table/Fig-4]: Categorisation of patients as per number of units transfused and prevalence of TTI.

In this study, no significant association was found between prevalence of TTI with age but prevalence of TTI increases with increasing number of transfusions and also there was increased prevalence seen in thalassemia as compared to other diseases may be due to more frequent need of blood products.

DISCUSSION

The TTIs are of great concern for safety of multi-transfused patients. Prevelance of TTIs varies from region to region depending on the units of blood transfused in that particular population. There were long list of pathogens, which can be transmitted through blood transfusions [16,17]. This was found to be an important issue due to high prevalence of asymptomatic carriers in the society, concealing medical history by paid professional blood donors, blood donations during the window period of infections and quality systems [18].

In present study, 15.42% of all multi-transfused patients were seropositive for TTIs. This result were close to result of Oza T et al., and Gugnani P et al., which was 11.38% and 14.2% [19,20]. In this study, 43 males and 31 females are seropositive for TTIs and ratio of M:F was 1.39. This result are slight more than the result of Gugnani P et al., where the ratio was one but lower than the result of Bhavsar H et al., where 22 were males and 11 where females, so the ratio was 2.0 [21]. Prevelance of HCV as compared to other TTIs was higher in both sexes in this study and in the study done by Gugnani P et al., which was 6.3% and 7.1% [20].

In the present study, prevalence, is higher in younger age group i.e., less than 10 years of age as this groups was mainly constituted by thalassemia patients. Prevalence was found to be (44, 22.56%). Similar results was found in the study of Mittal K et al., where maximum prevelance were seen in age group between 6-10 years i.e., 60 and between 0-5 years were 44 in thalassemia patients [22]. Highest prevalence was seen in younger age groups than rest of the other disease group as majority of the multi-transfused dependent patients were thalasaemic than rest of the disease category. Also, less study were available in the prevelance of TTIs in multitransfused patients of other disease category.

The prevelance of HCV was found to be highest in all age groups. This findings were similar to the study by Mittal K et al., who found (20% HCV, 1.25% HBV) in less than 10 years, 31.25% HCV, 1,25% HBV) in 11-15 years, all patients (HCV) in 16-20 years, 16.25% HCV, 3.75% HBV) in more than 20 years [22]. The highest prevalence of HCV was seen in all age groups that can be due to screening for HCV in donated blood bags started late as compared to that of anti-HIV and anti-HBV [23]. Anti-HCV test has been made mandatory by the Government of India from June 2001 [18]. The highest prevalence of HCV as compared to HBV might be due to non availability of vaccine against HCV and also the risk of infections is not obsolete as screening methods are not sensitive enough to detect infections in window period [16,24].

It is observed that, prevalence of TTIs infection was highest in thalassemia group 31.71% than in other disease category. This findings were similar to the study of Laguna-Torres VA et al., where majority of multiple transfused patients were thalassemia, haemophilics and haemodialysed patients associated with 13.1% HCV, 4.1% HBV seropositive. Haemodialysis was associated with an increase in risk of approximately 6.5 times for HCV, 2.9 times for HBV and 7.1 times for HIV. The probability of acquiring TTI is related to the probability of being exposed to the infected units of blood. This majority of positivity depends on the prevalence of carriers among the blood donors and the number of units transfused. The high prevalence of TTIs observed among patients of various disease categories could reflect the limitations for continued blood screening as well as safe practices for blood transfusions [3]. Continued attention to the universal screening of blood products for TTIs as well as a more scientifically-sound and rational approach to avoiding paid blood donors, as well as a more thorough screening and selection of potential blood donors, is necessary to limit the number and rate of TTIs in India in the future.

In present study, as per the number of units transfused, all the 480 multi-transfused patients were divided into groups. This is observed that with increasing number of transfusion, prevalence of TTIs also increases progressively. This finding is in accordance with Mittal K et al where number of transfusions between 10-100 was 38.57%, 201-300 were 55%, 301-400 were 48% and >400 were 66.6% [22]. A fact that contributes to the conclusion that in the population studied, the prevalence observed is predominantly associated with the risk of transfusion.

Measures can be taken for prevention of TTI like voluntary donation in place of replacement donation, proper selection of donors, use of adequate asepsis technique during transmission. A repeat voluntary blood donor will be one who follows safe lifestyle behaviours will be tested for infections and thus will be the safest choice for being a blood donor. Burden of TTIs needs to be frequently assessed both in the setting of health facility and general population to understand the functioning of blood safety programmes of India. High prevalence of HCV necessitates better methods of screening in the transfusion facilities. Periodic monitoring of multi-transfused subjects through heamovigilance should be made a part of the blood safety programmes.

This study has shown that there are still problems that need to be addressed in regard to infectious disease testing, possible nosocomial infections, better strategies for HBV immunisations, selection of the appropriate blood product for each patient, as well as an integrated approach and increased awareness of complication of blood transfusion for the clinical management of multiple transfused patients. The prevelance of TTIs shown a decreasing trend due to compulsory screening of products in blood bank, governed by the Drugs & Cosmetic Rule in India 1940. Highly sensitive and specific test like p24 antigen detection or RNA detection by RT-PCR is to be introduced for detection of HIV in window period. However, detection of the hepatitis B core antigen antibody serves as a sensitive and specific marker. And also limited studies report the burden of blood-borne infections following multiple transfusion of blood from central India. Majority of studies includes the thalassemia patients, rest of the multiple transfused patients were not included. The need to explore the burden of TTIs in these patients cannot be overemphasised.

Limitation(s)

Even after screening of blood, the transmission risk of infections is not obsolete as screening methods are not sensitive enough to detect infections in window period. The limitations of high prevelance of HBsAg may be due to the fact that it's not detected in blood during window period. The high prevalence of HCV may be due to late introduction of screening test for HCV as compared to HIV1/2 and no such vaccination is available till yet.

CONCLUSION(S)

This study highlighted the prevelance of transmission transmitted disease not only in thalasaemic patient's diseases but its prevelance were high in haemodialysis patients as well. In the present study transmission of TTIs were seen, majority of which were HCV seropositivity, this occur inspite of sensitive screening included in blood bank before transfusion. So, this transmission of TTIs in patients can be prevented by providing safe blood products screened for TTIs and following some recommended measures: vigilant donor screening, voluntary donor promotion, implementation of advanced technologies like higher generation ELISA. Also, HBV prevelance can be reduced by vaccination to patients requiring multiple transfusions, but no such vaccination was there for HCV. So, introduction of fourth generation ELISA and Nucleic Acid Amplification Testing (NAT) test can reduced the window period of TTIs.

Acknowledgement

The authors would like to acknowledge the team in blood bank at RD Gardi Medical College for the enormous amount of work done in collecting and processing the data, without which this study would not have been possible. The authors are thankful to the management and faculties of RD Gardi Medical College, Ujjain. The authors would also like to thank the families of the 480 patients who kindly chose to participate in this study. Funding from any sources were not involved in this study.

REFERENCES

[1] Vidja PJ, Vachhani JH, Sheikh SS, Santwani PM. Blood transfusion transmitted infections in multiple blood transfused patients of Beta thalassaemia. Indian J Hematol Blood Transfus. 2011;27(2):65-69.

[2] Remesar M, Gamba C, Kuperman S, Marcosa MA, Miguez G, Caldarola S, et al. Antibodies to hepatitis C and other viral markers in multi-transfused patients from Argentina. J Clin Virol. 2005;34(Suppl 2):S20-26.

[3] Laguna-Torres VA, Perez-Bao J, Chauca G, Sovero M, Blichtein D, Chunga A, et al. Epidemiology of transfusion-transmitted infections among multi-transfused patients in seven hospitals in Peru. J Clin Virol. 2005;34(Suppl 2):S61-68.

[4] Ballester JM, Rivero RA, Villaescusa R, Merlın JC, Arce AA, Castillo D, et al. Hepatitis C virus antibodies and other markers of blood-transfusion-transmitted infection in multitransfused Cuban patients. J Clin Virol. 2005;34(Suppl 2):S39-46.

[5] Bhasin R, Chatterjee K, Ramalingam V. Blood transfusion transmitted diseases. In: Saran R (ed) Transfusion medicine technical manual, 2nd edn. Director General, Directorate General of Health Services, New Delhi, India, 2003; Pp. 143-74.

[6] Government of India. Ministry of health and family welfare. Department of health. Schedule F. The Drugs and Cosmetics Act, 1940 and the Drugs and Cosmetics Rules, 1945, as amended upto 30th June, 2005. Part XIIB. Pp. 326. Available from: http://www.cdsco.nic. In/Drugs and Cosmetic Act.pdf.

[7] Kumar S. Indian supreme court demands cleaner blood supply. Lancet. 1996;347(8994):114.

[8] Ray VL, Chaudhary RK, Choudhury N. Transfusion safety in developing countries and the Indian scenario. Dev Biol (Basel). 2000;102:195-203. PMID: 10794106.

[9] Narayan S. Microbes and blood transfusion. Indian J Med Microbiol. 2001;19(3):119-26.

[10] Sen S, Mishra NM, Giri T, Pande I, Khare SD, Kumar A, et al. AIDS in multitransfused children with thalassemia. Indian Pediatr. 1993;30:455-60.

[11] World Health Organisation: New report highlights global progress on reducing HIV, viral hepatitis and sexually transmitted infections and signals need for renewed efforts to reach 2030 targets; https://www.who.int/news/item/20-05-2021-new-report-highlights-global-progress-on-reducing-hiv-viral-hepatitis-and-sexually-transmitted-infections-and-signals-need-for-renewed-efforts-to-reach-2030-targets.

[12] American Red Cross; History of Blood Transfusion; https://www.redcrossblood.org/donate-blood/blood-donation-process/what-happens-to-donated-blood/blood-transfusions/history-blood-transfusion.html.

[13] Choudhury N. Transfusion transmitted infections: How many more? Asian J Transfus Sci. 2010;4(2):71-72.

[14] Kapoor C, Muhammad H, Muhammad I. Poly transfused thalassemia patients; prevalence of viral markers and malaria parasite. Prof Med J. 2007;14:177-81.

[15] Blood Bank SOP, Transfusion Medicine Department, AIIMS. 2017.

[16] Chuodhury N, Saraswat S, Naveed M. Serological monitoring of thalassemia major patients for transfusion associated viral infections. Indian J Med Res. 1998;107:263-68. PMID: 9701894.

[17] Lee WS, Chan LL. Risk of seroconversion of hepatitis B, hepatitis C and HIV in children with multitransfused thalassemia major. J Paediatr Child Health. 2005;41(5-6):265-68.

[18] Jain R, Perkins J, Johnson Susan T, Desai P, Khatri A, Chudgar U, et al. A prospective study for prevalence of transfusion transmitted infection in multiple transfused thalassemia major patients. Asian J Transfus Sci. 2012;6(2):151-54.

[19] Oza T, Agrawat A, Dhruva G, Oza HV. A study of prevalence of HIV, HbsAg and HCV in thalassemia major children. NJIRM. 2012;3(4):114-17.

[20] Gugnani P, Oberoi L, Arora M. Prevalence of transfusion transmitted infections in multiple blood transfused β-Thalassemia patients from a tertiary care centre in North India. IJCMR. 2019;6(12):L14-17.

[21] Bhavsar H, Patel K, Vegad M, Madan M, Pandey A, Asthana A, et al. Prevalence of HIV, Hepatitis B and Hepatitis C infection in Thalassemia major patients in tertiary care hospital, Gujarat. Nat J Integr Res Med. 2011;2(3):47-51.

[22] Mittal K, Abrol P, Yadav J. Prevalence of transfusion transmitted infections amongst multiple blood transfused patients of βthalassemia major in a tertiary care hospital. Int J Res Med Sci. 2017;5(1):181-85.

[23] Choudhury N, Phadke S. Transfusion transmitted diseases. Indian J Pediatr. 2001;68(10):951-58.

[24] Singh H, Pradhan M, Singh RL, Phadke S, Naik SR, Aggarwal R, et al. High frequency of hepatitis B virus infection in patients with beta thalassemia receiving multiple transfusions. Vox Sang. 2003;84(4):292.

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor and Incharge Bloodbank, Department of Pathology, Ruxmaniben Deepchand Gardi Medical College, Ujjain, Madhya Pradesh, India.
2. Ex-Resident, Department of Pathology, Ruxmaniben Deepchand Gardi Medical College, Ujjain, Madhya Pradesh, India.
3. Ex-Resident, Department of Pathology, Ruxmaniben Deepchand Gardi Medical College, Ujjain, Madhya Pradesh, India.
4. Resident, Department of Pathology, Padmashri Dr. Vithalrao Vikhe Patil Foundation's Medical College, Ahmednagar, Maharashtra, India.
5. Professor and Head, Department of Pathology, Ruxmaniben Deepchand.Gardi Medical College, Ujjain, Madhya Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Swati Patel,
B6/4, RDGMC, Ujjain, Madhya Pradesh, India.
E-mail: drswatipatelsoni02@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Aug 26, 2021
- Manual Googling: Dec 02, 2021
- iThenticate Software: Dec 22, 2021 (21%)

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: **Aug 25, 2021**
Date of Peer Review: **Oct 01, 2021**
Date of Acceptance: **Dec 23, 2021**
Date of Publishing: **Apr 01, 2022**