ORIGINAL ARTICLE

Frequency of Thalassemia and Its Association with Hepatitis C Infection in Local Population of Pakistan

Ghulam Jilany Khan[1], Nadeem Reyaz[2], Muhammad Bahadur Baloch[3], Analisa Cassandra[4] Susanne Schlicht[5]

ABSTRACT

Background and Objective: Thalassemia major or βeta-thalassemia is a hereditary blood disorder, necessitating continuous blood transfusions. Frequent blood transfusions often result in the development of hepatitis, hepatocellular carcinoma, acquired immunodeficiency syndrome (AIDS) and many other infectious diseases. Incidence of infection of viral hepatitis in thalassemia patients is majorly associated with infections in the donor population. The objective of this study was to ascertain the frequency of HCV infection in patients with thalassemia in Pakistan.

Methods: This study was conducted from January 2015 to September 2018 in various sections of Punjab and Sindh, Pakistan. A total number of 439 patients were enrolled in this study, samples of blood from the patients were collected and their Anti-HCV antibodies were evaluated by enzyme-linked immunosorbent assay (ELISA); HCV-RNA genome presentation was assessed by HCV-nested-real time-polymerase chain reaction (RT-PCR) for the confirmation.

Results: It was found that major population of the thalassemia patients were male (63%). While the female thalassemia population was almost twice in Sindh province as compared to Punjab. In present study, n = 187 were detected positive among those n = 168 were confirmed by PCR. Only 47% thalassemia patients were found vaccinated against hepatitis B whereas all HCV positive patients were not vaccinated, and (84.96%) thalassemia patients were from the family of first cousin marriage. The patients which are not vaccinated also had significant chance to acquire the viral infection ($P < 0.001$). Moreover there could be some gender and environmental or geographical factors associated with thalassemia that are yet to be explored in detail.

Conclusion: Thalassemia patients carry major risk for acquiring transfusion associated infection.

KEYWORDS: β-thalassemia, Hepatitis C Virus, Hepatitis B vaccination, Human immunodeficiency virus, Enzyme-linked Immunoessay, Real time-polymerase chain reaction.

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INTRODUCTION

Thalassemia major or β-thalassemia is a congenital blood disorder. Repetitive blood transfusions are mandatory to overcome its consequent hemolytic anemia. Frequent blood transfusions are never risk free.[1,2] It is a significant issue in Pakistan conceivably because of first cousin marriages.[3] By and large, the normal existence of βeta-thalassemic patients in Pakistan approximately around 5%.[1] This disease load was 90,000 to 100,000 patients all through the nation. It is expounding at the pace of 5000 youngsters each year.[1,4] Amongst the different blood transfusion based risks, the blood-borne viruses, for example, Hepatitis B and C viruses are the commonest infectious agents transmitted via a transfusion.[1] HCV is liable for approximately 80 to 90% of hepatitis following transfusion.[5] Since several transfusions of blood in thalassemia affected are vital for the existence, therefore, such patients are consistently at the risk of transfusion-related infectious diseases for instancehepatitis.[1]

World Health Organisation (WHO) perceives thalassemia as a foremostinherited blood disorder worldwide, having a bearer population of over 360 million. It has additionally been evaluated that about 5.2% of the total population conveys a thalassemic gene-disorder.[6] Pakistani population has the utmost frequency of thalassemia in children. It is probably owing to the wide occurrence of the defective alpha-beta chain gene, cousin marriages and high nationwide birth rate.[1,4]

The predominance of viral hepatitis manifestations in thalassemic patients is associated with the infection in the donor. Consequently, the prohibition of blood-borne viral transmission continues to be a key obsession for transfusion administrations overall.[1] The existence of HCV in thalassemia patients had been found altogether high. Documentation from various geographical regions of Iran likewise exhibited the HCV infection up to 55.5% in thalassemia patients.[7]

Studies of Hsu et al.[8] and Jia et al.[9] evaluated infectious viral proteins of HCV and HBV comprehensively, and concluded that these viral proteins have a complicated and multidimensional role in the hepatocellular carcinoma development by means of promoting angiogenesis, activation of oncogenes, stimulation of epithelial mesenchymal transition, and increasing the vascular invasion.

There are insufficient studies about this transfusion associated deadly hazard in Pakistan. Also the fatal consequences towards hepatocellular carcinoma in thalassemia patients in Pakistan proved a major motivation for this study. The existence of HCV infection following thalassemia associated transfusion was, therefore, assessed in this present study.

METHODS

This prospective observational study was piloted from January 2013 to September 2018, in five thalassemia centers of Punjab and Sindh provinces of Pakistan. The patients encompassed in the study were of more than 5 years of age, which had undertaken transfusions of blood (at least 40 times), with no known history of hepatitis viral infection to their parents, were considered for study after gaining consent from the parents/ guardians and by themselves. Whereas the patients aged less than 05 years, the patients who underwent transfusions of blood less than 40 times, with a former history of liver associated disease, with any antibiotic or antiviral drug therapyor whose parents have a known history of HCV infection, were not included in this study.

After obtaining the approval from ethical committee of University of Central Punjab, Lahore, Pakistan (Letter No: UCP/FOP/139) and the consent of the patients and/or guardians/parents, blood samples were collected as per the guidelines of WHO.

Venous blood samples were collected, some amount of the sample was taken as it is for rapid strip test (rapid testing of serum with 3.5mm strip), and remaining blood samples were treated with ethylene di-amine-tetra-acetic acid (EDTA) for the further analysis. Demographic information like age, interval andnumber of blood transfusions, history of vaccination against hepatitisand other data were acquired through thorough interview of the patients and/or guardians/parents. A complete flow gram of the study design is described in Fig:1 below.

Serum was isolated and every sample was cautiously screened. The presence of hepatitis C virus was confirmed through standard methods. Immediately after sampling rapid testing through

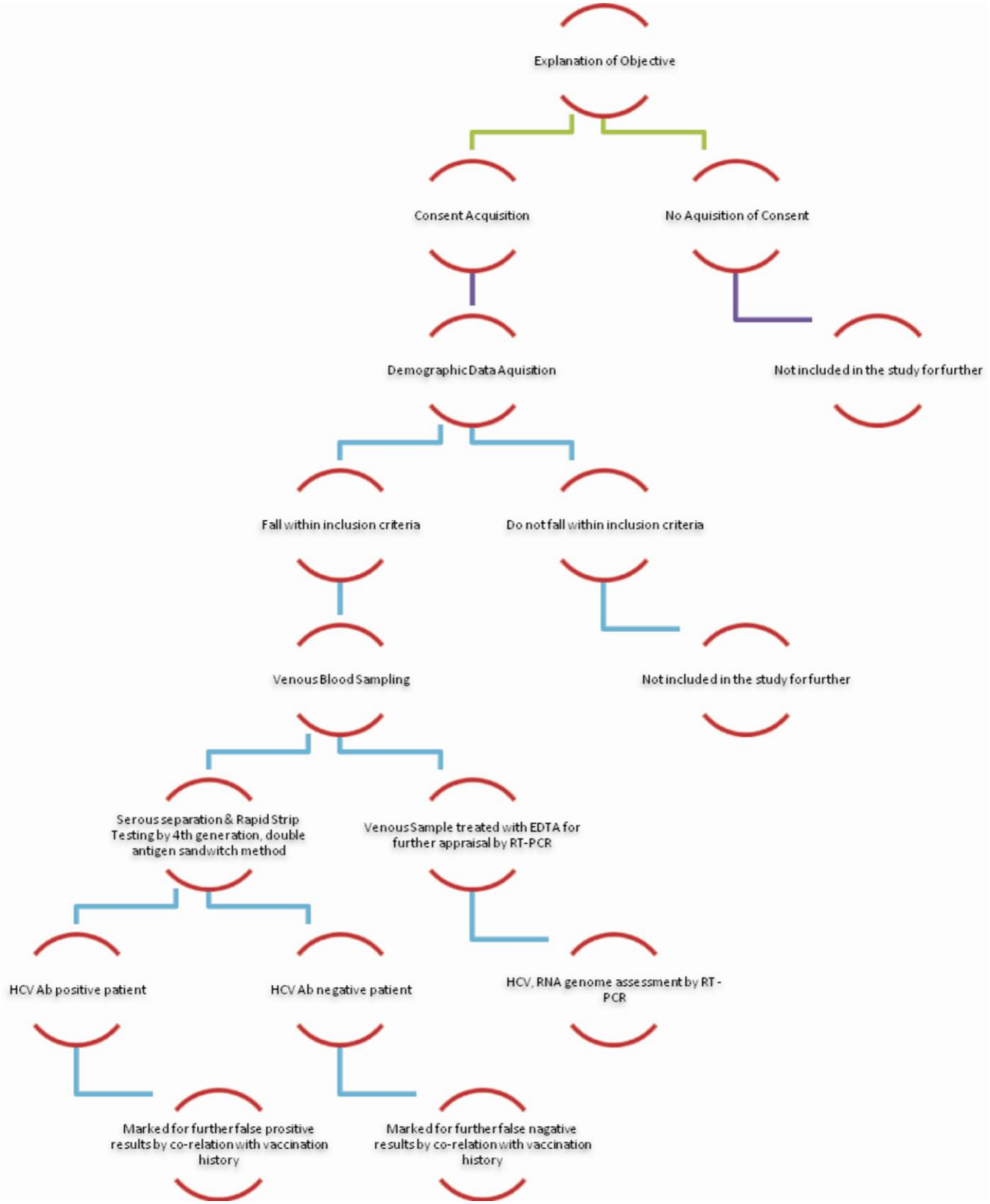

Fig.1: Flow gram of the study conducted from January 2013 to September 2018 in two provinces (Punjab and Sindh) of Pakistan.

double antigen Sandwich method, 4th generation was conducted.

The positive sample was then subjected to Anti-HCV antibody (HCV-Ab) evaluation in the plasma samples by third generation ELISA kit. As described earlier by Yeom et al.[10] The samples with positive results were additionally evaluated. Confirmation of HCV-RNA genome was confirmed by HCV-real time-polymerase chain reaction (RT-PCR). RNA was isolated from cell by using TRIzol method.[11] The PCR tested samples were seen on gel doc system to see the bands of DNA.[12]

STATISTICAL ANALYSIS

Chi square test was applied to see the frequency of HCV positive thalassemia patient's thalassemia amongst those vaccinated and non-vaccinated. A P-value of ≤ 0.05 was set as statistically significant.

RESULTS

In these analyses, all the persons were evaluated, but the extreme age of the persons was 21.5 ± 2 years and no patient with thalassemia was testified above this age on ground reality, although some of the individuals claimed to be familiar with thalassemia patient of older age, but no such patient responded to current study. Maximum respondents (n=71) were amongst the of 9.5 – 10.5 years age group (**Fig:2**).

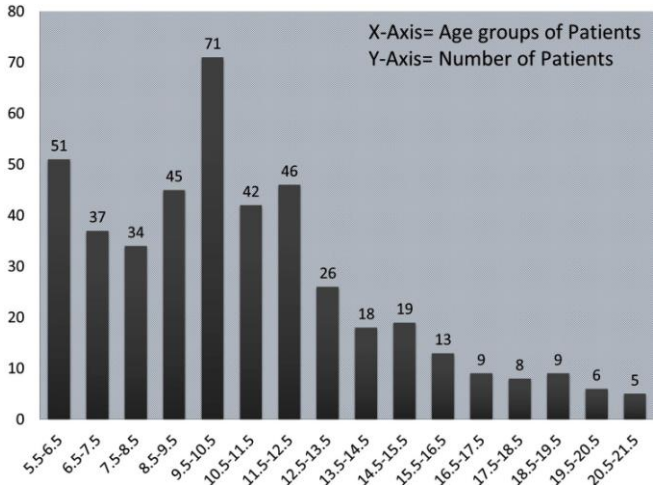

Fig.2: Frequency of thalassemia patients in different age groups.

Out of 439 antiphonals, 278 patients were male and 161 were female. This indicated that the thalassemia occurrence was more in males (63%) when contrasted with females (37%). It was additionally been recognized that the commonness of female patients was similar with males up to the age of 14.5 years (Fig:3).

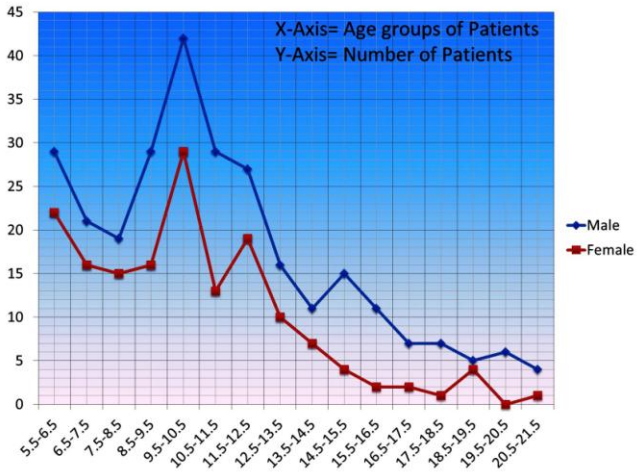

Fig.3: Gender-wise prevalence of disease along with age groups.

It was observed that the patients from Sindh (n=236) province were more affected as compared to Punjab (n=203). Besides the fact that the population of Sindh was little more affected than the population of Punjab, still the male patients of Punjab region were more affected (n=145) as compared to the male population of Sindh province (n=133); moreover male to female ratio is also quite different in both provinces. In Punjab, male to female ratio is around 2.5**:**1 where as in Sindh it is around 1.29**:**1. Another interesting observation is the number of female patients in both regions; in Sindh province female patients reported were almost the twice (n=103) of the female patients in Punjab (n = 58) (Fig:4).

By stepwise thorough HCV initial screening of 439 samples of the patients, 187 samples were found positively infected while 252 samples of the patients indicated negative response (Table-1, Fig:5).

Maximum numbers of patients were recognized in the 9.5 – 10.5 years age group which was about 16% of the whole respondents (Table-2).

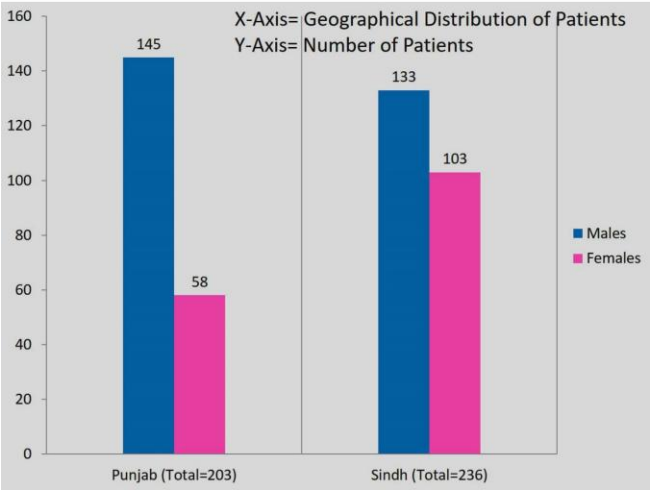

Fig.4: Geographical distribution of patients within Pakistan in two most populous provinces, Sindh and Punjab.

Table-1: Screening and PCR conformational results.

HCV Antibody	HCV RNA	HCV Infection
Negative/ Non-Reactive (n = 252)	Not Detected	Either there was no infection, or it was too soon for test
Positive/ Reactive (n = 187)	Not Detected	Either previous infection, or screening result false positive (n = 19)
	Detected	Current infection (n = 168)

Table-2: Step wise screening, of the blood samples from the patients, positive samples from the rapid strip testing, were further subjected to RT-PCR for the confirmation.

Age Groups	HCV Screening Through Rapid Strip Test (HCV-Ab)		PCR Confirmation RT-PCR	
	Negative	Positive	Positive	Not Confirmed
5.5-6.5	24	27	24	3
6.5-7.5	19	18	16	2
7.5-8.5	18	16	14	2
8.5-9.5	27	18	15	3
9.5-10.5	46	25	23	2
10.5-11.5	26	16	14	2
11.5-12.5	29	17	15	2
12.5-13.5	14	12	12	0
13.5-14.5	11	7	6	1
14.5-15.5	12	7	6	1
15.5-16.5	7	6	6	0
16.5-17.5	4	5	5	0
17.5-18.5	4	4	3	1
18.5-19.5	5	4	4	0
19.5-20.5	4	2	2	0
20.5-21.5	2	3	3	0
Total	252	187	168	19

Just 208 patients came out vaccinated against hepatitis B whereas 223 patients were observed without any history of vaccination against hepatitis. Out 223 non-vaccinated patients, 168 (75.3%) were detected HCV positive while 36 non-vaccinated patients were HCV negative (P < 0.001). It was discerned that large number of the hepatitis C virus infected patients had never been vaccinated which could have conducted the patients towards HCV infection (**Fig:5**).

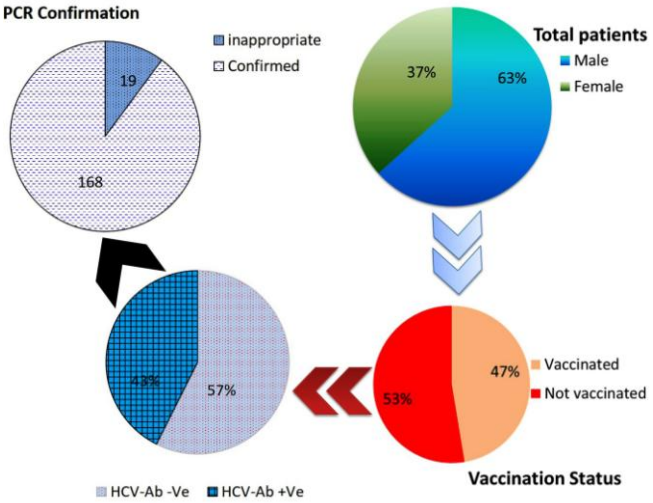

Fig.5: Schematic representation of study for prevalence of HCV in thalassemia patients in Pakistan with vaccination status against HBV (P < 0.001).

DISCUSSION

Thalassemia is a genetic blood disorder and βeta-thalassemia is the most prevalent autosomal single–gene disorder worldwide, distributed in above 60 countries with up to 100 million carrier population.[6] The combined frequency of βeta-thalassemia predictable in Gaza strip was 4.3%.[13] Iran reported occurrence of anti-HCV antibodiesin patients with thalassemia as 4.2%.[14] An earlier random survey based study which was conducted in locality of Faisalabad in 2016, showed 77.6% positive results in thalassemic patients together with HCV infection.[15] The results of present study indicated that prevalence of HCV among β-Thalassemia patients was 38.27% by RT-PCR. The nations with a higher HCV occurrence in overall population also had a greater incidence rate amongst thalassemia patients.[16]

Ahmadi Vasmehjani et al,[14] has documented high seropositivity in the thalassemia patients aged 21–30 years. This is contrary to this study with mean age around 10.80 ± 1.9 years of the individuals in the survey. Although some of the individuals claimed to be familiar with thalassemia patient of older age, but no such patient responded to the current study. Furthermore, the increase in number of blood transfusions was in proportion with an increase in age, the possibility of acquiring the disease was also raised. This showed a direct relationship between HCV infection and blood transfusions. Out of 439 samples, anti-HCV antibodies were not detected in 252 samples, this was conceivableowing to two reasons that is, either the patient had no exposure to HCV or it was too prompt to be diagnosed.

Out of 187 initially found infected respondents, 168 patients confirmed the disease when their blood samples were further analyzed for HCV-RNA genome by RT-PCR. The maximum respondents were from age group 9.5 – 10.5 years (n = 71), and counts 16% of the total studied population (**Table-2**). Moreover, it was observed that the patients beyond 15.5 years of age had additional probabilities to procure the infection as compared to individuals of younger age. The higher the number of transfusions, sexual procuring and environmental exposure can be attributed to the probabilities.[14]

Another interesting finding of the present study was vaccination of hepatitis B. It was observed that the patients who received vaccine against HBV had fewer chances to develop HCV infection compared to those who had not been vaccinated. Screening test of hepatitis C showed 187 positive cases and all of them didn't receiveany vaccine against the viral disease. It can be attributed to influence of HBV on HCV replication.[17]

The findings in present study featured transfusion of blood as the major risk factors for HCV infection amongst patients of beta-thalassemia. The higher occurrence of HCV infection was observed in older thalassemia patients. Moreover, the patients with a progressive number of units transfused and increasingly number of transfusions every month, were found more prone towards the infection that spectacles the importance of safe and healthy blood donations which consequently may help to reduce the incidences of HCV infections. Besides the disconsolation and helplessly tiring routine of blood transfusions and its associated therapy, it becomes quite alarming when thalassemia patients get infected with hepatitis virus.[1,7] Because of precarious CBC values, decreased and continuously fluctuating hemoglobin level and very young age of the patient, it is not appreciable for the accustomed interferon therapy.[19]

CONCLUSION

A low incidence of HCV prevalence has been seen in β-thalassemia major patients undergoing regular transfusion. However, the prevalence of disease is almost three times more in males than females. Nevertheless, the patients who are not vaccinated against hepatitis B, may have significant chance to acquire the viral.

LIMITATIONS OF THE STUDY

There could be a regional/atmospheric and/or gender effect on the development of hepatitis viral infection in the patients of thalassemia. This association of atmosphere, gender and thalassemia, with hepatitis infection is further needed to be explored and requires a thorough investigation.

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CONFLICT OF INTEREST

None to declare.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

None to disclose.

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Author's Contribution

GJK: Conception and design of study, critical analysis with intellectual output.
NR: Analyzing the data, final approval of the manuscript.
MBB: Acquisition of data, drafting the manuscript, statistical analysis.
AC & SS: Conception and design of the study.
ALL AUTHORS: Approval of the final version of the manuscript to be published.