DOI: https://doi.org/10.54393/pjhs.v5i11.2294

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (P): 2790-9352, (E): 2790-9344
Volume 5, Issue 11 (November 2024)

Original Article

OPEN ACCESS

Types of Inherited Hemoglobin Disorders among the Patients Attending a Tertiary Care Hospital

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ARTICLE INFO

Keywords:
Thalassemia, Hemoglobin, Reticulocyte, Ferritin Level

How to Cite:
Anwar, R., Arzoo, K., Riaz, A., Ali, R., Saghir, S., & Habib, B. Types of Inherited Hemoglobin Disorders among the Patients Attending a Tertiary Care Hospital: Types of Inherited Hemoglobin Disorders among the Patients. Pakistan Journal of Health Sciences, 5(11). https://doi.org/10.54393/pjhs.v5i11.2294

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Received Date: 20th September, 2024
Acceptance Date: 17th November, 2024
Published Date: 30th November, 2024

ABSTRACT

The genetic conditions known as hemoglobinopathies, which include thalassemia impact the synthesis and structure of Hemoglobin, the red blood cell protein that carries oxygen. **Objectives:** To investigate the prevalence of different types of thalassemia associated with age, gender and Hematological parameters. **Methods:** The total number of participants was n=139. The cross-sectional study was conducted at Rai Medical College Sargodha. The study was conducted for six months, from July 2023 to Dec 2023. Biochemical parameters investigated such as Hemoglobin, mean corpuscular hemoglobin MCH, reticulocyte and ferritin were done in the Hematology lab. Collected data were analyzed by SPSS version 25.0. **Results:** The gender distribution among the participants included male 50.3% and female 49.6%. The mean age of the patients was approximately 34.8 years. This study of 139 participants found Hb-E Beta Thalassemia (25.4%) to be the most common type, especially among those with a history of cousin marriage. Severe forms like Hb-E Beta Thalassemia and Beta Thalassemia Major had low hemoglobin and MCH levels, indicating severe anemia, while milder forms showed near-normal levels. In mean corpuscular Hemoglobin E Beta Thalassemia (29.7 ± 4.1), p=0.001, mean corpuscular Hemoglobin A levels are considerably lower than Beta Thalassemia Trait. There was no association with age and gender, p>0.005. **Conclusions:** It was concluded that our investigation offered important insights into the biochemical profiles linked to various thalassemia types, even though it did not identify any appreciable variations in thalassemia prevalence by age or gender.

INTRODUCTION

The term "Hemoglobinopathies" refers to a class of hereditary diseases that affect the production of globin chains, which are vital constituents of Hemoglobin (Hb)—the oxygen-transporting protein found in red blood cells. A variety of clinical problems are brought on by genetic changes in these illnesses that change the normal production or structure of Hb [1]. Thalassemia is a disorder marked by diminished synthesis of one or more globin chains, such as beta or alpha chains. Two such instances are the alpha and beta types of thalassemia. Those involve atypical Hb, which is brought about by mutations which alter the globin chains' internal structure. Here are Hb-S, Hb-C, and Hb-E (Hb-E) as examples [2]. About 1,200 different genetic modifications impacting the DNA sequences of the human β-like (Hb-Z, Hb-A2, Hb-A1, and Hb-Q1) and β-like (Hb-E1, Hb-G2, Hb-G1, Hb-D, and Hb-B) globin genes are the primary root cause of the noted clinical variation. These variants have been catalogued in the Hb-Variants database, a locus-specific repository of Hb variants and their associated clinical phenotypes [3]. Accurate DNA diagnosis of thalassemia can be achieved by family research and thorough Hematological tests. For this reason, a variety of methods can accurately, quickly, and affordably identify the underlying genetic abnormality in afflicted individuals [4]. The majority of these are the result of one or more globin chains having a single amino acid

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substituted. An estimated 320,000 infants are born annually with major Hb disorders: 83% with sickle cell disease and 17% with thalassemia. these births, about 80% take place in poor nations. Five of the most cautious projections indicate that more than 100 million people with a global frequency of 1.5% for beta-thalassemia and at least 5.2% of the world's population (over 360 million) carry a substantial Hb variation [5, 6]. The human body uses Hb for a variety of purposes, including buffering hydrogen ions, metabolizing nitric oxide, carrying carbon dioxide from tissues to the lungs, and transporting oxygen from the lungs to the tissues. Adult blood contains between 11.5 and 18 g/dL of hemoglobin [7]. The two main categories of Hb disorders are hemoglobinopathies, which are caused by abnormalities in the Hb gene structure, and thalassemia, which are caused by mutations that impact the expression and synthesis of the Hb chain. Thalassemia is an inherited hematological disorder categorized by a decrease or absence of one or more of the globin chain synthesis. Beta-thalassemia is caused by one or more mutations in the beta-globin gene. The absence or reduced amount of beta-globin chains causes ineffective erythropoiesis which leads to anemia [8, 9]. A thalassemia phenotype and a functional deficit in the globin chain are the results of some structural Hb variants that are also ineffectively produced or globin chain variants that are so unstable that they cannot form tetramers [10]. The first group, known as thalassemia hemoglobinopathies, comprises the δβ fusion variants (Hb-Lepore) and Hb E, β26 (Glu→Lys). In this case, the substitution at β-codon 26 (GAG→AAG) also results in alternative splicing of the β globin mRNA, which lowers the normally spliced β message encoding the Hb-E variant. Hereditary persistence of fetal haemoglobin (HPFH) is a subset of hemoglobinopathies that cause varying elevations in Hb-F in otherwise healthy individuals [11]. Owing to their simultaneous elevation in Hb-F levels, the δβ- and γδβ-thalassaemias are sometimes categorized as part of the syndrome of elevated Hb Fs, creating a continuous range within the HPFHs. Nonetheless, the differentiation between HPFH and δβ-thalassemia must be maintained for pragmatic and medical purposes [12]. There may be a gap in comparing the research population's Hb problem prevalence and features to those of other genders and ages in Pakistani communities may offer important context and insights.

This study aims to investigate the prevalence of different types of thalassemia associated with age, gender and Hematological parameters.

METHODS

A cross-sectional study was conducted at the Department of Physiology at Rai Medical College Teaching Hospital, Sargodha, from July 2023 to Dec 2023. This study was approved by the institutional review board (IRB) reference number (RMCS/ERC/3/22). The study participants were attending the Physiology and Hematology outpatient department and the sample size was determined to be n=139. The formula for calculating the sample size when estimating a proportion is n= Z2 x P x (1-p) 2/E2, where n=sample size, Z=Z score confidence level (95%), P=prevalence of Hb disorder 10% (p=0.10) and E=margin of error 5% (E=0.05) [13]. Inclusion criteria were participants aged between 15-54 years, male and female gender, and patients suffering from hemoglobinopathies previously diagnosed according to Hb electrophoresis. Exclusion criteria included critically ill patients needing immediate hospitalization, extremes of age, patients already admitted, and those with Hematological malignancy. Informed consent was obtained from all participants or their guardians. The Hematological parameters were examined through blood sampling to diagnose, including Hb, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Red Cell Distribution Width (RDW). The morphological abnormalities, such as microcytosis, hypochromic, and target cells, that are suggestive of thalassemia were investigated in a peripheral smear. Hb Electrophoresis tests were used to detect and quantify abnormal Hb fractions, including Hb-A2 and Hb-F levels. Data were collected through interviews, using a questionnaire including demographic characteristics and relevant information related to Hb disorder [14]. The patient's demographics, clinical state, and laboratory findings were all reported at the time of presentation. Standard deviation, mean, numerical frequencies, and percentages (%) were used to display data. The data were statistically analyzed using descriptive statistics for frequencies of each Hb disorder, One way ANOVA was used for comparing the mean of different groups, and chi-square tests were used for association between categorical data by SPSS version 25. There was statistical significance among the variables (p-value<0.05).

RESULTS

The demographic characteristics of the participants in your study (n=139) reveal important insights into the composition of your sample. The gender distribution among the participants included male 50.3% and female 49.6%. The mean age of the patients is approximately 34.8 years. The majority of the participants 46.7% fell within the age group 15-24 as compared to another age group. The majority of participants included rural area 57.5% and urban area 42.4%. Most participants 56.8% have had their illness for more than 5 years. Of the interviewees, 55 (39.5%) said they were married to a relative. A total of 84 participants, or 60.4%, were not wed to a cousin. Of the participants, 72 (51.7%) were married, and 67 (48.2%) were single. Of the subjects, 84 (60.4%) had no family history of

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Hb problems and 25 (17.9%) had thalassemia, 30 (21.5%) had sickle cell disease in family history (Table 1).

Table 1: Demographic Characteristics of Participants

Characteristics		Number of Participants (n=139)
Gender	Male	70 (50.3%)
	Female	69 (49.6%)
Age	15-24	65 (46.7%)
	25-34	35 (25.1%)
	35-44	20 (14.3%)
	45-54	19 (13.6%)
Residence	Urban	59 (42.4%)
	Rural	80 (57.5%)
Period of Illness	2 Years	22 (15.8%)
	3 Years	38 (27.3%)
	>5 Years	79 (56.8%)
Cousin Marriage	Yes	55 (39.5%)
	No	84 (60.4%)
Marital Status	Yes	72 (51.7%)
	No	67 (48.2%)
Family History	Thalassemia	25 (17.9%)
	Sickle Cell Disease	30 (21.5%)
	No	84 (60.4%)

The distribution suggests that while milder forms (Hb-E Trait and Beta Thalassemia Trait) are present, they are less common compared to the severe form of Hb-E Beta thalassemia (Figure 1).

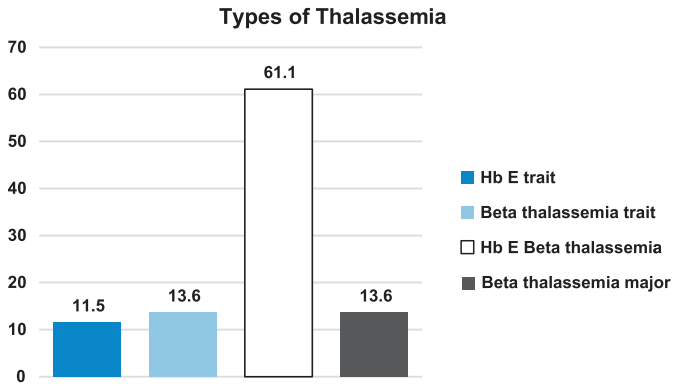

Figure 1: Different Types of Thalassemia in the study Participants

The distribution of inherited hemoglobinopathies among patients with a history of cousin marriage is shown in the table. According to the data, the beta thalassemia trait accounts for 20% of cases, whereas the Hb-E trait is present in 21.8% of cases. Furthermore, the greatest percentage of these conditions—25.4%—are Hb-E Beta thalassemia, a compound illness that involves both Hb-E and Beta thalassemia features. Lastly, 20% of cases are known to have beta thalassemia major, a severe form of thalassemia. With the highest prevalence observed in those with the combined Hb-E Beta thalassemia phenotype, this distribution points to a significant correlation between cousin marriage and several Hb diseases. The possible hereditary hazards are reflected in the comparatively high percentages for all disorders (Figure 2).

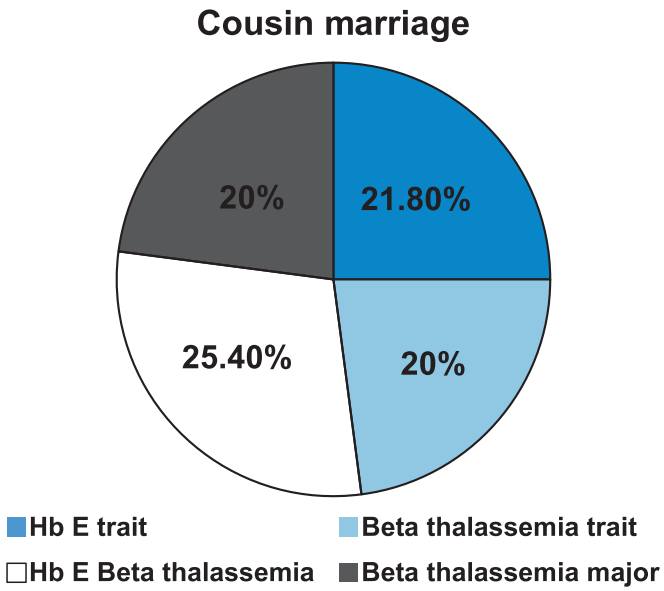

Figure 2: Percentage of Different Types of Thalassemia Associated with Cousin Marriage

Our current study indicates that the various forms of thalassemia differ considerably in terms of electrophoretic profiles and biochemical characteristics. In Hb-E Beta-thalassemia (6.81 ± 1.1 g/dL) and Beta-thalassemia major (5.9 ± 0.4 g/dL), Hemoglobin (Hb) levels were significantly reduced, indicating severe anemia; in contrast, Hb-E trait (14.1 ± 2.2 g/dL) and Beta-thalassemia trait (13.8 ± 0.1 g/dL) showed near-normal levels, suggesting a mild clinical presentation (p=0.001). A similar pattern was seen in the Mean Corpuscular Hemoglobin (MCH), with the lowest values being found in Hb-E Beta-thalassemia (7.7 ± 0.6 pg) and Beta-thalassemia major (5.5 ± 1.3 pg), indicating microcytic hypochromic anemia. MCH readings were lower than normal levels (p=0.001). However, they were greater in the Hb-E trait (15.9 ± 2.9 pg). In Hb-E beta-thalassemia (13.4 ± 4.1%) and beta-thalassemia major (12.8 ± 2.2%), there was a significant rise in the reticulocyte percentage, a measure of red cell formation, suggesting greater hemolysis and red blood cell turnover. The beta-thalassemia trait, on the other hand, had the lowest reticulocyte count (1.5 ± 0.1%), which indicated limited hemolysis (p=0.001). Due to frequent blood transfusions and iron overload, ferritin levels were highest in Hb-E Beta-thalassemia (12.9 ± 2.2 mg/ml) and Beta-thalassemia major (12.5 ± 1.1 mg/ml), while ferritin levels were lowest in Beta-thalassemia trait (2.1 ± 0.2 mg/ml) (p=0.001). Affected by defective beta-globin synthesis, Hb-A levels in the Hb electrophoresis profile were highest in Beta-thalassemia trait (79.4 ± 8.9%) and much lower in Hb-E Beta-thalassemia (22.5 ± 6.2%) and Beta-thalassemia major (29.7 ± 4.1%) (p=0.001). Hb-A2 levels were significantly higher in Hb-E beta-thalassemia (29.3 ± 3.1%) and Hb-E trait (26.9 ± 3.7%), which is consistent with aberrant Hb-E synthesis (p=0.009). As

DOI: https://doi.org/10.54393/pjhs.v5i11.2294

anticipated, the percentage of Hb-E was highest in Hb-E Beta-thalassemia (30.1 ± 5.9%) and lowest in Hb-E trait (17.8 ± 5.2%). Nevertheless, p=0.067 indicates that there was no statistically significant difference in the Hb-E percentages between the groups. Hb-F levels were higher in every group, particularly in Hb-E Beta-thalassemia (19.6 ± 7.3%), which indicates the continued presence of fetal Hb. The groups' differences in Hb-F levels, however, did not reach statistical significance (p-value=1.899) (Table 2).

Table 2: Hematological Parameters and Electrophoretic Profile Associate with Different Types of Thalassemia through Blood Sampling

Parameters	Hb-E trait	Beta Thalassemia Trait	Hb-E Beta Thalassemia	Beta Thalassemia Major	p-value
Hb (g/dL)	14.1 ± 2.2	13.8 ± 0.1	6.81 ± 1.1	5.9 ± 0.4	0.001
MCH (pg)	15.9 ± 2.9	13.5 ± 0.2	7.7 ± 0.6	5.5 ± 1.3	0.001
Reticulocytes %	4.5 ± 3.2	1.5 ± 0.1	13.4 ± 4.1	12.8 ± 2.2	0.001
Ferritin (mg/ml)	4.7 ± 1.4	2.1 ± 0.2	12.9 ± 2.2	12.5 ± 1.1	0.001
Hemoglobin					
Hb-A %	44.8 ± 5.5	79.4 ± 8.9	22.5 ± 6.2	29.7 ± 4.1	0.001
Hb-A 2 %	26.9 ± 3.7	3.0 ± 0.1	29.3 ± 3.1	6.2 ± 0.3	0.009
Hb-E %	17.8 ± 5.2	1.5 ± 0.2	30.1 ± 5.9	10.7 ± 1.1	0.067
Hb-F %	16.3 ± 0.1	15.8 ± 0.4	19.6 ± 7.3	16.9 ± 1.1	1.899

Our study indicates that the Hb-E trait, Beta thalassemia trait, Hb-E beta-thalassemia, and Beta-thalassemia major show that there are no gender differences that are statistically significant in this study sample (0.119, 0.881, 0.055, and 0.121, respectively). These p-values were marginally higher than the 0.05 cutoff (Table 3).

Table 3: Gender based Association with Types of Thalassemia

Types of Thalassemia	Male (n=70)	Female (n=39)	Chi-square (x^2)	p-value
Hb-E Trait	5 (7.1%)	3 (7.69%)	4.1503	0.119
Beta Thalassemia Trait	2 (2.85%)	4 (10.25%)	4.1503	0.881
Hb-E Beta Thalassemia	61 (87.14%)	29 (74.35%)	4.1503	0.055
Beta Thalassemia Major	2 (2.85%)	3 (7.69%)	4.1503	0.121

In this study there are no significant differences between the Hb-E trait, Beta thalassemia trait, Hb-E Beta thalassemia, and Beta thalassemia major, suggesting a potential but non-conclusive age-related difference in prevalence. These p-values were marginally higher than the 0.05 cutoff (Table 4).

Table 4: Age based Association with Types of Thalassemia

Age	15-24	25-34	35-44	45-54	Chi-square (x^2)	df	p-value
Hb-E Trait	6 (37.5%)	3 (18.75%)	4 (25%)	3 (18.75%)	3.364	5	0.009
Beta Thalassemia Trait	5 (26.31%)	6 (31.57%)	3 (15.78%)	5 (26.31%)	3.364	5	0.061
Hb-E Beta Thalassemia	46 (54.11%)	32 (37.64%)	3 (3.5%)	4 (4.7%)	3.364	5	0.006
Beta Thalassemia Major	4 (21.05%)	5 (26.31%)	7 (36.84%)	4 (21.05%)	3.364	5	0.007

Certain types of thalassemia are slightly more common in individuals from cousin marriages. For example, 21.8% of those with the Hb-E trait and 25.4% with H-E beta-thalassemia were born of cousin marriages, compared to 20% for both the beta thalassemia trait and major. These results suggest a modestly increased risk of inheriting thalassemia in consanguineous unions, likely due to a higher chance of recessive gene transmission in genetically similar parents (Table 5).

Table 5: Association of Cousin Marriage with Different Types of Thalassemia

Types of Thalassemia	Cousin Marriage (%)	No Cousin Marriage (%)	Chi-Square Value	p-value
Hb-E Trait	21.8%	78.2%	0.000	1.000
Beta Thalassemia Trait	20.0%	80.0%	0.106	0.744
Hb-E Beta Thalassemia	25.4%	74.5%	0.426	0.514
Beta Thalassemia Major	20.0%	80.0%	0.106	0.744

DISCUSSION

In the present study to found that, the gender distribution is almost equal, with male comprising 50.3% and female 49.6% of the participants. The mean age of the participants is approximately 34.8 years, with the majority (46.7%) falling within the 15-24 age groups. The larger proportion of participants (57.5%) are from rural areas compared to urban areas (42.4%). This indicates that a significant number of patients are living with long-term conditions related to their Hb disorders. The relatively high percentage of participants with a duration of illness exceeding 5 years highlights the chronic nature of these disorders and suggests a need for long-term management strategies. This balanced distribution suggests that both genders and ages are similarly affected or seek medical consultation at a tertiary care hospital for inherited Hb disorders. We agreed that the previous study by Ata *et al.*, and Mairbäurl *et al.*, reported a slightly higher prevalence of male in their sample, with male constituting around 55% of the study population. The balanced gender ratio in this study could suggest that there is increasing awareness and access to healthcare services for female, or it might reflect a true equal prevalence among genders in the region [15, 16]. The current study indicates that severe forms of thalassemia, such as Hb-E Beta Thalassemia and Beta Thalassemia Major, exhibit significantly lower Hb levels (6.81 ± 1.1) and MCH (5.5 ± 1.3) compared to milder forms like Hb-E Trait and Beta Thalassemia Trait. This finding is statistically significant with a p-value of 0.001. Significant differences were observed in biochemical parameters across different types of thalassemia. The patients with Beta Thalassemia Major consistently have lower Hb levels due to ineffective erythropoiesis and increased hemolysis. The low MCH in severe thalassemia reflects microcytic anemia, which is a hallmark of these conditions. These findings emphasize the severe impact of the disease on red

DOI: https://doi.org/10.54393/pjhs.v5i11.2294

blood cell morphology and functionality. Similar results were observed in a study by Wasim *et al.*, and Borai *et al.*, who also reported that patients with Beta Thalassemia Major exhibited significantly lower Hb levels compared to those with Beta Thalassemia Trait, supporting the notion that the severity of hemolytic anemia is a distinguishing factor between these conditions [17, 18]. Significantly elevated ferritin levels (12.9 ± 2.2) and reticulocyte count (13.4 ± 4.1) in severe thalassemia forms, such as Hb-E Beta Thalassemia and Beta Thalassemia Major, indicate higher erythropoietin activity and an excessive amount of iron. This result is also notable with a p-value of 0.001. The results presented here support the hypothesis that in people who suffer from severe thalassemia, the body attempts to combat persistent hemolysis by raising the reticulocyte count. Elevated ferritin levels have been linked with poor erythropoiesis as well as repeated transfusions of blood, which may contribute to iron exhaustion, which is an important concern for patients with chronic thalassemia. Hb-A concentrations in Hb-E beta thalassemia are significantly reduced (29.7 ± 4.1) than in the beta thalassemia trait, with an independent p-value of 0.001. This is suggestive of the abnormal output of Hb caused by the substance's heterozygous status of the Hb-E and beta thalassemia genes. The identical findings have been reported by Ahmad *et al.*, who determined that due to Hb- E being an Hb variant with a structural defect that inhibits the development of normal Hb-A, those with Hb-E beta-thalassemia had significantly decreased Hb-A levels. This outcome emphasizes the complicated Hb makeup in compound heterozygotes and its potential effects on therapy [19]. Understanding the gender disparity in the frequency of these conditions makes it easier to tailor genetic counselling and public health campaigns. While no significant differences were found, it remains important to consider other factors that could impact the occurrence of these disorders, such as cultural background, consanguinity rates, and environmental influences. Extensive research undertaken across diverse populations has revealed significant variations in the gender distribution of thalassemia. For example, an earlier study conducted by Mir *et al.*, did not detect any significant differences in thalassemia prevalence between genders, which is consistent with your findings. However, according to some local studies, there may be a higher frequency in men, possibly due to cultural or genetic reasons [20]. Severe variations of thalassemia, like Beta Thalassemia Major, typically show symptoms in early childhood, although milder versions may go years without symptoms being seen. The lack of notable age-related changes observed in the study sample may be due to the inclusion of individuals at varying stages of the disease progression. According to Shafique *et al.*, some populations may have more thalassemia characteristics in younger age groups due to recent improvements in awareness and diagnosis, whereas more severe variants may appear later as problems occur [21]. A current study showed that hematological indices, such as Hb, MCH, reticulocytes, ferritin, and levels of Hb-A and Hb-A2, are critical indicators for differentiating between different thalassemia syndromes. These markers' noteworthy variations demonstrate their therapeutic value. The non-significant results for Hb-E and Hb-F, however, imply that these metrics might not be enough to distinguish between these circumstances on their own. Programs to raise public awareness about the genetic dangers of consanguineous marriages must be centred in the community.

CONCLUSIONS

It was concluded that even though the analysis did not find any discernible differences in the prevalence of thalassemia by age or gender, it did provide valuable insights into the biochemical profiles associated with different kinds of the disease. Further research is required to fully comprehend the complex links that exist between demographic factors and thalassemia and to enhance the diagnosis, treatment, and management of this inherited illness.

Authors Contribution

Conceptualization: RA
Methodology: KA, AR, RA
Formal analysis: KA, SS
Writing review and editing: SS, BH

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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