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IMPACT OF IRON OVERLOAD AND HAMETOLOGICAL IN TRANSFUSION DEPENDENT THALSSEMIA PATIENTS

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Abstract

Background: In order to investigate the demographic, hematological and renal failure variables that affect TDT patients in Hyderabad and adjacent regions, the current study was conducted.

Methods: A total of 142 individuals were interviewed throughout the research period, comprising 71 TDT patients and 71 control subjects (age and gender matched with no personal or family history of blood disorders). Serum ferritin, hepatitis C and B, blood groups, hematological parameters, complete blood count have been compared with control groups.

Results: When comparing TDT patients to the control group, we discovered that their serum haemoglobin (HB), mean corpuscular value (MCV), or mean corpuscular hemoglobin (MCH). In contrast to male control participants, red blood cells (RBC), HB, HCT, and MCV were shown to be considerably increased ($p<0.05$) in male patients and dramatically decreased ($p<0.05$) in both genders. It is a significant serum creatinine level was noted high among 5-10 years and 11-15 years in comparison to all other age groups. the higher level of serum urea at the age of 4-10 years and non-significant variation ($p>0.05$) was found among all other age groups of TDT patients.

Conclusions: Transfusion-related blood abnormalities in TDT patients include iron excess and its repercussions, which include injury to the kidneys and spleen. TDT patients also frequently have hepatic viral infections. It is important for medical professionals to carefully monitor and treat patients in order to lessen their suffering and help them return to a balanced way of life.

Keywords: Iron overload, renal chemistry, transfusion, thalassemia.

INTRODUCTION

Thalassemia is a diseases caused by genetic disorder in blood leading to defective hemoglobin concentration. Since the first case of Thalassemia was reported from Mediterranean source the name Thalassemia was given after Greek word "Thalassa' which means "The Sea"[1]. During 1925, Italian children with this disorder showed extreme weakness, structural deformities in bone marrow and splenomegaly. This disease is more commonly found in those countries surrounding the Mediterranean Sea[2]. One of the most prevalent autosomal recessive haemoglobin diseases in the world is thalassemia, which is inherited. The thalassemia gene is present in over 250 million people in the Middle East and south Asian nations. Every year, over 60,000 infants worldwide suffer from severe cases of thalassemia related to their genes[3].Thalassemia illness is one of the primary causes of paediatric mortality and morbidity in Pakistan[4]. An estimated 5,000 individuals, primarily children, are diagnosed with hereditary thalassemia major disease each year[5]. With a carrier ratio of 5 to 8% and a variety of clinical symptoms, thalassemia is a disease that is often growing in Pakistan[6]. Thalassemia is a type of heterogeneous genetic condition caused by mutations or abnormalities in the genes that produce the α or β globin chains. A malfunction in the β-globulin chain's synthesis causes β thalassemia, which in turn causes major transfusion-dependent thalassemia patients and small clinically silent heterogeneous thalassemia[7]. In order to maintain their haemoglobin level, patients with thalassemia major require regular blood transfusions, which affects several organs and significantly increases their morbidity and mortality. As previously mentioned, the high level of iron in their blood from repeated blood transfusions for the purpose of managing haemoglobin levels causes haematological and biochemical complications, including transfusion hemosiderosis and excessive gastrointestinal iron absorption. Many bodily organs suffer significant damage as a result of iron accumulation in the liver and other endocrine glands, which leads to endocrine system failure[8]. This variety of complications in thalassemia patients leads to death; consequently, better and proper handling of thalassemia treatment is needed. To prevent thalassemia, a combination of data measures including genetic research, parental investigation, and disease carrier identification are needed. An estimated 5,000 infants in Pakistan are born with thalassemia major annually. Pakistan is home to an exceptionally high number of thalassemia patients: around 150 million people with a 5-8% prevalence of being a general carrier of the β-thalassemia gene[9].Thalassemia comes in two varieties: α- and β-thalassemia. These are brought on by a person's decreased ability to combine their particular hemoglobin's beta and alpha chains. Alpha–thalassemia is further divided into silent carrier, alpha thalassemia trait, which causes hemoglobin H-disease and Hydrops fetal or major alpha thalassemia. Beta thalassemia is subdivided into β-thalassemia minor, β-thalassemia intermediate and β-thalassemia major. Study showed that more than 180 gene mutations in major β-thalassemia patients were identified around the world and fifty β-thalassemia gene mutation in eastern part of the Mediterranean countries[10]. Thalassemia major has different clinical manifestations, such as iron overload, splenectomy, renal and hepatic damage, dyslipidemia, viral infections and oxidative stress[11,12].

MATERIAL AND METHODS

Setting: Hematology and biochemical features involved in TDT patients compared with control participants were the two sections of the cross-sectional investigation that was undertaken. Haematology and biochemistry procedures for TDT patients in Hyderabad, Sindh province, Pakistan, were completed in accordance with the current study's defined methodology. The study was carried out at the Fatimid Foundation Centre (F.F.C.) in Hyderabad, Sindh Pakistan, on transfusion-dependent thalassemia TDT patients undergoing routine blood transfusion treatment.

Patient Selection

In this prospective study, 142 patients and control participants were interviewed. Of the 71 thalassemia patients, consent was obtained for blood samples to be used periodically for haematology and biochemical analysis. TDT patients ranged in age from 5.98 to 15.34 years on

average. TDT patients with a minimum age of less than 4 years and a maximum age of 26 years have been selected for this study.

Data collection

Patients were given information and awareness about the objective of this disease and its benefits and a prior permission, consent and willingness was obtained from patient for use of their blood samples for this study. An informed consent was filled and signed in from all participants. Data was collected on pre- prepared questionnaire. The questionnaire was divided into three sections: nutritional analysis, clinical investigation that served as a guide, demographic characteristics, and patient interviews to gather background information on personal histories.

Sample collection

Samples were gathered at the Fatimid Foundation Center in Hyderabad for the current investigation. The study involved obtaining two types of samples: blood samples with anticoagulant EDTA for hematology and serum for biochemical analysis from both thalassemia patients and control subjects. A 5 ml blood sample was extracted from the veins. The samples were placed in a blood CBC bottle containing 0.2% EDTA to prevent blood clotting. These samples were intended for cell count, hemoglobin concentration, and participant analysis. All participants who provided consent had 5 ml of their blood collected in a sterilized tube, which was allowed to clot. The samples were then transported to the Institute of Biochemistry, University of Sindh, Jamshoro. Afterward, the blood samples were centrifuged for 20 minutes at 2000 rpm, and the resulting supernatant was separated for biochemical analysis. The serum was stored at -40 degrees Celsius in a biomedical freezer until it was analyzed.

Laboratory methodology

The Sysmex KX-21 Auto analyzer is utilized to determine hematological parameters.

Statistical analysis

The data was reported and statistical analysis was conducted using SPSS (V.22 Inc., Chicago, IL, USA) and MS EXCEL 2013. Single-factor ANOVA was utilized to measure comparisons between groups and assess differences among age groups. The results were expressed as mean ± standard deviation. Student's t-test was performed to compare the hematology. A p-value of less than 0.05 was considered statistically significant for all parameters.

RESULTS

The aim of this study was to evaluate 71 chronic blood transfusion-dependent thalassemia patients (TDT) who were receiving regular blood transfusion therapy. The patients' ages ranged from 5.98 to 15.34 years, and their socio-demographic characteristics, personal information, and clinical examination for signs and symptoms were analyzed. Furthermore, 71 healthy individuals with no medical history were included in the study. The results are presented in the following subsection.

Table 1. Comparison of socio-demographic characteristics of Transfusion-Dependent Thalassemia Patients and Control Subjects

Socio-demographic characteristics	TDT Patients *n*=71	Control subjects *n*=71
Mean age range	5.98-15.34	6.38-17.5
Gender		
Male	(*n*=46 or 64.78 %)	(*n*= 29 or 40.47 %)
Female	(*n*=25 or 35.21 %)	(*n*=42 or 59.52 %)
Age Group		
Up to 4 years	(*n*=4 or 5.63 %)	(*n*= 8 or 10.71 %)
5-10 years	(*n*= 41 or 57.74 %)	(*n*=30 or 42.57 %)
11-15 years	(*n*= 11 or 15.49 %)	(*n*=15 or 21.42 %)

16-20 years	(*n*= 9 or 12.67 %)	(*n*= 8 or 10.71 %)
21-26 years	(*n*=6 or 8.45 %)	(*n*=10 or 14.28 %)
Origin		
Migrants	(*n*= 62 or 87.32 %)	(*n*= 64 or 90.47 %)
Non-migrants	(*n*=9 or 12.67 %)	(*n*= 7 or 9.52 %)
Education		
Graduates	(*n*=1 or 1.40 %)	(*n*= 25 or 35.71 %)
Intermediate	(*n*=2 or 2.81 %)	(*n*= 5 or 7.14 %)
Matriculation	(*n*=4 or 5.63 %)	(*n*= 3 or 3.57 %)
Middle	(*n*=6 or 8.45 %)	(*n*= 5 or 7.14 %)
Primary	(*n*=25 or 35.21 %)	(*n*= 16 or 22.61 %)
Illiterate	(*n*=33 or 46.47 %)	(*n*= 17 or 23.80 %)
Life Style		
Sedentary	(*n*=42 or 59.15 %)	(*n*= 14 or 19.04 %)
Physically Active	(*n*=29 or 40.84 %)	(*n*= 57 or 80.95 %)
Family profession		
Labor	(*n*=28 or 39.42 %)	(*n*= 30 or 41.66 %)
Govt. Job	(*n*=17 or 23.94 %)	(*n*= 12 or 16.66 %)
Land lord	(*n*=2 0r 2.81 %)	(*n*= 4 or 5.95 %)
Shopkeeper	(*n*=24 or 33.80 %)	(*n*= 25 or 35.71 %)
Family history of thalassemia patients		
Yes	(*n*= 47 or 66.19 %)	(*n*= 3 or 4.76%)
No	(*n*= 24 or 33.8 %)	(*n*= 68 or 95.23 %)

TDT= Transfusion dependent thalassemia

The findings of the current study suggest that socio-demographic factors, such as occupation, literacy rate, and socio-economic condition, play a significant role in the development of thalassemia. Our results indicate that out of the 71 TDT patients examined, the majority were males, accounting for 64.7% of the cases, while females accounted for 35.2%. Furthermore, a significant proportion of these patients fell within the age group of 5-10 years, with a frequency of 57.74%. This age group was found to have the highest survival rate among TDT patients during the period of 2018-2019. Conversely, there were very few TDT patients who were older than 10 years of age. The recent study conducted has provided new insights into the demographics of Transfusion Dependent Thalassemia (TDT) patients. It was observed that a significant proportion of TDT patients were migrants, accounting for 87.32% of the total cases. Furthermore, a considerable number of these patients were found to be illiterate, making up 46.47% of the population.

Additionally, a sedentary lifestyle was prevalent among 59.15% of the TDT patients. The study also shed light on the occupational distribution of TDT patients. Among the TDT patients, the majority belonged to the labor community, comprising 39.42% of the cases. Following closely were individuals working as shopkeepers, accounting for 33.8% of the patients. It was also noted that a significant proportion of TDT patients had a family history of thalassemia, with 66.19% of the cases having a familial connection. Analyzing the age groups affected by thalassemia, the study found that the 5-10 years age group was the most commonly affected. This highlights the vulnerability of children within this age range to thalassemia.

Furthermore, a comparison of blood groups between TDT patients (71) and control subjects (71) in Hyderabad was conducted. The results, as shown in Table 4, revealed that the highest number of TDT patients (23) had blood group B+, followed by 16 patients with blood group O+. Overall, this study provides valuable insights into the demographics and characteristics of TDT patients, emphasizing the need for targeted interventions and awareness campaigns to address this health issue effectively.

Table 2: A Comparative Analysis of Blood Parameters in Transfusion-Dependent Thalassemia Patients and Control Individuals/Subjects.

Groups	**Ferritin** (20-200) g/ml Mean ± SDV	**RBCs** (4.3-5.9) 10^6/ LMean ± SDV	**HB** M-11-14 g/dL Mean ± SDV	**MCV** (76-96) FL Mean ± SDV	**MCH** (27-32) PG Mean ± SDV	**p-Vaule** (<0.005)
Iron Over Load TDTPatients(N=71)	5092.9± 3402.4	2.81 ± 0.78	7.16 ± 2.08	77.75 ± 4.96	25.07 ±2.35	**(<0.005)**
Controls (N=71)	32.12 ± 26.97	4.37 ± 0.62	11.56 ± 1.3	81.42±5.77	26.40 ±2.26	
Gender wise						
Male TDT patients (N=46)	5796 ± 3193.8*	2.76 ± 0.69*	7.01 ± 1.83*	77.36 ± 3.71*	25.05 ± 2.04*	**(<0.005)**
Male TDT Controls (N=46)	33.06 ± 13.59	4.37 ± 0.51	11.57 ± 1.01	81.62 ± 4.66	26.45 ± 1.65	
Female TDT patients (N=25)	4877.5 ± 1830*	2.94 ± 0.54*	7.45 ± 1.41*	78.24 ± 3.87	25.6 ± 1.53	**(<0.005)**
Female TDT Controls (N=25)	31.39 ± 22.70	4.32 ± 0.57	11.29 ± 0.66	80.92 ± 5.16	26.22 ± 1.73	
Age wise groups TDT Patients						
< 4 years (N=04)	2418.12 ± 1376.36*	2.54 ± 0.39*	6.22± 0.55*	73.8 ±3.84	21.6± 2.09	0.12
5-10 years (N=41)	4033.3± 2037.3*	2.93 ±. 080*	7.54 ± 2.15*	77.96± 5.81	25.61±2.59	0.35
11-15 years (N=11)	7042.18 ± 2953.76*	2.55± 0.63*	2.55 ± 0.64*	77.63±3.2	24.78±1.71*	0.29
16- 20 years (N=9)	5213.38±718.40*	2.57±0.49*	6.52±1.41*	77.66±2.75	25.3±1.6	0.49
>20 years (N=6)	9305.28±5889.31*	3.11±0.65	7.68±1.85*	78.68±2.35	25.46±0.78*	0.1

p<0.05 (p-value was calculated by t-test) TDT. RBC= Red blood Cells; HB=hemoglobin; MCV= Mean corpuscular value; MCH= Mean corpuscular hemoglobin.

The study conducted a comparison of blood parameters between transfusion-dependent thalassemia patients and control subjects. The findings revealed a significant decrease (p<0.05) in RBC, HB, MCV, and MCH in TDT patients as compared to the control group. Additionally, the study showed a significant increase (p<0.05) in serum ferritin levels in TDT patients, indicating severe iron load in these patients. Furthermore, the study found that male TDT patients had higher serum ferritin levels than females, reflecting the chronic condition of TDT patients. The gender-wise comparison of blood parameters showed that TDT male patients had significantly increased (p<0.05) serum ferritin levels, while RBCs, HB, MCV, and MCH were observed to be decreased when compared to control subjects. TDT female patients exhibited an elevated serum ferritin level and a decrease in RBCs and HB. In comparison to female control subjects, female patients showed non-significantly altered variations (p>0.05) in MCV and MCH. Our findings revealed that the majority of TDT patients had significantly decreased (p<0.05) mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) compared to control subjects. The significantly decreased hemoglobin level in TDT patients had a severe impact on spleen function, leading to splenomegaly in these individuals. Additionally, the premature destruction of red blood cells (RBCs) contributed to the occurrence of hemolytic anemia in the patients, further deteriorating their condition. Furthermore, our study demonstrated a notable reduction (p<0.05) in RBCs and hemoglobin levels in the blood of male TDT patients when compared to female TDT patients. This signifies a severe anemic condition in male TDT patients, while male controls do not encounter such a problem. Hemolysis was observed in nearly all thalassemia patients who were dependent on blood transfusions in our recent study. The blood parameters indicated a significant reduction in MCV, MCH, and HB levels in all TDT patients, regardless of gender. Furthermore, serum ferritin levels were significantly higher (p<0.05) in all age groups of TDT patients compared to control subjects, while remaining normal in the latter. Additionally, RBCs and HB levels were significantly lower (p<0.05) in all age groups of TDT patients compared to control subjects. There were notable differences in hematology parameters when comparing genders. However, it was observed that serum ferritin levels were significantly higher (p<0.05) in both males and females across all age groups compared to the control subjects. Upon further examination of Table 2, it was found that there was a significant decrease in mean corpuscular volume (MCV) in the age group older than 20 years. Additionally, when comparing the age groups of 16-20 years, it was observed that there was a significant decrease in mean corpuscular hemoglobin (MCH) in patients with transfusion-dependent thalassemia (TDT) compared to the control subjects of the same age groups. The aforementioned findings reveal disrupted hematology in patients with transfusion-dependent thalassemia (TDT), suggesting the presence of chronic infection, inflammation, tissue injury, and

chronic iron overload. Although there was no significant variation in serum ferritin levels when comparing different age groups of TDT patients, elevated levels were observed in certain age groups when compared to control subjects. The medical history of TDT patients at the Fatimid Foundation's Hyderabad center has revealed that iron overload and its effects have contributed to the development of diabetes mellitus, hypothyroidism, and hyperparathyroidism in these patients. Our findings suggest that the disturbed hematology of TDT patients in Hyderabad may lead to chronic infection, inflammation, tissue injury, and chronic iron overload.

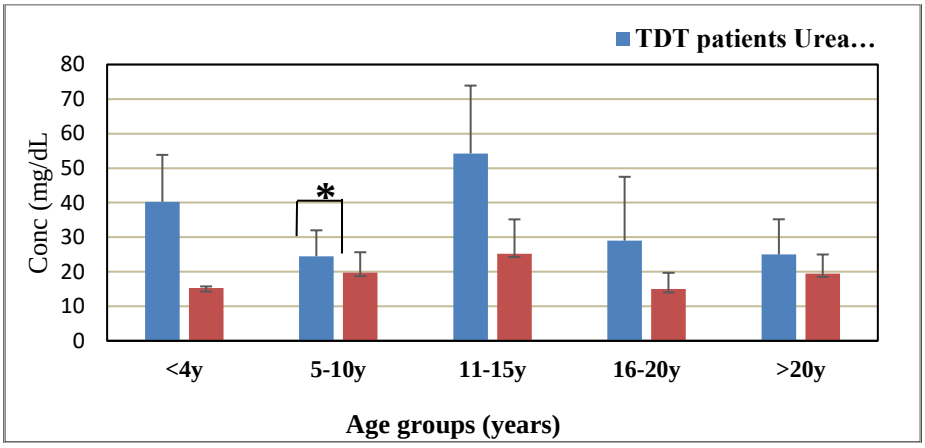

Figure 1. Age Wise Comparison of Urea between Transfusion Dependent Thalassemia Patients and Controls

In this figure Age Wise Comparison of Urea between Transfusion Dependent Thalassemia Patients and Controls. Present finding indicated that the higher level of serum urea at the age of 4-10 years and non-significant variation ($p>0.05$) was found among all other age groups of TDT patients.

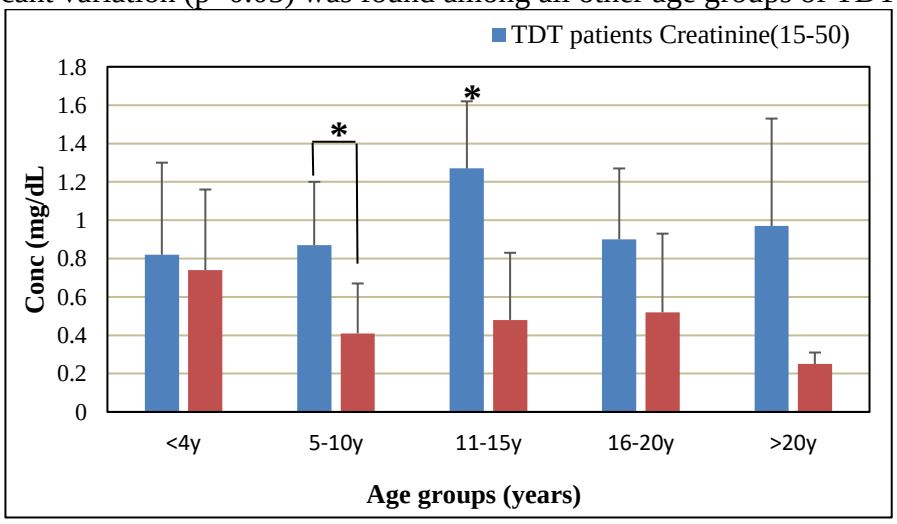

Figure 2. Age Wise Comparison of Serum Creatinine between Transfusion Dependent Thalassemia Patients and Controls Subjects.

In this figure Age Wise Comparison of Serum Creatinine between Transfusion Dependent Thalassemia Patients and Controls Subjects result is described. It is a significant serum creatinine level was noted high among 5-10 years and 11-15 years in comparison to all other age groups.

DISCUSSION:

Transfusion Dependent Thalassemia patients experience various abnormalities and complications related to blood transfusions, including iron overload and its effects on the spleen, liver, and kidneys. Additionally, dyslipidemia and viral infections in the liver are commonly found in TDT patients. In this particular study, a total of 140 individuals were chosen for observation. This group

consisted of 71 Transfusion Dependent Thalassemia patients and 71 control subjects who were matched in terms of age, gender, and had no personal or family history of any blood disorders. The aim of the study was to examine the complications arising from blood transfusions in Thalassemia patients and compare them with those of the control group. All participants were interviewed using a self-structured questionnaire to gather information on sociodemographic characteristics, lifestyle, and dietary habits. The results of present research showed thatEducation, lifestyle, and career (sociodemographic features) played vital role in the severity of complication of TDT patients. Previous researchers have also find sociodemographic features responsible for complications in thalassemia patients[13,14]. This study showed that majority of TDT patients were male and some comparable results are also reported elsewhere[15]. While few studies have reported the larger part of TDT patients as females[16, 17, 18]. Our results are comparable with the reported ones. As Thalassemia is a gene mutation blood disorder therefore in present study the consanguineous relationships were found one of the primary sources of thalassemia transmission. It was also discovered that thalassemia was most common in the age groups ranged 5-10 years, we found life-threatening complications after 10 years age in the TDT patients of Hyderabad hence cannot survive at a later age, whereas other study showed most common affected age group of >15 years old. The study showed that the TDT patients were illiterate but non migrant[19]. Present study also showed the illiterate patients in majority but migrant. Thalassemias are a group of hematological disorders affecting synthesis of α or β globulin chains in haemoglobin. The majority of TDT patients still depend on continues blood transfusion and chelation therapy[20]. Regular chelation therapy and blood transfusionimproved the span and quality of their lives, but had some side effects too. Results showed that RBCs decreased severely in TDT patients who caused anaemia to these patients and the same results were reported by some scientists[21]. Furthermore, significantly decreased (<0.05) HB concentrations were reported to be lower in TDT patients by other researchers[22]. Which we confirmed in our investigation too. All TDT patients had lower MCV, MCH, and HB. Same findings were reported by another investigator that MCH became low in TDT patients[23]. All studies and our research indicate that frequent blood transfusion changed the hematology of TDT patientsand developed a condition called hypochromic microcytic. The iron overload in thalassemia patients can be attributed to multiple life-long transfusions and enhanced iron absorption resulting in increased the serum ferritin level[24]. This is in agreement with our study. Presently we found high serum ferritin level indicated severe iron over load in TDT patients. Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and hemoglobin were significantly decreased ($p < 0.05$) in TDT patients as compared to controls almost same investigations were found by other researchers[25]. The current research further validates the occurrence of chronic renal failure in TDT patients. It is crucial for clinicians to closely monitor these patients to alleviate their discomfort and help them regain a semblance of normalcy in their lives. Future studies can explore effective treatment options that can potentially extend their lifespan and alleviate their suffering.

Conclusion:

The findings of this study indicate that individuals with transfusion-related blood abnormalities, such as severe hypochromic microcytic anemia and reduced red blood cells, are commonly observed in patients with transfusion-dependent thalassemia (TDT). While a positive family history of thalassemia is a major contributing factor to the disease, factors such as age, socioeconomic conditions, and education also play a significant role in its occurrence. It is worth noting that poor socioeconomic conditions and lack of knowledge among thalassemia patients can exacerbate the severity of the disease. The continuous blood transfusion treatment given to TDT patients has an impact on their hematological parameters. The present study found that RBCs, MCV, MCH, HB, and HCT were significantly lower in TDT patients compared to control subjects, indicating a complete disruption in RBC indices. Although chelating therapy is administered to TDT patients to address the iron overload, further research is necessary to address specific challenges and develop alternative treatment options.

Conflict of Interest

The authors declare that there is no conflict of interest.

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