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CD72 gene expression in children suffering from primary immune thrombocytopenia and its correlation with the disease activity

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Abstract

Background Immune thrombocytopenia is a condition in which the immune system attacks platelets, leading to a low platelet count. CD72 is a co-receptor found on B cells during all developmental stages except those of plasma cells. Activation of CD72 promotes B cell growth and differentiation. We aimed to investigate the expression and role of CD72 in pediatric immune thrombocytopenia, as well as its correlation with disease pathogenesis and activity. The study involved 60 children diagnosed with ITP and 40 healthy controls, who were matched accordingly. All participants underwent a thorough medical history assessment and clinical examination. The RT-PCR method was utilized to determine the level of CD72 expression.

Results CD72 expression level was considerably higher in cases than in controls (*P*-value < 0.001). Within the cases group, we detected a significant inverse correlation between CD72 expression and platelet count (*P*-value < 0.03). Also, there was a notable rise in CD72 expression among those experiencing active ITP compared to those in remission. Moreover, autoantibody-positive patients exhibited greater levels of CD72 expression than their autoantibody-negative counterparts did. While there was no discernible association detected between CD72 expression and the duration of the disease.

Conclusion CD72 expression is linked to the pathogenesis of ITP. Also, expression elevation is associated with disease activity. In addition, it is not related to disease chronicity. CD72 can be considered a new approach to the diagnosis, treatment, and follow-up of pediatric ITP.

Keywords ITP, B lymphocytes, CD72, RT-PCR

1 What is known

- Immune thrombocytopenic purpura is an autoimmune disorder distinguished by a decrease in platelet count.
- Data about the function of the CD72 gene in ITP is scanty.

2 What is new

- We discovered a noteworthy correlation between CD72 gene expression and both ITP pathogenesis and activity, but no link with disease chronicity.
- This study may help a better understanding of the ITP pathophysiology that can help in the advancement of new therapeutic strategies.

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3 Background

Immune thrombocytopenia is a condition where platelets are covered with autoantibodies that target platelet membrane antigens. This leads to their sequestration in the spleen and phagocytosis by mononuclear macrophages. The outcome is a shortened lifespan of platelets in the bloodstream, along with insufficient compensation by bone marrow megakaryocytes that produce more platelets, reducing the number of circulating platelets [1]. The diagnosis is usually established by ruling out the recognized origins of thrombocytopenia. Platelets in circulation are sensitized by IgG autoantibodies. This results in hastened elimination by antigen-presenting cells (such as macrophages) in the spleen, and occasionally in the liver. The bone marrow counterbalances the platelets' destruction by boosting their production. [2] ITP typically manifests in pediatrics and adolescents within a few weeks after a viral ailment. Some medicines can trigger immune thrombocytopenia, which is identical to ITP [3]. Most kids achieve an automatic recovery within a few weeks or months, and a splenectomy is seldom necessary. It's uncommon for young adults to experience spontaneous recoveries that make splenectomy necessary within years of diagnosis [3]. B lymphocytes exhibit various surface molecules apart from B cell receptors (BCR) that act as indicators of differentiation and also regulate BCR signaling. Recent studies have revealed the functionality of most antigens that were formerly categorized as lymphocyte differentiation markers. CD72 is an example of such a molecule that was initially identified as a B cell differentiation marker through traditional serological and genetic methods. With the advent of monoclonal antibodies, it was further characterized as a molecule that can, independently or in conjunction with BCR signaling, influence B cell growth and differentiation [4]. CD72 is a trans-membrane protein found on B cells that possesses a lectin-like domain of the C type in its extracellular region and an immune-receptor tyrosine-based inhibition motif in its cytoplasmic region [5]. CD72 is typically regarded as a suppressor of BCR signaling. It is proven to have a two-fold impact on B lymphocyte development and function. In mature B cells, CD72 has a positive impact by briefly recruiting CD19 [6]. In immature B cells, CD72 has a negative impact on BCR signaling through its concurrent phosphorylation and enlistment of SHP-1 [7]. Appropriate B cell homeostasis necessitates the connection between CD72 and its ligand, CD100 and propagated B cell initial activation and differentiation into plasma cells [8]. This communication additionally stimulates interferon and tumor necrosis factor production [9]. As a result, CD72 has a crucial role in the survival and function of B cells in vivo. Earlier investigations show that there is an association between CD72 polymorphism and the onset of ITP in children [10]. In active ITP, the upregulation of CD72 in CD27+ memory B cells is linked to platelet levels and anti-platelet antibody concentrations [10].

In our study, we aimed to evaluate the expression and role of CD72 in pediatric immune thrombocytopenia, as well as its relation to disease pathogenesis and activity.

4 Methods

This observational case–control study was carried out from May 2022 to June 2023 and had the participation of 60 pediatric patients with ITP from the Department of Hematology. All pediatric age groups were involved, from 1 to 13 years old, with 26 male and 34 female individuals. The identification of primary ITP was based on American Society of Hematology 2019 guidelines for ITP [11], which defined it as a condition characterized by isolated thrombocytopenia, a platelet count $< 100 \times 10^9$/L, and absence of other conditions linked to thrombocytopenia. [9]. Individuals with diabetes, severe or long-term diseases, high blood pressure, autoimmune disorders, cancerous growths, and heart ailments were excluded. Active ITP was identified as primarily diagnosed patients with a platelet count of $< 50 \times 10^9$/L (36 patients). 24 ITP patients who attained remission (platelet count $> 50 \times 10^9$/L) after treatment were categorized as ITP in remission. Based on their response to steroid therapy, patients were divided into 3 groups: (1) Complete response (6.7%): platelet levels that are equal to or greater than 100×10^9/L, with no signs of bleeding. (2) Partial response (45.0%): platelet levels that are equal to or over 30×10^9/L with at least a two-fold increase from the baseline count and no bleeding signs. (3) No response (48.3%): platelet levels that are less than 30×10^9/L, less than a two-fold increase from the baseline count, or bleeding symptoms. A control group of 40 healthy children from general outpatient clinics, matched for age and gender, involving 22 males and 18 females ranging from 4 to 12 years old, with a normal platelet count and negative antiplatelet antibodies, was included. The average platelet levels among fit individuals were $(275.9 \pm 70.0) \times 10^9$/L. Approval for this research was granted by the Medical Ethics Committee. Every participant in the study provided informed consent. Every patient underwent a comprehensive medical interview, which included obtaining information on their age, gender, consanguinity, history of fever, infection, or medication use prior to the condition, platelet and blood transfusions, and details of bleeding symptoms such as location, severity, and frequency. Additionally, treatment details such as type, duration, and clinical response were recorded. Furthermore, a thorough clinical examination and laboratory investigations were conducted, involving a complete blood count, direct platelet count, bone

marrow aspiration or biopsy in selected cases, antiplatelet antibody testing, and CD72 gene expression analysis via RT-PCR.

4.1 Anti-platelet antibody evaluation

EDTA-treated whole blood samples were gathered and stored at 4 °C before being evaluated within a week of collection. The GTI Pak Auto kit was utilized for the creation of eluates as per the manufacturer's guidelines (GTI Diagnostics, Brookfield, WI, USA). Microwell strips having 3 diverse immobilized glycoprotein complexes, GPIIb/IIIa, GPIb/IX, and GPIa/IIa, were added with acid eluates generated by elution at pH 3.0 from thrombocytes. The standard ELISA was employed to identify antibodies attached to the immobilized GPs.

4.2 CD72 gene expression

CD72 gene expression was applied to peripheral blood mononuclear cells (PBMCs). Three mL of venous blood samples were collected using ethylenediaminetetraacetic acid (an anticoagulant), and centrifugation at 800 rpm for 15 min was done. PBMCs were obtained by density gradient centrifugation at 2000 rpm for 20 min using.

Ficoll-Hypaque. RNA isolation and RT-PCR analysis were followed. The whole RNA was extracted by the Trizol reagent of the Qiagen mRNA Easy kit (QIAGEN GmbH, Germany) and transcribed into cDNA by a reverse transcription kit. For amplification, an initial denaturation was applied for 2 min at 50 °C and then for 10 min at 95 °C, followed by 40 cycles of (95° C for 15 s, 60 °C for 60 s). The amplification primers used were CD72 forward 5¢ -CAG CTCCGCCTCAAG ATAAC-3; reverse 50 -TTGCAAGGTCTCC TTCGTC T-3; GAPDH, an endogenous control was used to normalize gene expression of CD72. GAPDH amplification primers were forward (5¢ TGAAGGTCGGAGTCA ACGGATT- 3¢, reverse (5¢-CCTGGAAGATGGTGA TGGGATT- 3¢). For quantitative PCR, the comparative threshold cycle method was utilized for mRNA quantification according to a software tool on Step One™ Detection System (Applied Biosystems, Thermo Fisher, USA), was used, with ™SYBR ™ Green (Applied Biosystems, Thermo Fisher, Inc, Lithuania) as a binding dye for double-stranded DNA. Gene expression was normalized to GAPDH mRNA levels by the 2DDCt method.

4.3 Statistical analysis

- The data was coded and entered into SPSS version 25 (Statistical Package for Social Science) for analysis.
- For categorical variables, descriptive statistics were performed using frequency and percentage, while numerical variables were presented as mean ± standard deviation (SD).
- Appropriate statistical tests were used, including an independent sample *t*-test for two groups
- Chi-Square ($\chi 2$) test for categorical data
- Pearson correlation (Spearman for non-parametric) analysis was utilized to detect the correlation between scale variables.
- *R*-values ranged from 0 to 0.3 (either positive or negative, indicating a weak correlation), 0.3 to 0.6 (moderate correlation), and more than 0.6 to 1 (strong correlation).
- *P*-values equal to or less than 0.05 were considered statistically significant.
- Graphs were utilized to visually represent certain information.
- An ROC curve was used to detect the optimal cut-off of the CD72 marker in predicting Purpura.

5 Results

The analysis involved a total of 60 individuals diagnosed with ITP. The average age was 5.64 ± 2.33 years. Of the participants, 43.3% were male and 56.7% were female. Additionally, 3.3% had confirmed consanguinity, and 63.3% had a history of fever. Platelet transfusions were administered to 5% of the patients who experienced life-threatening bleeding, and out of these, one individual had a decrease in hemoglobin levels, resulting in acute anemia and an impending risk of heart failure, necessitating a blood transfusion.

In relation to the bleeding symptoms, the prevalent one was ecchymosis (83.3%), pursued by purpura (80.0%) and then epistaxis (20.0%), whereas none of the individuals experienced bleeding from the gums, blood in urine, or bleeding in the brain. The clinical information of ITP patients is presented in Table 1.

Furthermore, a vast majority of our patients (81.7%) were prescribed oral corticosteroids, while 10% were taking Imuran, 44.8% were on revolade (eltrombopag), and 50% were administered IV steroids with no use of intravenous immunoglobulin. Notably, only 11 patients (18.3%) experienced spontaneous remission. The average duration of steroid treatment was 7 ± 5 months. Upon assessing the response to steroid therapy, we observed that 6.7% achieved complete remission, 45.0% reported partial remission, and 48.3% showed no response. In terms of disease activity, 60% of the participants (36 patients) had active ITP, while 40% (24 patients) were in remission.

The comparison between the laboratory results of the cases and controls indicated a significant dissimilarity in terms of platelet count (105.6 ± 100.5, $275.9 \pm 70.0 \times 10^9$ respectively) (*P*-value < 0.001). However, there was no variation in relation to RBCs or

Table 1 Clinical data of patients with ITP

Items	Cases (no = 60)
Positive consanguinity	2 (3.3%)
Positive family history	0 (0%)
History of drug intake	0 (0%)
Preceding infection	38 (63.3%)
Vaccination prior to presentation	0 (0%)
Platelets transfusion	3 (5%)
Blood transfusion	1 (1.6%)
Ecchymosis	50 (83.3%)
Purpura	48 (80.0%)
Epistaxis	12 (20.0%)
Hematuria	0 (0%)
Bleeding gums	0 (0%)
CNS bleeding	0 (0%)
Splenomegaly	0(0%)
Hepatomegaly	1 (1.6%)
Lymphadenopathy	3 (5%)

WBCs. The presence of anti-platelet antibodies was detected in 18 patients, out of which 12 (60%) had GPIIb/IIIa autoantibodies, 4 (22.2%) had autoantibodies against both GPIIb/IIIa and GPIb/IX, and 2 (11.1%) had autoantibodies against both GPIIb/IIIa and GPIa/IIa.

Concerning the expression of the CD72 gene in the study, it was significantly elevated in cases as opposed to controls (2.52 ± 1.19, 1.14 ± 0.42, respectively), with a *P*-value < 0.001. (See Fig. 1). Among the patients group, a significant negative correlation was observed between CD72 expression level and platelet count (*P*-value < 0.03). However, there was no significant correlation with either RBCs or WBCs. Additionally, no links were found between CD72 expression and any of the following variables: disease duration (*P*-value < 0.53), age (*P*-value < 0.98), or gender (*P*-value < 0.6) of the patients. Furthermore, no correlation was observed with the response to steroid therapy (*P*-value < 0.26). The correlations between CD72 gene expression and other study variables in ITP patients are presented in Table 2.

Furthermore, the level of expression demonstrated a notable increase in active cases of ITP compared to cases in remission (3.06 ± 1.16 and 1.51 ± 0.31, respectively), with a *P*-value below 0.009. Additionally, a valuable difference in CD72 expression was observed among patients who tested positive for autoantibodies and those who tested negative (*P*-value below 0.023). Our findings also indicated that CD72 expression can discriminate between cases and controls at a cut-off greater than 1.46, with a sensitivity of 83.3% and a specificity of 85% (Fig. 2). Moreover, the CD72 marker played a significant role in predicting disease activity (active ITP versus ITP

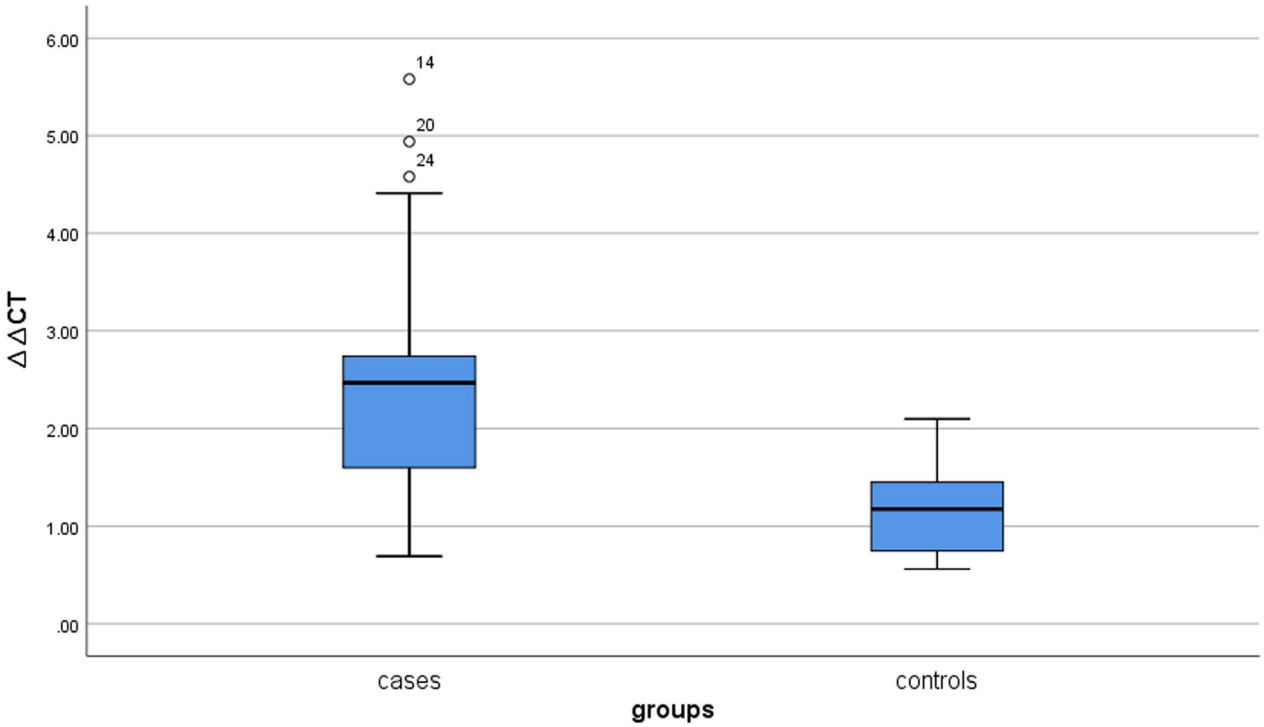

Fig. 1 CD72 gene expression in both groups (cases versus controls)

Table 2 Correlations between CD72 gene expression and other study variables of ITP patients

Variables	*R*	*P*
Age (years)	– .004	.982
Duration of disease	– .015	.938
RBCs (million/mm^3)	– .102	.591
WBCs (× 10^9/L)	.098	.606
Plts (× 10^9/L)	– .397	.030
Sex	.096	.600
Response to steroid therapy	–	0.266

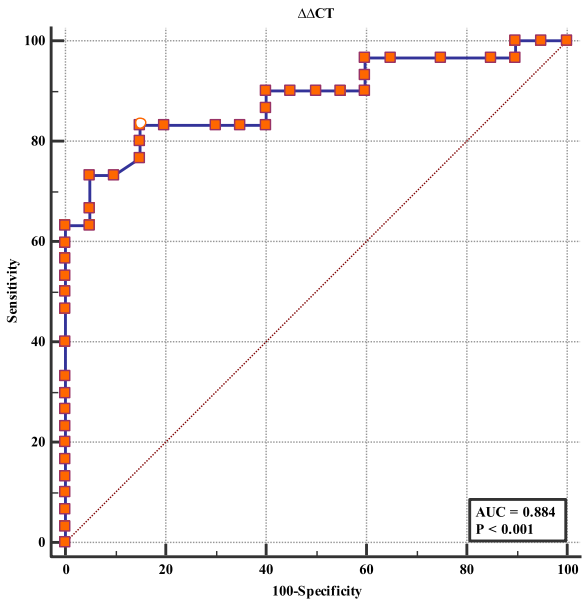

Fig. 2 Receiver operating characteristic curve for prediction of ITP from CD72 quantitative gene expression

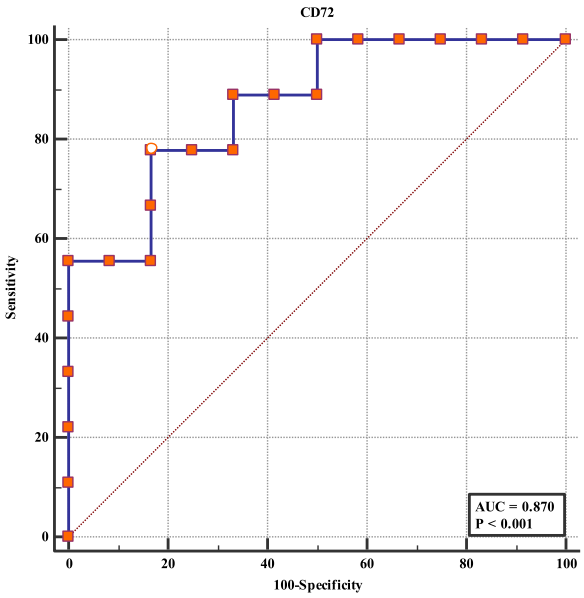

Fig. 3 Receiver operating characteristic curve for prediction of disease activity (active ITP) from CD72 quantitative gene expression

in remission) at a cut-off greater than 2, with a sensitivity of 83.33% and a specificity of 77.78% (Fig. 3).

6 Discussion

CD72 serves as a co-receptor of BCR and is identified as a significant regulator in the development of various autoimmune disorders [12]. Upon CD72 binding, several initial signaling processes are initiated, including the activation of non-src kinase Btk and Src kinases Lyn and Blk, resulting in the activation of mitogen-activated protein kinases, which are typically linked with affirmative signaling. CD72 signaling is capable of enabling B cells that are deficient in Btk to skip their unresponsiveness to BCR signaling. Conversely, signals mediated by BCR are amplified in CD72-deficient cells but dampened in CD100-deficient cells. The effects of CD72 on B cells are dual due to its interaction with both positive and negative signaling molecules. CD72 binds with SHP-1, a protein tyrosine phosphatase containing the SH2 domain that regulates signaling negatively, as well as with Grb2, an adapter protein linked to the Ras/MAPK pathway. Furthermore, CD72 association with CD19, a positive modulator of B cell receptor signaling, is triggered by ligation. Therefore, a dual signaling hypothesis illustrates how CD72 promotes the growth and differentiation of B cells [12].

Unusual expression and function of CD72 on B cells have been documented in certain autoimmune ailments, like sjogren's syndrome and systemic lupus erythematosus [8]. The concentration of soluble CD72 in the bloodstream of systemic lupus patients is higher in comparison to rheumatoid arthritis patients or individuals in good health [13].

In our investigation, by assessing the expression of the CD72 gene, we observed a marked inverse correlation between CD72 expression and the platelet count of cases. Furthermore, patients with ITP exhibited significantly elevated CD72 expression relative to controls. These results indicate that CD72 may have a role in the development of ITP and support its involvement in immunity. This is due to the CD72 positive impact on B cell response, which may arise from the sequestration of negative signals from BCR [14]. Similarly, Lyu et al. [10] reported that upregulation of CD72 expression on CD27$^+$ memory B cells might take part in ITP pathogenesis. However, Xu et al. [15] did not discover a substantial

increase in CD72 expression in patients compared to controls, suggesting that the rise in CD72 expression is more linked to the development phase of ITP than its pathogenic features.

Furthermore, we observed a significant increase in expression levels among active ITP patients compared to those in remission. These findings align with those of Xu et al. [15], whereby they noted heightened CD72 expression in the B lymphocytes of active ITP patients relative to those in remission. Additionally, Lyu M et al. [10] found that elevated CD72 expression on the CD27+B memory subset was linked to ITP disease activity. Conversely, Zhou et al. [16] reported lower mRNA expression of CD72 in active ITP cases than in remission and controls. These opposing effects on B cells are attributed to CD72 dual roles in the development and function of B lymphocytes, which depend on interactions with different BCR signaling modulators [17]. Several co-receptors, including CD72 and FccRIIb, have been observed to regulate B cells negatively, thereby maintaining self-tolerance [18]. However, some studies suggest that CD72 may act as a positive regulator, triggered by CD100 overexpression on activated CD4+ T cells [19].

Moreover, the expression of CD72 was notably higher in patients who tested positive for antiplatelet antibodies compared to those who tested negative. These findings indicate that the increased gene expression is linked to the activity of ITP disease and the production of autoantibodies, which agrees with the findings of Lyu et al. [10]. Additionally, we did not report any significant correlation between CD72 expression and the age or sex of ITP patients, which was also reported by Lyu et al. [10]. Despite the limited data available on the role of the CD72 gene in disease chronicity, we found no association between CD72 expression and the duration of the disease, indicating that disease chronicity is not associated with this gene. By measuring CD72 expression, we could differentiate cases from controls at a cut-off > 1.46 and active from in remission ITP with a cut-off > 2. Therefore, monitoring CD72 expression levels could provide valuable insights for diagnosing ITP and developing new strategies for its management.

7 Conclusions

Our research has shown that CD72 gene expression could play a role in ITP pathogenesis and is related to disease activity and positive antiplatelet autoantibodies. Therefore, by evaluating the expression of the CD72 gene, we can distinguish between active and in remission cases. Nevertheless, CD72 expression does not have a connection with the chronicity of the illness. Ultimately, CD72 may be a novel approach in the follow-up, prophylaxis, or treatment of ITP.

7.1 Limitations

The small sample size of the study can affect the results, so further studies are needed on a larger group of children to confirm the results.

Abbreviations

ITP	Immune thrombocytopenia
RBCs	Red blood cells
WBCs	White blood cells
RT- PCR	Real-time polymerase chain reaction
BCR	B cell receptor

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Not applicable.

Author contributions

YA and RA analyzed and interpreted the patient data. DS supervised data collection and analysis. ME helped in data analysis. ME and YA collected the data. All authors have read and approved the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Beni-Suef University, Faculty of medicine and the ethics code was FWA00015574 FMBSUREC/01102022. Also, written consent was obtained from the care givers of the participating children.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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