



Original Article

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# Iron deficiency anemia in pediatric children at Kalmunai North Base Hospital, Sri Lanka

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### Abstract

**Objective:** This study's aim was to estimate association between the iron deficiency anemia (IDA) and associated risk factors among children at Kalmunai north in Sri Lanka during COVID-19.

**Material and Methods:** During the pandemic, 101 children aged between land 14 years were involved in the cross sectional study. Hemoglobin levels, serum ferritin and C-reactive protein (CRP) were measured to determine anemia. Additionally, dietary intake, socioeconomic status, and demographic information were collected through interviews with the caregivers. Chi-square tests and Pearson correlation were used to investigate connections between IDA and other factors such as demographic characteristics and eating habits. In addition, a multivariate regression analysis was performed to identify independent predictors of IDA.

**Results:** 7.9% of children were found to be anemic at Kalmunai Base Hospital. Low dietary iron consumption was revealed as a significant risk factor for IDA. Meat, liver, fish, chicken, fresh milk, dark green leafy vegetables, and black tea were found to be independent predictors of IDA, explaining 57.7% of the variation in IDA frequency (R<sup>2</sup> value = 57.7%; P< 0.001). Consuming dark green leafy vegetables, poultry, liver, beef, and fish are linked to a lower incidence of IDA in children. On the other hand, a higher risk of IDA appears to be associated with increased consumption of fresh milk and black tea. In conclusion, this study focuses on Dietary factors, especially the consumption of particular foods, were found to be significant contributors of IDA, even if demographic parameters did not differ significantly from IDA. Strategies to increase iron intake and dietary diversity, particularly among young children, are critical in the fight against IDA.

**Keywords:** Pediatric children, Hemoglobin, Iron deficiency anemia and Dietary habits, Iron rich food

## Introduction

Iron deficiency anemia (IDA) is still a global public health concern that affects people of all ages. However, its frequency in pediatric groups is particularly concerning, given the potential for long-term developmental and health effects [1]. In recent years, healthcare providers and academics have focused more attention on understanding and resolving this condition, realizing the enormous impact it can have on children's growth, cognition, and overall well-being [1].

(IDA) is a condition in which the body's iron stores are depleted, resulting in insufficient production of hemoglobin, the protein responsible for transporting oxygen in the blood [2]. The degree of deficiency has little bearing on the non-specific symptoms of ID and IDA. Clinically, children may exhibit symptoms like exhaustion, shortness of breath, palpitations, vertigo, headaches, and restless legs [3]. It can lead to serious public health problems, including a rise in childhood illnesses and deaths, as well as impaired immune system, mental, and physical development, decreased physical activity, low endurance, and poor learning in both infants and adolescents, as well as a lifelong reduction in serotonin levels and dopamine receptors [3–5].

Despite significant advances in healthcare and child well-being in recent decades, the frequency of IDA among Sri Lankan pediatric patients remains a major concern. Although there aren't many studies on nutritional deficiency anemia in the nation, the Ampara district of Sri Lanka hasn't been the subject of any research on IDA in children. This study was aimed in order to determine the severity of IDA and the demographics and eating habits associated with the presence of IDA among children at Base Hospital, Kalmunai North.

This study is significant on several levels. For starters, it adds to the body of knowledge on iron deficiency anemia by shining light on its specific patterns and issues in the context of Sri Lankan pediatric healthcare. Second, it has the potential to inform healthcare policies and interventions aimed at lowering the burden of IDA among Sri Lankan children, hence increasing their overall health and well-being. Third, it is hoped that the findings of this study will help with the development of suitable strategies to enhance complementary feeding and consumption of iron-rich diets, hence reducing the risk of IDA in children.

### Materials and Methods

A cross-sectional study design was used in this study to investigate the risk factors for IDA in pediatric children at the pediatric clinic and ward at Base Hospital, Kalmunai North in the Ampara district, which is located at latitude 7°25'12.1"N and longitude 81°49'20.4"E. Kalmunai Hospital, which acted as a primary healthcare centre for paediatric children in the Kalmunai region of Sri Lanka. The paediatric section at the hospital was the major location for data gathering. Pediatric patients aged 1 to 14 years who were hospitalized to or received outpatient care at Kalmunai Hospital during the study period from January to April 2022 were included in the study. The sample was chosen at random, with attempts taken to guarantee representation from various age groups and genders.Using the sample size calculator, the sample size was determined.

There were two population statistical formulas used:

$$(ss = t2 \times p \times (1-p)/e2$$

i.e. t – Coefficient for confidence level, p – Prevalence rate, e –precision rate.

The second formula:

### ss final = ss theoretical\* 1/% response \*1/% eligibility \*1/% valid

A 6% prevalence of anemia [6], a 5% margin of error, a 95% confidence level, and a 5% expected non-response rate were used. The study's final required minimum sample size was 101. Potentially fewer children were hospitalized for treatment or diagnosis as a result of the COVID-19 pandemic's dynamic impact on the healthcare system. The sample size represents the subset of children who could access healthcare services during this challenging period.

Excluded from the data collection were children getting anemia treatment, those who had undergone blood transfusions prior to the four-month period, and parents who chose not to voice their concerns.A questionnaire that was administered by an interviewer was utilized to gather socio demographic, socioeconomic, and clinical data. Two knowledgeable nutrition professionals evaluated the surveys.

An experienced phlebotomist used aseptic techniques to collect 5 ml of blood in plain tubes (2 ml) and EDTA tubes (3 ml) from each study participant. Following collection, the samples were delivered within two hours to the Aqsha Medical Laboratory. Serum ferritin, Hb levels, and CRP (C-reactive protein) were measured. Hemoglobin was estimated using automated hematology analyzer (BC6800, Mindray, China). A blood sample of two milliliters was taken up into the simple tubes and centrifuged. Blood and serum were clearly separated from one another. Following that, serum was added to the fully automatic biochemical analyzer (BS480 China). Serum ferritin and CRP levels were then assessed. HB levels, serum ferritin, and CRP were used to determine an IDA status.

In order to evaluate anemia (Hb <11 g/dl) and iron deficiency (SF< 15  $\mu$ g/l), WHO established cut-off levels were utilized. When CRP is more than 5.0 mg/l, as determined by the assessment, an acute phase of infection has been detected [7]. Detected infected children were treated in the ward and after their recovery they were included in our study. Children were classified as having severe, moderate, mild and normal iron level according to WHO [8]. The classification of Hb is below in table1.

Table 1		Classification of IDA according to WHO (Hb level are in the table)					
Children	Non-anaemia	a Anaemia (g/dl)					
		Mild	Moderate	Severe			
6–59 months	11	10.0-10.9	7.0-9.9	<7.0			
5–11 years	11.5	11.0-11.4	8.0-10.9	<8.0			
12–14 years	12	11.0-11.9	8.0-10.9	<8.0			

All laboratory activities were subject to strict adherence to specific standard operating procedures and manufacturer's instructions. In keeping with this, monitoring of samples were used. The expiration dates on all chemicals and quality control samples were examined. An individual identification number was used to record laboratory results on typical report formats.

The Social Package for Social Science (SPSS) version 21 (IBM Corp., Chicago, Illinois, USA) was utilized for accurate data entry. Based on HB levels, serum ferritin, and CRP, IDA status was determined. To ascertain the relationship between IDA and dietary consumption of foods high in iron, Pearson correlation was performed. In order to assess the link between IDA and demographic characteristics, the Pearson Chi-square test was utilized. Multi regression analysis was then utilized to evaluate the relationship between dietary habits, demographic features and IDA. For statistical significance, a p-value of less than 0.05 was utilized as the threshold.

#### Results

In this study, we noticed that 60% of patients came to the clinic primarily for chronic condition management and follow-up care, whereas the remaining 40% were admitted to the ward for acute illnesses or acute on chronic illness (A sudden worsening of a pre- existing chronic condition). Some (No:14) children in the study had co-morbidities, such as asthma and epilepsy which complicated their clinical profile. There were 54% male and 47% female among the children. Eight children in all (7.9%) were found to have IDA.

Table	e 2	Proportion o <sup>.</sup> Base hospita	5	0	
Age group (Years)	No of childre	Mild en (10-10.9 g/d %	Moderate ll) (7-9.9 g/dl %	Severe ) (<7 g/ dl) %	Total Anaemic %
1 - 3	38	2	1	0	37.5
4 - 6	24	0	3	0	37.5
7 - 10	26	0	1	1	25
11-14	13	0	0	0	0
Total IDA %		25	62.5	12.5	100

Age, sex, family income, sector, and mothers' level of education were found that did not differ significantly with IDA from other children. However, it was shown that there was a significant relationship between IDA and child bearing interval (P < 0.001) and number of children (P< 0.005) as given in Table 3.

variableIDAMean Hb $\pm$ SD (g/dl)P value SD (g/dl)No%Yes%Age1-33892.7 %37.9%12.58 ± 1.10.6204-62488.9%312.5%12.38 ± 1.4710-1413100%00%12.79 ± 0.9GenderMale4990.7%59.3%12.35 ± 1.20.593Female4493.6%36.4%12.62 ± 1.45SectorUrban4896.0%24.0%12.76 ± 1.00.149Rural4588.2%611.8%12.18 ± 1.412.18 ± 1.4Educational level of mother612.27 ± .40.675grade 6-116390%710%12.28 ± 1.30.118*Children number14397.7%12.3%12.45 ± 1.031990.5%250%10.97 ± 1.80.018*22291.7%28.3%12.64 ± 2.555250%250%10.65 ± 2.40.000**4787.5%112.5%12.06 ± 2.555250%250%10.65 ± 2.40.000**4787.5%112.5%12.60 ± 1.324787.5%112.5%12.61 ± 1.3231990.5%250%10.65 ± 2.40.000**1 year4 </th <th>Table 3</th> <th colspan="5">Relationship between IDA with independent demographic variables</th>	Table 3	Relationship between IDA with independent demographic variables					
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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	higher studies	4	100%	0	0%	12.83±1.1	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Children number						
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1	43	97.7%	1	2.3%	12.78±.9	0.018*
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	2	22	91.7%	2	8.3%	12.45±1.0	
4  7  87.5%  1  12.5%  12.06±2.5    5  2  50%  2  50%  10.97±1.8    Child bearing interval  2  50%  10.65±2.4  0.000**    1 year  4  50%  4  50%  12.58±1.2    3 year  20  100%  0  0%  12.71±0.6    4 year  15  93.8%  1  6.2%  12.60±1.3    > 4 year  28  100%  0  0%  12.63±0.8    Monthly income	3	19	90.5%		9.5%	12.30±1.2	
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Child bearing    interval    1 year  4  50%  4  50%  10.65±2.4  0.000**    2 year  26  89.7%  3  10.3%  12.58±1.2    3 year  20  100%  0  0%  12.71±0.6    4 year  15  93.8%  1  6.2%  12.60±1.3    > 4 year  28  100%  0  0%  12.63±0.8    Monthly income							
interval    1 year  4  50%  4  50%  10.65±2.4  0.000**    2 year  26  89.7%  3  10.3%  12.58±1.2  3    3 year  20  100%  0  0%  12.71±0.6  4    4 year  15  93.8%  1  6.2%  12.60±1.3  5    > 4 year  28  100%  0  0%  12.63±0.8  0    Monthly income		2	5070	2	5070	10.97±1.0	
1 year  4  50%  4  50%  10.65±2.4  0.000**    2 year  26  89.7%  3  10.3%  12.58±1.2    3 year  20  100%  0  0%  12.71±0.6    4 year  15  93.8%  1  6.2%  12.60±1.3    > 4 year  28  100%  0  0%  12.63±0.8    Monthly income	0						
2 year  26  89.7%  3  10.3%  12.58±1.2    3 year  20  100%  0  0%  12.71±0.6    4 year  15  93.8%  1  6.2%  12.60±1.3    > 4 year  28  100%  0  0%  12.63±0.8    Monthly income		4	50%	4	50%	10.65+2.4	0.000**
3 year  20  100%  0  0%  12.71±0.6    4 year  15  93.8%  1  6.2%  12.60±1.3    > 4 year  28  100%  0  0%  12.63±0.8    Monthly income	5						0.000
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> 4 year    28    100%    0    0%    12.63±0.8      Monthly income    -							
Monthly income      <20 000	5						
<20 000    27    84.4%    5    15.6%    11.92±1.3    0.118      20 000-40 000    52    94.5%    3    5.5%    12.59±1.3		-		-			
20 000-40 000 52 94.5% 3 5.5% 12.59±1.3		27	84.4%	5	15.6%	11.92±1.3	0.118
>40 000 14 100% 0 0% 13.26±0.8	20 000-40 000				5.5%		
	>40 000	14	100%	0	0%	13.26±0.8	

\* Refers to Significance at 0.05

\*\* Refers to significance at 0.01

Hb- Hemo globin, SD- standard deviation

When food intake is taken into account, there is a significant relationship between Hb level and foods like orange guava and dates, green leafy vegetables, chicken, meat, liver, fish, prawns, and eggs, dairy products like chocolate and cheese, and beverages like black tea and fresh milk as given in Table 4.

According to the findings of the multiple regression analysis (R2 value = 57.7%; P< 0.001, Dark green leafy vegetables, meat, liver, fish and chicken were decreased the IDA . Fresh milk, and black tea were the positive factors of IDA as given in Table 5. None of the other characteristics under analysis were discovered to be independent predictors of IDA.

Estimated multiple regression equation from table 6.

 $\label{eq:Y} \begin{array}{l} Y = 17.394 \ \text{-}0.402 X1 \text{-} \ 0.422 X2 \ \text{-} \ 0.421 X3 \ \text{-} \ 0.427 X4 \ \text{-} \\ 0.343 X5 \text{+} \ 0.157 X6 \ \text{+} \ 0.287 X7. \end{array}$ 

Consumption of dark green leafy vegetables, poultry, liver, beef, and fish was connected with a negative coefficient, indicating that increasing consumption of these foods is linked to a lower risk of IDA. Black tea and fresh milk consumption have a positive coefficient, indicating that increasing use of black tea and fresh milk is connected with an increased risk of IDA. Overall, the findings imply that eating dark green leafy vegetables, poultry, liver, beef, and fish is related with a lower risk of IDA in children. Higher consumption of black tea and fresh milk, on the other hand, appears to be connected with an increased risk of IDA.

### Discussion

Age, gender, family income, sector, and mothers' level of education did not influence the link with IDA, according to the study's findings, which is in line with the findings of other earlier research [9, 10]. However, according to other studies, socioeconomic level significantly affects the prevalence rates of IDA [9, 10] there does not appear to be agreement regarding the importance of socioeconomic status as a risk factor for IDA [11]. Although children between the ages of 4 and 6 had a higher IDA turnout (11.1%). In this research, there was no evidence of a significant association between a child's gender and their hemoglobin level (p=0.593). However, male children had a slightly greater prevalence of IDA. The outcomes of earlier research are supported by this result [11, 12]. In comparison to urban areas, where IDA prevalence was just 4%, rural areas had an incidence rate of 11.8%. The prevalence of IDA was found to be lower in urban samples than in rural ones, according to Rashid's research [13], and this result is in line with his findings. Little education was held by the mother of anemic children. Children were found to frequently have anemia, and the majority of their parents had little to no college education [14], findings that were similar to those of the current study. In a study of children aged 6-59 months conducted in Bangladesh, those with higher education were a smaller amount likely to be anemic than those with lower education, elementary school, or secondary education. The significant IDA factor was the number of children per family (p = 0.018). Hb level and childbearing interval showed a significant correlation (p < 0.001). Although the number of children and Childbearing interval had a significant correlation with IDA in chi square analysis (P = 0.018, p < 0.001), the multiple regression analysis revealed no effect after correcting for other variables.

Meat, poultry, fresh milk, fish, dark green leafy vegetables, black tea, and liver products were all discovered to be strongly and independently related with IDA. Which agrees with earlier results published. The study found a significant association between green leafy vegetables and Hb levels. This is because it has been shown that a rich source of iron is found in green leafy vegetables [15]. Similar research shown that when children had a composite green leafy vegetable powder as opposed to just the typical stew and soup provided by the school food program, their mean hemoglobin concentration improved considerably [15]. Fish, liver, poultry, beef, shrimp, and eggs were strongly negatively correlated with IDA. This might be because foods containing animal meat, like cattle, pork, lamb, chicken, and fish, are a good source of dietary iron [16]. According to studies by [17, 18] irregular meat and vegetable diet was found to be a significant predictor of anemia in school-aged children. This fact backs up the study's findings. Fresh milk and black tea exhibited a substantial and favorable relationship with IDA. This might be because tea polyphenols create insoluble iron-tannin complexes in the gastrointestinal lumen [19]. How effectively iron is absorbed may be impacted by the fact that cow's milk has roughly four times as much calcium as human milk [20]. Time separating consuming cow milk and black tea with main meal is good for healthy life.

ble 4 Relationship between IDA with food consumption among the children

			-			
Fruits	Daily	3Time/week (%)	Weekly (%)	Monthly (%)	Rare/Never (%)	P value
Orange			(,,,)	(,,,)	(,,,)	Pearson correlation-0.214*
Normal children	0	9.7	34.4	55.9	0	Significant (2 tailed) 0.032
IDA children	0	0	0	100	0	
Banana						
Normal children	12.9	25.8	30.1	26.9	4.3	Pearson correlation0.098
IDA children	37.5	12.5	25	25	0	Significant (2 tailed) 0.329
Рарауа						
Normal children	0	1.1	22.6	63.5	12.9	Pearson correlation-0.170
IDA children	0	0	0	62.5	37.5	Significant (2 tailed)0.090
Guava	0	2.2	40.4	40.0	0.6	D
Normal children IDA children	0 0	2.2 0	48.4 0	40.9 50	8.6 50	Pearson correlation-0.272** Significant (2 tailed) 0.006
IDA cilitaren	0	0	0	30	30	
Dates						
Normal children	4.3	4.3	4.3	77.4	9.7	Pearson correlation-0.226*
IDA children	0	0	0	75	25	Significant (2 tailed) 0.023
	-	-	-			
Star gooseberry						
Normal children	1.1	23.7	34.4	19.4	21.5	Pearson correlation-0.098
IDA children	0	0	12.5	50	37.5	Significant (2 tailed) 0.328
Pomegranate						
Normal children	1.1	23.7	34.4	19.4	21.5	Pearson correlation-0.195
IDA children	0	0	12.5	50	37.5	Significant (2 tailed) 0.050
vegetables	Daily	3Time/week (%)	Weekly (%)	Monthly (%)	Rare/Never	
	(%)				(%)	
Green leafy						
vegetable Normal children	10.8	54.8	28	4.3	2.2	Pearson correlation -0.282**
IDA children	0	0 0	37.5	4.5 62.5	0	Significant (2 tailed) 0.004
Beans	0	0	37.3	02.5	0	
Normal children	2.2	16.1	23.7	46.2	11.8	Pearson correlation-0.046
IDA children	0	0	37.5	25	37.5	Significant (2 tailed) 0.647
Tomato						
Normal children	11.8	31.2	37.6	15.1	4.3	Pearson correlation-0.099
IDA children	12.5	0	37.5	50	0	Significant (2 tailed) 0.323
Potato						
Normal children	5.4	19.4	52.7	22.6	0	Pearson correlation 0.067
IDA children	12.5	12.5	62.5	12.5	0	Significant (2 tailed) 0.505
Beets						
Normal children	0	0	14	50.5	35.5	Pearson correlation 0.066 Significant (2 tailed) 0.511
IDA children	0	12.5	12.5	50	25	Significant (2 taneu) 0.511
Leeks Normal children	0	0	20.4	66.7	12.9	Pearson correlation -0.149
Normal children	0	0	20.4	00.7	12.9	Significant (2 tailed) 0.137
fish						
Normal children	30.1	60.2	7.5	1.1	1.1	Pearson correlation -0.379**
IDA children	0	0	87.5	12.5	0	Significant (2 tailed) 0.000
Chicken						
Normal children	1.1	10.8	60.2	26.9	1.0	Pearson correlation -0.463**
IDA children	0	0	25	62.5	12.5	Significant (2 tailed) 0.000
Liver						
Normal children	0	0	21.5	59.1	19.4	Pearson correlation-0.371** Significant (2 tailed) 0.000
IDA children	0	0	0	37.5	62.5	Significant (2 tailett) 0.000
Red meat	0	7 5	0.7	22	E0 1	Deerson correlation 205**
Normal children	0	7.5	9.7 0	23	59.1 100	Pearson correlation395** Significant (2 tailed) 0.000
IDA children shrimp	0	0	0	0	100	
Normal children	0	5.4	31.2	37.6	25.8	Pearson correlation-0.280**
IDA children	0	0	0	25	75	Significant (2 tailed) 0.005
Egg	-	•	J			
00						

Normal children IDA children Vershurt	33.3 0	48.4 12.5	14 62.5	2.2 12.5	2.2 12.5	Pearson correlation-0.202* Significant (2 tailed) 0.043
Yoghurt Normal children IDA children	4.3 0	22.6 37.5	37.6 62.5	31.2 0	4.3 0	Pearson correlation 0.157 Significant (2 tailed) 0.117
Chocolate	0	0110	0210	Ũ	Ũ	
Normal children	14	26.9	28	29	2.2	Pearson correlation 0.274**
IDA children	75	25	0	0	0	Significant (2 tailed) 0.006
Cheese						
Normal children	0	5.4	7.5	25.8	61.3	Pearson correlation 0.229*
IDA children Black tea	0	12.5	37.5	37.5	12.5	Significant (2 tailed) 0.021
Normal children	28	21.5	7.5	18.3	24.7	Pearson correlation 0.283** Significant (2 tailed) 0.004
IDA children Fresh milk	62.5	25	12.5	0	0	
Normal children	3.2	2.2	3.2	43	48.4	Pearson correlation 0.283** Significant (2 tailed) 0.004
IDA children Coffee	0	12.5	62.5	25	0	
Normal children		2.2	1.1	33.3	63.4	Pearson correlation 0.131 Significant (2 tailed) 0.193

\*Correlation is significant at 0.05 levels (2-tailed)

\*\*Correlation is significant at 0.01 levels (2-tailed)

Table 5	The estimated multiple regression result is presented below					
Variables	R value	R <sup>2</sup> value	Adjusted R Square	P value	F value	
Predictors: (Constant), fresh milk, meat, Dark green leafy vegetable, Black tea, liver, fish, chicken	0.760a	0.577	0.545	0.000a	18.130	

Table 6The coeffici

The coefficient values of selected food items

	(	Coefficie	entsa		
Model	Unstand Coeffici		Standardized Coefficients	t	Sig.
	В	Std. Error	Beta		
(Constant)	17.394	0.912		19.073	< 0.001
Dark green leafy vegetable (X1)	-0.402	0.104	-0.268	-3.873	< 0.001
Chicken (X2)	-0.422	0.146	-0.224	-2.897	0.005
Liver (X3)	-0.421	0.143	-0.213	-2.935	0.004
Meat (X4)	-0.427	0.105	-0.303	-4.079	< 0.001
Fish (X5)	-0.343	0.126	-0.203	-2.716	0.008
Black tea (X6)	0.157	0.058	0.192	2.715	0.008
fresh milk (X7)	0.287	0.099	0.207	2.887	0.005
. D l V					

a. Dependent Variable: IDA

Sig – significant

## Conclusion

The results highlight the role that dietary factors play in the development of IDA, with inadequate iron consumption being found to be a major risk factor. Interestingly, eating dark green leafy vegetables, chicken, liver, beef, and fish were linked to a decreased frequency of IDA. This highlights the importance of varied and nutrient-rich diets in preventing IDA in children. In contrast, consuming more black tea and fresh milk was associated with a higher risk of IDA. Even though there were no statistically significant differences between demographic factors and IDA, the study emphasizes the need for focused efforts to increase intake of iron-rich foods and dietary diversification, particularly for young children.

Author Contributions: Conceptualization, Dr. Muneeb and Dr. Rajavarthani; methodology, Mrs. Afreen and Dr. Rajavarthani; validation, Dr. Muneeb and Dr. Rajavarthani; formal analysis, Mrs. Afreen, Dr.Muneeb, and Dr. Rajavarthani; investigation, Mrs. Afreen; resources, Mrs.Afreen and Dr. Roshanth; data curation, Mrs. Afreen; writing – original draft preparation, Mrs. Afreen; writing – review and editing, Dr. Rajavarthani and Dr. Muneeb; visualization, Mrs. Afreen; supervision, Dr. Muneeb, Dr.Rajavarthani and Dr. Roshanth; project administration, Dr. Muneeb, Dr. Rajavarthani and Dr. Roshanth; funding acquisition, Mrs. Afreen. All authors have read and agreed to the published version of the manuscript.

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