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Pathology Section

Laboratory and Etiological Profile of Febrile Thrombocytopenia Cases- A Cross Sectional Study

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ABSTRACT

Introduction: Fever is a symptom which is caused by a variety of illnesses and it usually occurs in response to an infection or inflammation. Patients presenting with fever in tropical country like India usually have an infectious etiology and many have associated thrombocytopenia. Common causes of febrile thrombocytopenia include infectious etiology like, dengue, malaria, typhoid, Leptospirosis etc. Such study was not conducted before in the given region to know various causes of febrile thrombocytopenia, so this study was done.

Aim: To evaluate the laboratory and etiological profile of patients with febrile thrombocytopenia in patients admitted to Sapthagiri institute of medical sciences and research centre (SIMS, RC) Bangalore.

Materials and Methods: A cross sectional study was carried out on 580 patients aged > 18 years admitted to medicine ward, SIMS, RC, Bangalore between the period August-October 2015.

Results: From the 580 patients studied, the commonest infectious etiology was dengue (48.28%) followed by septicemia (19.83%) and typhoid fever (15.52%). Among 580 cases, 376 cases (64.82%) were males and 204 cases (35.18%) were females. The most common age group affected was between 18-30 years. Among 280 cases of dengue, 91 cases showed leucopenia and 60 cases showed deranged liver function tests. In malaria, 4 cases showed features of thrombocytopenia with leucopenia and 1 case showed icteric hepatitis.

Conclusion: The uncommon causes of severe thrombocytopenia in our studies included DIC, septicemia, UTI, apart from usual causes like dengue, malaria and typhoid fever. All the cases of febrile thrombocytopenia should be evaluated and subjected for detailed platelet studies, as it is a very important basic investigation to establish the correct diagnosis and also to prevent fatal outcome from the disease.

Keywords: Dengue, Infections, Platelets, Seasonal variation

INTRODUCTION

Fever is the most ancient hallmark of any disease. It is also known as pyrexia which is derived from the Greek word, “pyretus” meaning “fire”. Febrile is also originated from the Latin word “febris”, meaning “fever” [1]. It has been considered as one of the most common manifestation of diseases since ancient times. It was even recorded by many ancient scholars like Hippocrates [2].

“Fever” is characterized by increase in the body temperature, usually by 1° to 4°c. It is one of the most prominent clinical manifestations of acute phase response, especially when inflammation is associated with infection [3]. Thrombocytopenia is defined as a platelet count less than normal range usually below 1, 50,000 per microlitre [2]. Fever is produced due to production of substances called pyrogen. Pyrogen acts by stimulating the synthesis of prostaglandins which is secreted in the vascular and perivascular cells of the hypothalamus. Lipopolysaccharides which are the bacterial products, also called as exogenous pyrogen, stimulates the leukocytes to release cytokines such as TNF and IL-1. They are also called as endogenous pyrogens; they then increase the enzymes\ cyclooxygenases that convert arachidonic acid into prostaglandins. PGE2 stimulates the production of cyclic adenosine monophosphate (c-AMP), which has a function to reset the temperature set point at a higher level [3]. It was thought that fever is due to a product of polymorphonuclear leukocyte initially. IL-1 has now shown to have a major role in thermoregulation in our body This endogenous pyrogen is generated by mononuclear phagocytes which is identical or very similar in composition to substances previously identified mononuclear cell factor, lymphocyte activating factor (LAF), and leukocyte endogenous mediator collectively called as Interleukin 1(IL-1)[4].

Patients with history fever in tropical country like India usually have an infectious etiology and many of them have associated thrombocytopenia. Infections like dengue, typhoid, malaria, and leptospirosis are some of the common causes of febrile thrombocytopenia [5].

Thrombocytopenia cases are considered mild if counts are between 60,000 and 1.5 lakh, moderate if between 20,000 and

60,000, and severe if less than 20,000 per microlitre. Patients with platelet count less than 10,000/microlitre have increased risk of spontaneous bleeding, petechiae and bruising [6].

Even though malaria is endemic in tropical region, dengue has higher fatality rate than malaria which is above 1% over the last 10 years [7]. In humans, Dengue fever (DF) is the most common cause of mosquito borne arboviral infections. Many undifferentiated febrile illnesses are very common in tropical countries like India which may mimic like dengue, salmonella typhi, malaria, Leptospirosis, influenza etc., [8]. According to WHO, annually 50 million cases of DF occur worldwide with a mortality rate of 2.5% [9]. Reduced platelet count might also be due to pseudo thrombocytopenia, which may be caused by EDTA is a common laboratory phenomenon [10]. Sometimes non infectious causes such as primary hematological disorders may also present with febrile thrombocytopenia [11].

AIM

To evaluate the laboratory and etiological profile of patients with febrile thrombocytopenia and to determine the relative frequency of different diseases in these patients belonging to this region.

MATERIALS AND METHODS

A cross sectional study was carried out on total of 580 patients aged > 18 years with fever and thrombocytopenia admitted to medicine ward, Sapthagiri Institute of Medical Sciences and Research Centre (SIMS, RC), Bangalore between the period August 2015-October 2015 were included in the study.

Study Design

Patients who were admitted with a history of fever, and those who had associated thrombocytopenia, for them, a detailed history was taken, general and systemic examination findings were noted. Routine investigations like complete hemogram, urine routine, serum electrolytes, PCV, ESR, PS for malarial parasite, aPTT, etc., were done. Special investigations like blood culture, Liver function tests, Renal function tests, urine culture, Widal, card antigen test for malaria, IgM ELISA for leptospira, IgM ELISA for Dengue, C-reactive protein, Bone marrow aspiration were done whenever necessary. As the given study is a cross sectional study, statistical analysis is not applicable for this study.

For platelet count, three methods were used.

- A 3 part cell counter is an automated cell counter with features of counting RBC's, WBC's, platelets and hemoglobin estimation along with blood indices all together.
- **Crude Method:** A blood film was made from EDTA mixed blood and was stained with Leishman's stain. The count was considered adequate if there was 1-2 platelet per 20/30 RBCs. At 1000x magnification 7-20 platelets/oil immersion.
- **Direct Visualization:** 0.02 ml EDTA blood was diluted with 2ml of diluting fluid followed by charging the Neubaur's chamber with the fluid and number of platelets was counted.

The ethical committee of Sapthagiri Institute of Medical Sciences and Research Centre, Bangalore has approved to conduct this study.

The various inclusion and exclusion criteria for our study were:

1) **Inclusion criteria:** Patients of both sexes aged > 18 years admitted to SIMS, RC with history of fever i.e. morning body temperature > 37.2°C (>98.9°F) and evening body temperature of >37.7°C (>99.9°F), with a platelet count <1.5 lakh/cumm.
2) **Exclusion criteria:** Patients of both sexes aged < 18 years of age.

- Diagnosed cases of platelet disorders and dysfunction.
- Patients on antiplatelet therapy.
- Pregnant patients.
- Patients with cirrhosis and chronic liver diseases.

A cut off age limit of 18 years was considered for this study because most of the febrile thrombocytopenia cases in children were self limiting and no definite cause was established in most of the cases. Hence, febrile thrombocytopenia cases in pediatric age group were not included in this study.

RESULTS

Out of 580 patients who were recruited in this study, the commonest infectious etiology of febrile thrombocytopenia was Dengue 48.28%, followed by Septicemia 19.83%, Typhoid fever 15.52% and DIC 5.69% [Table/Fig-1]. In our study it was noted that, males (64.82%) were commonly affected than females, which can be attributed to outdoor activities among men [Table/Fig-2]. [Table/Fig-3] shows, the

Disorders	Number of cases	Percentage (%)
Dengue	280	48.28%
Septicemia	115	19.83%
Typhoid	90	15.52%
DIC	33	5.69%
Viral fever other than dengue*	30	5.17%
Malaria (*P. falci, P. vivax*)	13	2.25%
Megaloblastic Anemia	10	1.72%
UTI	5	0.86%
Hematological Malignancies	04	0.68%
Total	580	100%

[Table/Fig-1]: Proportion of various disorders presenting with febrile thrombocytopenia
*Viral fever other than dengue included, HIV, Chikungunya and unknown viral fevers.

Male	376	64.82%
Female	204	35.18%
Total	580	100%

[Table/Fig-2]: Gender distribution of fever with thrombocytopenia cases

Lab parameters	Dengue	Malaria	Typhoid	Septicemia	Urinary tract infection	DIC	Megaloblastic anemia	Viral fever(other than dengue)	Leukemia
Anemia	46	7	21	35	03	14	10	13	4
Leucopenia	91	4	8	18	0	9	3	10	1
Leucocytosis	9	0	6	20	05	15	0	15	3
Thrombocytopenia	280	13	90	115	05	33	10	30	4
Renal function tests	20	3	3	24	1	28	0	4	1
AST/ALT*	60	1	0	26	0	28	0	6	1
Bilirubin	45	1	0	0	0	15	0	0	0

[Table/Fig-3]: Laboratory parameters in various diseases.
*Aspartate aminotransferase, Alanine aminotransferase.

various laboratory parameters in various diseases. Dengue fever showed maximum number of anemia (46 cases), leucopenia (91 cases), thrombocytopenia (280 cases), along with deranged liver functions (60 cases), whereas septicemia showed maximum cases of leucocytosis (20 cases). DIC showed maximum number of deranged renal function tests (28 cases). The diagnosis of all cases of leukemia and 7 cases of megaloblastic anemia were confirmed by bone marrow aspiration study. [Table/Fig-4] shows, the commonest age group affected was between 18 to 30 years (48.27%) followed by 31 to 40 years (18.97%). This can be once again attributed to the outdoor activities of the younger age group and exposure to mosquitoes.

Age	No. of cases	Percentage (%)
18-30 years	280	48.27%
31-40 years	110	18.97%
41-50 years	90	15.52%
51-60 years	73	12.58%
>60 years	27	4.66%

[Table/Fig-4]: Distribution of febrile thrombocytopenia with relation to age.

DISCUSSION

Fever is one of the commonest manifestations of illness. Thrombocytopenia is very common in malaria, especially in falciparum type and the pathogenesis for this include sequestration of platelets due to immune mediated destruction of platelets along with elevated platelet activated immunoglobulin in the body. Hence, in dengue fever, destruction of platelets is due to immune mediated mechanisms. Transient thrombocytopenia occurs with many systemic infections. Thrombocytopenia usually occurs in 50-75% with bacterial or with fungal infections. It occurs in 50% cases of gram negative bacterial infections and also in sepsis. It is even seen in other viral infections including HIV [2].

In the present study, 580 patients were included, who were admitted with a history of fever and on peripheral smear examination showed thrombocytopenia. All patients were aged > 18 years. Among 580 cases, the commonest etiology for fever with thrombocytopenia was Dengue (48.28%), followed by septicemia (19.83%), typhoid (15.52%). It was more common among males (64.82%) than in females (35.18%). The commonest age group affected was between 18 to 36 years (48.27%), the reason for this increased incidence in males and younger age group in the given locality has been attributed to the prolonged outdoor activities and increased

Disease	Present study	Nair PS et al., [11]	Lakum et al., [12]	Bhalara et al., [6]	Gandhi et al., [1]
Dengue	280(48.28%)	15(13.8%)	177(35.4%)	28(60.8 %)	30(26.79%)
Malaria (*P. falci, P. vivax*)	13(2.25%)	10(9.2%)	234(46.8%)	----	46(41.07%)
Typhoid fever	90(15.52%)	16(14.7%)	23(4.6%)	----	5(4.46%)
Septicemia	115(19.83%)	29(26.6%)	39(7.8%)	3(6.5%)	5(4.46%)
DIC	33(5.69%)			----	
Megaloblastic anemia	10(1.72%)	13(11.9%)	----	----	6(5.36%)
UTI	05(0.86%)	----	----	----	----
Leukemia	04(0.68%)	04(3.7%)	07(1.4%)	2(4.3%)	2(1.79%)
Other viral fever	30(5.17%)	----	14(2.8%)	----	18(16.07%)

[Table/Fig-5]: Comparison of causes of thrombocytopenia with other studies.

chances of exposure to mosquitoes and also majority of the women being homemaker.

Dengue was the commonest cause of fever with thrombocytopenia in which 60.71% had severe thrombocytopenia, 23.22% patients had moderate thrombocytopenia, and 16.07 % had mild thrombocytopenia.

Compared to study done by PS Nair et al., [11], septicemia (26.6%) was the major cause of febrile thrombocytopenia, where as studies done by Gandhi et al., [1] and Lakum et al., [12], showed Malaria (41.07%) and (46.8%) respectively as the major cause, this difference was noted due to seasonal and regional variation. Study done by Bhalara et al., [6] showed Dengue (60.8%) as the major etiology which was comparable with our study [Table/Fig-5].

In our study, dengue was the commonest cause of fever with thrombocytopenia in which, 170 patients had severe thrombocytopenia (<50,000), 65 patients had moderate thrombocytopenia (50,000-1 lakh) and 45 patients had mild thrombocytopenia (1-1.5 lakh). It was followed by septicemia, in which 59 patients had severe thrombocytopenia, 43 had moderate thrombocytopenia and 13 had mild thrombocytopenia i.e. 48.45% of patients belonged to severe thrombocytopenia, 33.62 % to moderate thrombocytopenia and 17.93% to severe thrombocytopenia where as studies done by Nair et al., [11] and Bhalara et al., [6] showed similar results when compared to our study.

EDTA dependent pseudo thrombocytopenia may also be seen in many laboratories which is due to in vitro clumping at room temperature. These cases can be mistaken for thrombocytopenia if the patient is suffering from fever. The in vitro clumping is not related to age or sex nor associated with any particular pathology or usage of specific drugs, but was seen in both healthy subjects and in patients with various diseases. Studies have proven that this phenomenon is due to the presence of natural auto antibodies which have antiplatelet activity and has no pathological significance. The clinical interest resides in the need for its recognition in order to avoid unnecessary investigations and therapeutic interventions. Hence, the best and rapid technique for obtaining accurate platelet count in such individuals is to collect and then examine EDTA blood at 370 or even in citrated blood [10].

In our study, along with infections, Megaloblastic anemia, DIC and hematological malignancies also accounted for the etiology which was comparable with the studies done by Gandhi et al., [1] and Nair et al., [11]. But in Srinivas et al., study [13], 100% causes were infections.

The various laboratory parameters of dengue, malaria, viral infections other than dengue and typhoid fever did not correlate with the parameters in study done by Yogeesha KS et al., [8].

In our study, patients who showed < 50,000 to <10,000 were all transfused with platelets and a definite increase in the platelet count and better patient outcome was noted. Similar findings were also seen in study done by Dash et al., [14], who reported malaria as the commonest cause of febrile thrombocytopenia.

Though dengue was the most common infectious cause of febrile thrombocytopenia in our study, early diagnosis of dengue infection remains a challenge to all clinicians. Ho et al., [15] in his study calculated several laboratory parameters as predictors of laboratory confirmed dengue infections. No single lab test was good enough in terms of positive predictive value for acute dengue infection. Hence, a combination of several lab parameters such as leucopenia, elevated aminotransferase, TLC and low CRP gave a positive predictive value of about 89.5% The value of positive predictive value (ppv) of this given combination was increased to 93.1% by just adding prolonged aPTT and this combination served as a very good predictor marker for acute dengue infections. In our case, this value could not be calculated as all parameters were not done in all the cases.

LIMITATIONS

Firstly, the hospital based model might not reflect the whole dengue infected group of the given locality or population. Secondly, due to large sample size, the study could not be correlated with other clinical manifestations. Lastly, this study was not applicable to the pediatric age group, where the causes remained unknown and were self limiting.

CONCLUSION

The uncommon causes of severe thrombocytopenia in our studies included DIC, septicemia, UTI, apart from usual causes like dengue, malaria and typhoid fever. A definite seasonal variation was also noted, which was more commonly noted in the early winter. All the cases of fever should be evaluated for detailed platelet studies, because as it is very important basic investigation to establish the correct diagnosis and also it prevents the fatal outcome from the disease. Pseudothrombocytopenia due to EDTA should be ruled out in all cases. This was the first study conducted in this region to know the etiology of febrile thrombocytopenia. As dengue was the most common infectious etiology in this region, preventive measures such as avoidance of water pooling, spraying of insecticides and larvicides, along with personal protective measures can be undertaken.

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