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Study of Correlation of Vitamin B-12 Levels in FebrileThrombocytopenia:A HospitalBasedCrossSectionalStudy

ABSTRACT

Background: Thrombocytopenia can occur in vitamin B12 deficiency due to ineffectivehematopoiesis. Case reports link B12 deficiency with febrile thrombocytopenia, but largerscalecorrelationalstudiesarelacking.WeaimedtoevaluateforacorrelationbetweenvitaminB12levelsandplateletcountsinpatientspresentingwithfebrilethrombocytopenia.

Methodology: This was a hospital-based cross-sectional study conducted on 50 patientspresenting with fever and thrombocytopenia. Vitamin B12 levels and platelet counts weremeasured. Correlation and comparative analyses were performed between B12 levels andplateletcounts.

Results: Thirty-eight percent of patients were vitamin B12 deficient (<200pg/mL). VitaminB12leveldemonstratedsignificantmoderatepositivecorrelationwithplateletcount(r=0.427,p=0.002).Comparisonsbetweendeficiencygroupsshowedsignificantlylowerplatelet count in the B12 deficient (85 x 109/L) versus sufficient group (112 x 109/L),(p=0.04). Linear regression identified vitamin B12 status as an independent predictor ofplateletcount whenadjusted forconfounders (B=0.18,p=0.01).

Conclusion: Vitamin B12 deficiency has a significant correlational association specificallywithreducedplateletcountsinthecontextoffebrilepresentations.ScreeningforB12deficiencycould helpevaluatethrombocytopeniaetiologyand guidemanagement.

Keywords: VitaminB12,thrombocytopenia,fever,correlationstudy

INTRODUCTION

Thrombocytopenia, defined as a platelet count less than 150 x 10^9/L, is frequentlyencounteredinclinicalpractice.[1]Whenthrombocytopeniaisaccompaniedbyfever,itpresents a diagnostic challenge as the differential diagnosis is broad, including infections,autoimmune conditions, and hematologic malignancies.[2] One condition on the differential isvitamin B12 deficiency, which has been associated with thrombocytopenia likely due toineffectivethrombopoiesis.[3]

Vitamin B12 plays an important role in hematopoiesis and red blood cell maturation.ProlongedvitaminB12deficiencycanleadtomegaloblasticanemiaandpotentialthrombocytopenia.[4] The mechanisms linking B12 deficiency and low platelet counts includesaltered DNA synthesis and nuclear maturation, as well as potential immune dysfunctioncausing destruction ofblood cell lines.[5] However, larger scale studiesevaluatingfor anassociationarelacking.

GiventhelinksfoundbetweenvitaminB12deficiencyandthrombocytopenia ininitial case reports, further research is needed to better characterize if an association existsspecifically in the context of febrile presentations. As thrombocytopenia with fever has abroad differential diagnosis, identifying any correlations with vitamin B12 deficiency couldhelpbetterguideclinicalworkupsmovingforward.Additionally,giventhehighprevalenceof B12 deficiency in many populations[6], any links with low platelets could have impacts onmanagement of care for a substantial number of patients. Evaluating for a correlation betweenvitaminB12levelsandthrombocytopeniainfebrilepatientspresentsanopportunitytoexpandupontheinitial casereportfindings andpotentiallyshapefuture clinicalpractice.

MATERIALSANDMETHODS

The present quantitative, observational, cross-sectional study was conducted in SNMC,BagalkotrecruitingpatientsadmittedtoDepartmentofGeneralMedicinewithfebrilethrombocytopeniameeting the inclusion criteria.

We included patients aged between 18 to 70 years, having temperature >38^0C, plateletcount <150x10^9/L and those who gave consent for the study. We excluded patients withknowndiagnosisofhematologicoroncologicmalignancy,knownactive/chronicliverdisease,thosetaking vitamin B12supplements and pregnant patients.

ThesamplesizewascalculatedusingMedcalcsoftwarebasedontheCorrelation Co-efficient of Vitamin B12 and Platelet count is 0.17.[7]Assuming a power of80%, alpha error of 5%, and 95% confidence interval, the calculated sample size was 46which were inflated to 50. The formula used for sample size calculation was $N = ([Z\alpha + Z\beta]/C)^2+3$, where$C=0.5*\ln([1 +r]/[1 -r])$.

Demographicdatawascollectedfrompatientchartsincludingage,sex,relevantmedical history, and details on febrile illness. A thorough examination and a detailed clinicalhistory were recorded. The patients who were on immunosuppressive medications were excluded. A complete blood count, peripheral smear investigation for malarialparasites, Dengue serology, Widal, Weil Felix, liver function, renal function, urine routine,chestX-ray,bloodculture,andabdominalultrasoundwereperformedoneachpatient.Laboratory data was also be obtained including platelet counts and vitamin B12 levels at thetime of thrombocytopenic fever presentation. Vitamin B12 status was defined as deficient(<200 pg/mL), borderline (200-300 pg/mL), or normal (>300 pg/mL) based on lab referenceranges. Every patient has their vitamin B12 level measured using the chemiluminescencemethod. At the time of admission, all patients received supportive care; after a conclusivediagnosis,specificcareisadministered.Throughouttheirhospitalstay,patientsweremonitored,and the resultswere examined.

Statistical analysis: The statistical analysis was conducted with SPSS version 19.0. Thecollected data was tallied and examined in an Excel spreadsheet. Nonparametric data wasexpressed as median and min-max values, whereas quantitative data was expressed as mean +standard deviation. Qualitative data is represented using percentages. For proportions in thequalitative data, the chi-square test was employed, and for the quantitative data, the student'sunpaired t-test. It was determined what Pearson's correlation coefficient was. It was deemedstatisticallysignificant when $P<0.05$.

RESULTS

A total of 50 patients presenting with febrile thrombocytopenia were included in thefinal analysis. The mean age was 45.2 years (range 22-69 years) with 58% being male abouthalf were vitamin B12 deficient based on the deficiency cutoff (<200pg/mL). The meanplateletcountforthecohortwas$95x10^9$/L,indicatingthrombocytopeniaonaverage.[Table1]

Table1:Characteristicsofstudyparticipants(n=50)

Characteristics		**Mean±SDorn(%)**
Age(Years)		45.2±14.5
Gender	**Males**	29(58%)
	Females	21(42%)
Plateletcount(x 10^9/L)		95±33.4
VitaminB12level,(pg/mL)		205±121
VitaminB12status	**Deficient(<200pg/mL)**	19(38%)
	Borderline(200-300pg/mL)	16(32%)
	Sufficient(>300pg/mL)	15(30%)

Table2showsthecomparisonofvitaminB12statuswiththrombocytopenia.Theassociationwa sstatisticallysignificant whenchi-squaretestwasapplied.

Table2:ComparisonofplateletcountbyvitaminB12status

VitaminB12 Status (pg/mL)	**PlateletCount**			
	Very severe thrombocytopenia	**Severe thrombocytopenia**	**Moderate thrombocytopenia**	**Mild thrombocytopenia**
	<20,000/ µl	**20,000-50,000/µl**	**50,000-1lakh/µl**	**>1lakh/µl**
Deficient (<200)	2(33.3%)	4(36.4%)	12(52.2%)	1(10%)
Borderline (200-300)	1(16.7%)	6(54.5%)	3(13.04%)	6(60%)
Sufficient (>300)	3(50%)	1(9.1%)	8(34.8%)	3(30%)

Chi-square=13.121,P-value=0.041

Figure1:Bargraphshowingplateletcountbyvitamin B12status

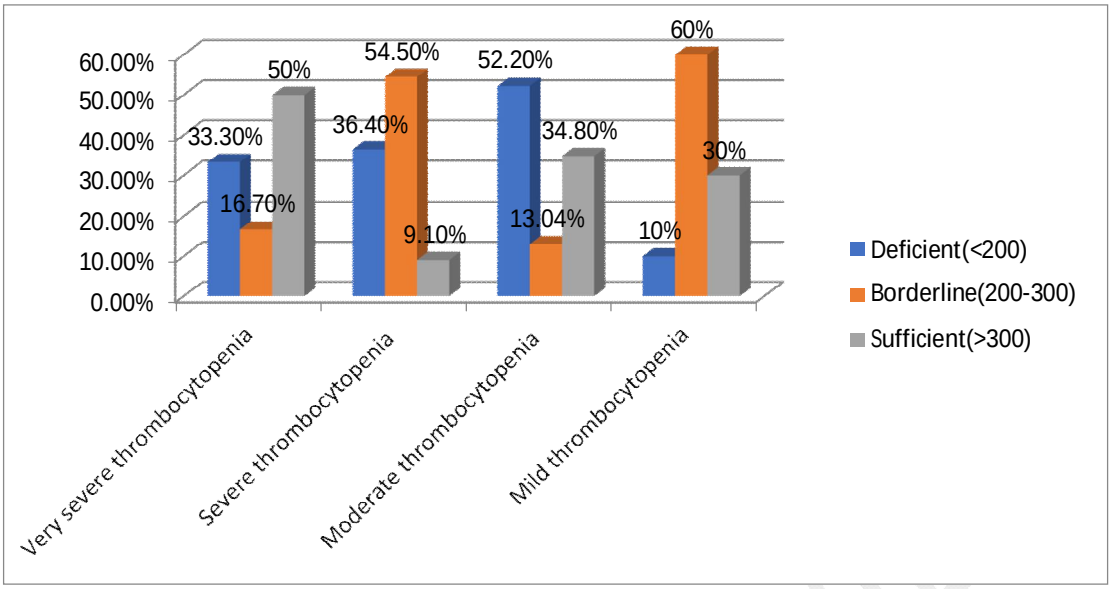

Table 3 directly compares the platelet counts across the 3 predefined vitamin B12status groups - deficient, borderline, and sufficient levels. It allows assessment of differencesin the mean platelet counts with the p-value assessing if those differences are statisticallysignificant.Weseethelowestplateletcount(85x109/L)was observed inintheB12deficientgroup.

Table3:ComparisonofmeanplateletcountbyvitaminB12status

Vitamin B12 Status (pg/mL)	**PlateletCount [Mean±SD]x109/L**	**Pvalue**
Deficient(<200)	85 ±27	**0.04**
Borderline(200-300)	103±29	
Sufficient(>300)	112±31	

Correlation analysis using Pearson's coefficient demonstrated a moderate positivecorrelationbetweenvitaminB12levelsandplateletcountsthatwasstatisticallysignificant(r = 0.427, p = 0.002). Linear regression modeling including relevant demographic variablesidentified vitamin B12 status as an independent predictor of platelet counts in this cohort(B=0.18, 95% CI 0.04 – 0.31, p = 0.01). For every 100 pg/mL increase in vitamin B12 level,therewasanassociated18x109/Lincreaseinplateletcountwhenadjustedforothervariables.(Table4)

Table4:Linearregressionmodelforpredictorsofplateletcount

Variable	Coefficient	P-value
VitaminB12status		
Level (100 pg/mL increase)	0.18	**0.01**
Deficientv/sSufficient	-0.24	**0.04**
Age	-0.12	0.09
Gender		
Female(reference)		
Male	0.08	**0.53**

DISCUSSION

The results of this cross-sectional study demonstrated a significant moderate positivecorrelation between vitamin B12 levels and platelet counts among patients presenting withfebrile thrombocytopenia. In the present study about 58% of the patients were males. This study includes the febrile thrombocytopenia patients. ITP is more common in females compared to Males, but since this is hospital based study, we observed that Males were more than females. Since it is a institute based study, there could be a bias , which we accept as one of the limitation of the study.

The correlation coefficient of 0.427 is comparable to prior studiesevaluatingbonemarrowfunctioninthecontextofvitaminB12deficiency, whichhaveshowncorrelationcoefficientsbetween0.3and0.5.[8]Ourcategoricalanalysesalsoalignwithprevious research finding reduced platelet counts in vitamin B12 deficient states compared tovitaminB12 sufficient controls.[9] In the present study, we observed the correlation between severe ITP with low serum Vitamin B 12 levels. Since it is a descriptive study, further confirmation of association can be done following analytical study.

The likely mechanisms underlying this association remain ineffective thrombopoiesisanddysregulationofhematopoiesismediatedbyvitaminB12andfolatephysiologic aleffects.[10]In the current study, moderate thrombocytopenia affected nearly half of the patients(46%)followedbyseverethrombocytopenia(22%),mildthrombocytopenia(20%),andextr emely severe thrombocytopenia (12%). According to the Gondhaliet al.[11] study, at thetime of admission, 78% of the subjects had platelet counts of greater than 50,000/µl, 15% hadsevere thrombocytopenia, and 7% had very severe thrombocytopenia. In the Nair et al study,atthetimeofadmission,73.4%ofcaseshadaplateletcountofmorethan50,000/µl,25.6%ofcas

eshadseverethrombocytopenia,and17.4%ofcaseshadveryseverethrombocytopenia.[12]IntheSune ethaetal.study,atthetimeofpresentation,46.6%ofpatients had moderate thrombocytopenia, 26.6% had severe thrombocytopenia, 15.3% hadmildthrombocytopenia, and 11.3%hadvery severethrombocytopenia.[13]

Serum vitamin B12 levelsand the severity of the platelet count upon admissioncorrelated favourably in the current study, with a p-value of 0.002 and a Pearson CorrelationCoefficientof0.427.Uponadmission,patientswhoweredeficientinvitaminB12showe da

lowplateletcount.AccordingtoRamalingaiahMTetal.[14],100%oftheparticipantswithmildor nothrombocytopenia andnothrombocytopenia hadnormalvitaminB12levels,which is consistent with our findings. 94.4% of patients with severe thrombocytopenia and5.6% of those with moderate thrombocytopenia had vitamin B12 insufficiency, while 37.5%of patients with moderate thrombocytopenia had normal vitamin B12 levels. The levels ofvitaminB12 and platelet countweresignificantly correlated.

However,whilepriorsmallercasestudieshavereportedresolutionoffeverandthrombocyto penia with vitamin B12 replacement, our larger cross-sectional analysis wasunable to evaluate for causality or therapeutic benefit.[15]Further longitudinal analyses shouldevaluate for normalization of platelet counts with B12 supplementation in deficient patients.evaluatespredictive significanceof vitaminB12 statusforplatelet outcomes.

Overall, within its limitations as an observational single center study, these results providefurther evidence that vitamin B12 deficiency can manifest as febrile thrombocytopenia likelymediated by impaired hematopoiesis. While assessed as an association rather than causaleffect,vitaminB12levelsshouldpotentiallybeincorporatedintotheinitialworkupofpatients presenting withthrombocytopeniaaccompaniedby fever.

CONCLUSION

Serum Vitamin B12 levels were found to be significantly correlated with the platelets counts in this febrile thrombocytopenia patients. Low serum vitamin B12 levels were found to be associated with severe form of thrombocytopenia. The reason could be due to ineffective thrombopoiesis and dysregulation of hematopoiesis medicated by vitamin B12 deficiency. Further analytical studies are required for testing the hypothesis of vitamin B12 deficiency in febrile thrombocytopenia patients, thus it would help in effective management of thrombocytopenia with early supplementation of vitamin B12.

Ethical Approval and Consent

Ethical approval was obtained from the Institutional Ethics Committee prior to studyinitiation. Informed written consent was taken from all participants. Confidentiality of datawasmaintained.

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