Clinico-Hematologic And Biochemical Profile Of Dimorphic Anemia With Bone Marrow Study

R Athar, Y Khonglah, V Raphael, A Pal, K G Lynrah

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Abstract

Introduction: Dimorphic anemia (combined iron and vit B12/Folate deficiency) has not been studied in this part of the country and the treatment may be ineffective if the dual deficiency is not diagnosed.

Material & Methods: A 1 year prospective hospital based study was conducted to correlate biochemical parameters with complete hemogram and bone marrow findings.

Results: The study group included 58 patients. The bone marrow correlated with peripheral smear in 49 cases (84.4%).Out of these 49 cases, only 12 cases showed low VitB12/folate with low ferritin. Therefore, there was complete correlation between the three parameters in 12/49(24.5%) cases only.

Conclusions: Bone marrow aspiration though invasive, provides a rapid and cost effective investigation for confirming the diagnosis of dimorphic anemia by reliably assessing the iron stores. Biochemical parameters though specific, are highly vulnerable to variation in their values. So, depending only on biochemical parameters may mislead the clinicians.

Keywords: Dimorphic anemia, ferritin, bone marrow

INTRODUCTION

Dimorphic anemia (DA) is characterized by two distinct red cell populations. The term is most often applied when there is one population of hypochromic, microcytic cells and another of normochromic cells, the latter being either normocytic or macrocytic. A dimorphic blood film can be seen in several circumstances, it can occur when iron deficiency anemia responds to iron therapy, after the transfusion of normal blood to a patient with a hypochromic anemia, sideroblastic anemia, macrocytic anemia post transfusion, unmasking of iron deficiency following treatment of megaloblastic anemia, delayed transfusion reactions and dual deficiency of iron and either vitamin B12 or folic acid which is the focus of our study [1].

DA is very common in India, but the exact incidence has not been specified in literature. A study conducted in north east Delhi among children of urban slums showed dimorphic anemia in 45.4% of 282 anemic children [2]. The DA needs to be recognized since the treatment may be ineffective if the dual deficiency is not diagnosed. It is also noticed that a dimorphic picture on peripheral smear may show normal to increased iron stores on bone marrow aspiration study, thereby, not correlating with the peripheral smear diagnosis and the biochemical parameters may not be as reliable [3]. Hence, this prospective study was planned to correlate and compare the clinico-hematologic, biochemical parameters with bone marrow study in cases diagnosed as DA on peripheral smear findings.

MATERIALS AND METHODS

The study period was between 01-01-2010 and 31-01-2011. During this period, there were a total of 13342 (41.4%) anemic cases. DA formed 12.5% of all cases of anemia. 58 patients who were diagnosed as DA on peripheral blood smear (PBS) and who consented for bone marrow aspiration (BMA) with relevant biochemical investigations were included in the study. Patients with DA and history of recent

blood transfusion, treatment with hematinics, pregnancy and with underlying hemoglobinopathy were excluded.

Grading of anemia - The patients were then grouped as mild (10-10.9 g%), moderate (7-9.9 g%) and severe (<7 g %) anemia based on their initial hemoglobin level [2]. Only those with moderate and severe anemia were subjected to BMA.

Clinical parameters - A detailed history which included dietary history, family history, fever, duration of illness, prior therapy or transfusion and bleeding tendency with clinical examination were carried out.

Hematological parameters – 2ml of EDTA blood was collected for complete blood counts using an automated 5 part Sysmex coulter machine. PBS and BMA smears were stained with Leishman's stain. Perl's stain for Iron was done on the bone marrow smears and iron stores were graded according to Gale [4].

Estimation of biochemical parameters – 6 ml of fasting blood sample in a plain vial was collected. After the sample has clotted completely, centrifugation was done at 3000 rpm for 6 minutes. The supernatant serum was collected. It was used for the estimation of Serum vitamin B12, folate, ferritin and Total iron binding capacity (TIBC). Estimation of serum B12, folate and ferritin was done by the Chemiluminescence method and estimation of TIBC was done by Ferrozine method in a semi automatic machine.

Statistical analysis – The results were analysed by Spearman's correlation coefficient method with SPSS software to find out the correlation between serum ferritin vs bone marrow iron stores and TIBC vs bone marrow iron stores.

RESULTS

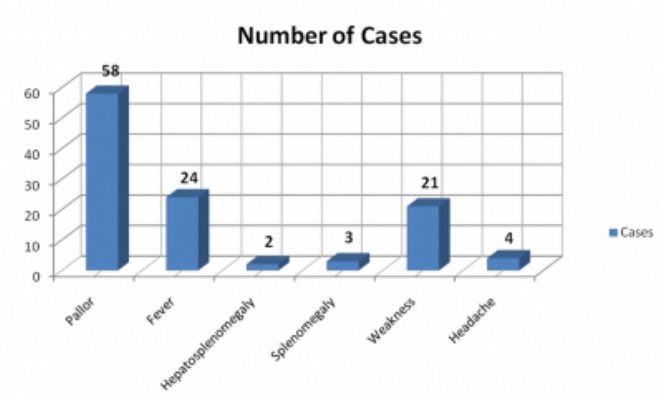
The ages of the patients ranged from 8 years to 81 years with a mean age of 35. The majority of the patients (32.75%) were in the age group 21-30. The male to female ratio is 1:2.

CLINICAL PRESENTATION

The most common clinical presentation was pallor followed by fever and weakness. The salient clinical features are summarized in Figure 1.

Figure 1

Number of Cases



HEMATOLOGICAL PROFILE

Most of the cases (75.8%) were that of severe anemia. Pancytopenia was seen in 18.96% of cases. The ranges and the mean values of all the hematological parameters studied are detailed in Table 1.

Table 1

HEMATOLOGICAL PROFILE (n = 58)

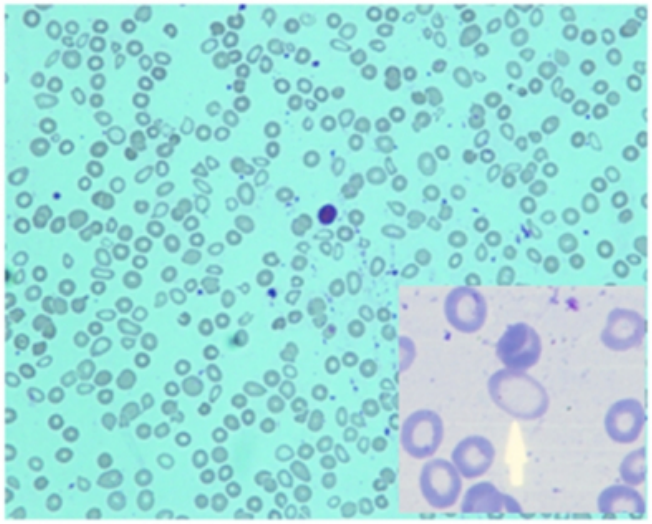
PARAMETERS	RANGE	MEAN
Hb(g%)	1.7-10.4	5.86
TLC /cu.mm	880-19,000*	5715.78*
Platelet Count $x10^3/\mu l$	10-890	204.57
RBC$x10^6/\mu l$	0.64-4.8	2.71
MCV(fl)	58.6-127	83.24
MCH(pg)	12.8-40	25.14
MCHC(%)	22.6-35	29.82
RDW(%)	13.2-26.9	18.68

*One case with leuco-erythroblastic picture had a high total count of 41,000/cu.mm and so was not included in the mean.

PERIPHERAL SMEAR FINDINGS - The PBS showed dual population of cells in all 58 cases. The dual population consists of macrocytes, macro-ovalocytes, hypochromic macroovalocytes with microcytic and hypochromic cells (Figure 2). Hypersegmented neutrophils were seen in 48 (82.75%) of cases. Leukopenia and Thrombocytopenia were seen in 19 (32.75%) and 15 (25.86%) cases respectively.

Figure 2

PBS showing both microcytic hypochromic cells and macroovalocytes (small lymphocyte for size comparison)(Leishmans stain, 40x). Inset shows a hypochromic macroovalocyte(Leishmans 100x)



BONE MARROW ASPIRATE FINDINGS – All marrows were megaloblastic with hypercellularity in 37(63.8%) cases (Figure 3). There was trilineage dyspoiesis in 14(24.1%) of cases. The iron stores were reduced (grade 0-1+) in 49(84.4%) and were normal to increased (grade 2+ to 4+) in 09(15.6%) cases (Figure 4).

Figure 3

BMA showing megaloblastic change with micronormoblasts(arrows)(Leishman stain,40x)

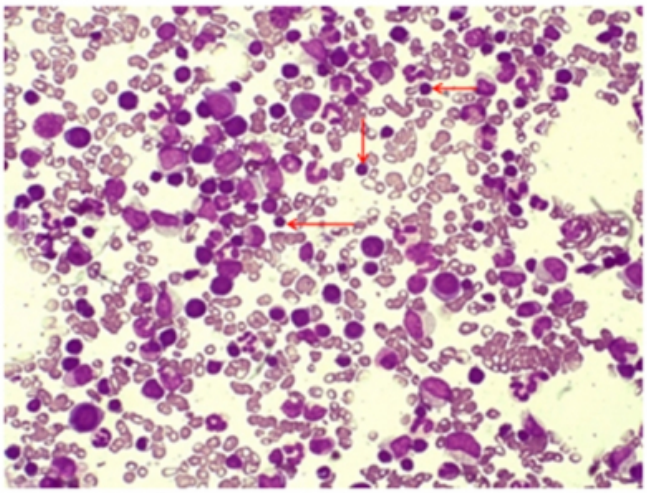
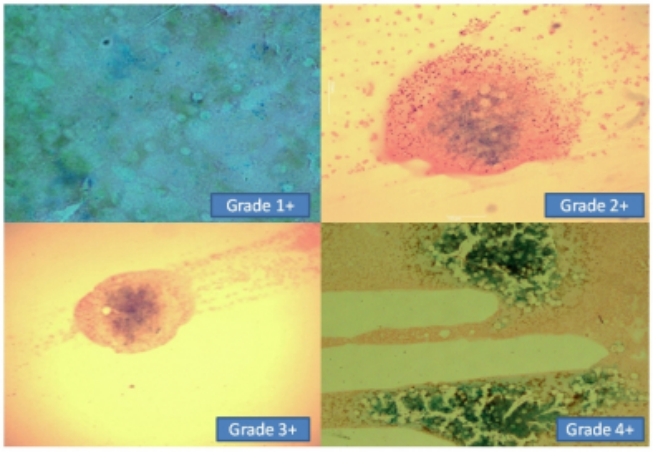


Figure 4

Grading of iron stores on BMA. Grade 1+ - Small iron-positive particles visible only under oil immersion (Perls stain,100x) ,Grade 2+ small iron particles visible under low magnification (Perls stain 10x) ,Grade 3+ numerous iron particles throughout marrow (Perls stain, scanner view) ,Grade 4+ clumps of iron particles(Perls stain, scanner view).



Correlation between PBS and BMA findings - Out of 58 cases, 49 cases (84.4%) showed features of DA on BMA, which correlated with the PBS findings.

BIOCHEMICAL PARAMETER FINDINGS

The results of the various biochemical parameters and their correlation with the PBS and BMA are summarized in Table 2:

Table 2

BIOCHEMICAL PARAMETERS WITH CORRELATION STUDY(n=58)

Biochemical Parameter	Normal Reference values	No. of cases with decrease value	No. of cases with increase value	No. of cases with normal values	Total no. of cases	% of cases correlating with PBS and BMA	% of cases not correlating with PBS and BMA
Vitamin B12	180-914 pg/ml	14	19	25	58	24.14	75.86
Folate	3-20 ng/ml	19	10	29	58	32.76	67.24
Ferritin	11-336.2 ng/ml	25	7	26	58	43.11	56.89
TIBC	250-400 µg/dl	28	6	24	58	10.4	89.6

Correlation between PBS, BMA and Biochemical parameters

Of the 49 cases showing dual deficiency on the bone marrow, 12 cases showed low VitB12/folate with low

ferritin. Therefore, there is complete correlation between PBS, BMA study and biochemical parameters in just 12/49 (24.5%) of cases. The rest 37 cases did not correlate with the biochemical parameters.

Correlation between bone marrow iron stores and serum ferritin

The bone marrow iron stores were reduced in 49 cases and the corresponding serum ferritin value was reduced in 25/49 cases. All 9 cases with normal to increase bone marrow iron stores also showed normal to increase serum ferritin level.

Correlation between BMA findings and Vit B12/Folate

All 58 cases showed megaloblastosis in the bone marrow, so it was expected that either serum Vit B12 or folate should have been low. But only 25 cases (43.1%) had either low serum Vit B12 or low folate. The rest 33 cases (56.89%) had either normal or high Vit B12 /Folate. So, there was a correlation between bone marrow findings and Vit B12/Folate in 43.1% cases only.

There was isolated B12 deficiency in 10.34%, isolated folate deficiency in 17.24% with combined deficiency in 15.5% of cases.

STATISTICAL ANALYSIS

The sensitivity and specificity of serum ferritin was found to be 51.02% and 100% respectively.

Table 3

STATISTICAL CORRELATION OF SERUM FERRITIN AND TIBC WITH BONE MARROW IRON STORES

VARIABLES	CORRELATION COEFFICIENT*	P-VALUE
Serum ferritin	0.4985	< 0.0001
TIBC	- 0.2439	0.0650

- Correlation analyses were carried out by Spearman's correlation test.

DISCUSSION

The present study included 58 patients who were diagnosed as DA on PBS along with BMA and biochemical parameters.

It is estimated that 30% of the world's population is anemic [5,6]. In India, a study done by ICMR district nutrition survey data reported prevalence of anemia in 84.2 %,with severe anemia in 13.1% population [7].In our study period of 13 months, there were 41.4% cases of anemia, of which dimorphic anemia was found to be 12.5% of the total anemic cases in all age groups. Fazlur rahman et al [8] found DA in 15 % of cases which is similar to our study. A study conducted in northeast Delhi showed DA in 45.4% of 282 anemic children [2]. In another study from Pakistan, DA was found in 42.70% of the patients [9]. The wide variation in the prevalence in different parts of India could be explained by the fact that the country has a very heterogenous population with diverse racial, ethnic, religious, cultural and socioeconomic differences which in turn influences the dietary habits.

The mean age of our patients is 35 years. Saira perwez et al [10] reported the mean age as 38.9 years. The majority of patients in our study were in the age group 21-30 years (32.75%). The male to female ratio was 1:2 in our study. Mussarrat et al [9] in his study which included mostly pediatric cases had a male to female ratio of 1.5:1.

The most common clinical presentation was pallor followed by fever and weakness. The presence of fever in 41.37% of the patients in our study is significant. An earlier series documented fever in 57% & 65.5% of patients in megaloblastic anemia [9]. The most common cause waas infection in which the individual is much more susceptible due to impaired intracellular killing of ingested bacteria by neutrophils and macrophages.

The mean hemoglobin in our study was 5.86 g/dl. Saira et al [10] and Iqbal et al [11] reported the mean hemoglobin as 6.8g/dl. In our study, thrombocytopenia was observed in 25.86% of patients and out of these 8.62% patients had platelet counts less than 40x109/L. Mussarat et al [9] reported thrombocytopenia in 72.20% of patients of which 22.06% had platelet counts less than 20x109/L. In yet another study from Zimbabwe, thrombocytopenia was detected in 48% of the patients [12].

Pancytopenia was seen in 18.96% and bicytopenia in 20.68% of the cases in our study. Mussarat et al [9] found pancytopenia in 30.09% and bicytopenia in 38.97% of their patients. Other studies have documented the incidence of pancytopenia and bicytopenia as 17.2% and 44.8% [13] and 43.8% and 80.5% [14] respectively.

Apart from pancytopenia, severe megaloblastic anemia may present with findings suspicious for malignant disorders

including pancytopenia, splenomegaly and leukoerythroblastosis [15]. In our study leukoerythroblastosis was reported in 6.89% of the patients. Mussarat et al found leukoerythroblastosis in 25.2% of his patients [9]. Prevalence of leukoerythroblastosis ranging from 1% to 20% has been reported in literature [16]. The leukoerythroblastic morphology is due to extramedullary hematopoiesis [17].

Our study showed macrocytosis and neutrophil hypersegmentation in 100% and 82.75% respectively, these are strong markers of cobalamin deficiency. In another study from Zimbabwe, macrocytosis and neutrophil hypersegmentation were reported in 54% and 32% respectively [12]. Mussarat et al found macrocytosis and neutrophil hypersegmentation in 68.9% and 51.5% of the patients [9]. The central pallor that normally occupies about one third of the normal blood cells is decreased in macro-ovalocytes. This contrasts with the finding of thin macrocytes, in which the central pallor is increased [18]. In our study, however, hypochromic macroovalocytes were found in 100% of the cases.

The bone marrow smears showed megaloblastic change and varying degree of dyserythropoiesis in all patients. Giant metamyelocytes were also seen. Similar findings were also found by Mussarat et al [9].

In our study, assessment of the bone marrow iron stores by Perl's Prussian blue reaction revealed increased stores in 15.6% of the patients and depleted stores in 84.40%. Mussarat et al found increased iron deposit in 57.30% of their patients (megaloblastic anemia) and depleted stores in 42.70% of the patients (megaloblastic anemia combined with iron deficiency) [9].

The pathogenesis of microcytic hypochromic cells on PBS and the presence of micronormoblast in the bone marrow of cases with normal/increase bone marrow iron stores remain unclear. There is possibly ineffective utilization of iron as parenteral administration of cobalamin to deficient patients' leads to rapid fall in iron levels and a decrease in plasma iron turnover with fecal urobilinogen reflecting cessation of ineffective erythropoeisis [19].

Ferritin is the intracellular storage form of iron found chiefly in the cytoplasm of the cells of the reticuloendothelial system. It can be quantitated in serum using immunoenzymatic assays. Serum ferritin concentrations have been documented to give an accurate indication of the amount of storage iron in healthy individuals and in patients with iron deficiency or iron overload [20]. It is the most specific biochemical test for iron deficiency anemia (IDA) because it correlates with total body iron stores and a low serum ferritin concentration reflects depleted iron stores. When compared with other iron status parameter, serum ferritin is one of the lowest biologically varying iron status markers, thus making it one of the most useful parameters. However there are many problems associated with the interpretation of serum ferritin levels. Apoferritin, a precursor of ferritin is an acute phase reactant protein and is therefore elevated in inflammatory process under the influence of IL-1 & TNF. Thus making it unreliable in the presence of inflammation [21,22]. In addition, there are gender differences (normally lower in women) that makes ferritin less than an ideal test for determining iron deficiency [23]. It is also unreliable as an index of iron stores when there is tissue damage.

Serum ferritin rises with aging. According to Hughes, the gold standard for the diagnosis of IDA has been considered to be the assessment of a BMA stained with Perl's stain [24]. Ali et al [20] in his study of 248 patients found lack of iron stores in 69 patients. Of these, the serum ferritin was elevated in 20 patients (29%) despite lack of demonstrable iron in the marrow specimen. They concluded that a low serum ferritin value probably indicates iron depletion, while an elevated value does not exclude that possibility. In their study, they could attribute prior iron therapy and blood transfusion in 2 cases each and certain chronic disease in other 16 cases as cause of increased ferritin [20].

We found in our study 24/49 cases (48.9%) showing elevated serum ferritin despite lack of bone marrow iron stores. Of these 24 cases, 15 had an underlying inflammatory disease. 5 patients had chronic liver disease, 3 had upper respiratory infections, 2 had acute gastro-enteritis and there was 1 case each of chronic kidney disease, splenic abscess, cor-pulmonale, pneumonia and rheumatoid arthritis. However, in 9 cases no specific cause for increased serum ferritin could be determined. So, our findings are in concurrence with their conclusion.

All our cases showed good response to treatment with iron, vit B12 and folate supplements following the report based on bone marrow. Even those nine cases which had megaloblastic changes with good iron stores in the marrow were given a low dose of iron along with vit B12 and folate as is practiced in our hospital, as sole vit B12 and folate supplements will result in rapid utilization of the iron stores,

thereby resulting in relative deficiency of iron and persistent overt microcytic hypochromic anemia. However, no long term follow up could be carried out as the patients were lost to follow-up.

Even though the most precise tool to evaluate iron status is the measurement of bone marrow iron content which requires an invasive technique [25], serum ferritin and transferrin saturation are currently the main surrogate markers used in daily clinical practice for assessing iron status [26]. The TIBC reflects transferrin, the protein to which virtually all iron in the blood is bound. Transferrin may be elevated in the setting of inflammation and may be low in the setting of malnutrition and chronic disease because of decreased transferrin synthesis. There are significant (17-70%) diurnal fluctuation that make it difficult to interpret its value if the time of day at which the test is obtained varies from test to test [23]. In our study, we found no correlation between TIBC and bone marrow iron stores (p=0.0650). We, however, could not find any similar study correlating TIBC with bone marrow iron stores for comparison.

The prevalence of megaloblastic anemia due to nutritional deficiency in developing countries including India is increasing [13,27]. Nutritional deficiency is expected to be more common in vegetarians than in non-vegetarians. Refsum et al reported that 75% of selected urban population from India showed metabolic evidence of hyperhomocysteinemia and increase in serum methylmalonic acid, consistent with cobalamin deficiency which he attributed to vegetarian diet [27]. However, studies have shown that the non-vegetarians in developing countries have a vit B12 status that is only marginally better than that of lacto-vegetarians and only daily meat eaters have vit B12 status similar to that of the non-vegetarians in the West [28].

The tribal population of Meghalaya, which constitutes 85% of the total population in our study has a traditional method of food consumption which includes mainly rice and meat, fermented foods, green leafy vegetables and fruits on a daily basis. Dairy products and pulses are not part of daily diet. Alcohol, fermented beetle nut and tobacco are widely consumed by both men and women irrespective of urbanization, higher education and income [29].In some cases, the cause of nutritional anemia may be due to the possibility of B12 and folate malabsorption. Giardia infection has been demonstrated to cause folate malabsorption [30]. Similarly, H. pylori infection has been incriminated to cause malabsorption of vitamin B12 [31]. Since folate and vitamin B12 deficiencies are quite common among Indian population, these could be the major factors for megaloblastic anemia in our population.

All 58 cases showed megaloblastosis in the bone marrow. But only 25 cases (43.1%) had either low serum Vit B12 (n=6) or low folate (n=10)or both low vit B12 and folate (n=9). The rest 33 cases (56.89%) had either normal or high Vit B12 /Folate. So, there was a correlation between bone marrow findings and Vit B12/Folate in 43.1% cases only. This discrepancy maybe because most of our patients were hospitalized due to severe anemia for evaluation. Serum folate/VitB12 levels are known to be highly labile, to the extent that a low serum folate concentration can be rapidly normalized shortly after consumption of a single nutritious folate rich meal during hospitalization.

Our study showed isolated B12 deficiency in 10.34%, combined deficiency in 15.5% and isolated folate deficiency in 17.24% of the cases. The Indian series from 1965 shows that isolated B12 or combined deficiency was present in nearly 7% and 5% of the instances while folate deficiency accounted for nearly 55% of the cases [32]. The low percentage of isolated folate deficiency in our study could be due to exclusion of pregnant ladies from this study. However, Sarode et al from Chandigarh reported B12 deficiency in nearly 85% of the cases with megaloblastic anemia [14]. Clinically, it is important to know whether the anemia is due to folate or vit B12 or combined deficiency, so that the appropriate treatment may be given. However, as both these tests lack in sensitivity and specificity, it has always been stressed to consider these results in the context of clinical data [18].

CONCLUSION

Bone marrow aspiration though invasive can be easily performed even in the presence of severe thrombocytopenia with little or no risk of bleeding. It provides a cost effective investigation for confirming the diagnosis of dimorphic anemia by reliably assessing the iron stores. Among the biochemical parameters, serum ferritin is more specific in predicting the iron deficiency. Other biochemical parameters such as Vitamin B12 and serum folate are also specific but not sensitive and are not cost effective. Normal values should be interpreted in the clinical context as the values can vary even by a single vitamin rich diet, overcooking of food or a single dose of treatment. We found that TIBC is not a reliable indicator of iron deficiency and hence has no significance in the evaluation of anemia. Depending only on

biochemical parameters may mislead the clinicians. In a country like India, hematological parameters are still the most reliable and cost effective way of evaluating anemia, considering that nutritional anemia is the most common cause of anemia. Bone marrow aspiration may be reserved for those cases with severe anemia, bicytopenia or pancytopenia and refractory anemia, as it will add a lot of value in the further specific diagnosis and management.

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Author Information

Rizwan Athar
Postgraduate Student, Department of Pathology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences
Shillong, India

Yookarin Khonglah
Associate Professor Department of Pathology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences
Shillong, India

Vandana Raphael
Professor & Head of Department Department of Pathology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences
Shillong, India

Arnab Pal
Ex-Assistant Professor (Presently Assistant Professor in PGIMER, Chandigarh) Department of Biochemistry, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences
Shillong, India

Kyrshanlang Giri Lynrah
Assistant Professor Department of Medicine, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences
Shillong, India