Fetal Hemoglobin, Serum Total Cholesterol, Plasma Phosphate and Serum Calcium Levels in Patients with Leukemia and Lymphoma

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In this study, fetal hemoglobin (HbF), blood total cholesterol, phosphate and calcium levels of leukemia and lymphoma cases in Mandalay were determined. It was a cross-sectional, comparative study. Thirty adult cases of leukemia and lymphoma and equal number of control subjects of comparable age (14 to 80 yrs) and sex were studied. Mean HbF of leukemia group (n=9), acute myeloid leukemia (AML) subgroup (n=6) and controls (n=30) were 2.72±0.47%, 3.17±0.69% and 1.39±0.25% of total Hb, respectively. The HbF levels of leukemia group and AML were significantly higher than that of controls ($p<0.02$). Mean serum total cholesterol levels of leukemia group, AML subgroup and controls were found to be 126.5±17.38 mg%, 137.96±24.66 mg% and 177.18±7.68 mg%, respectively. Cholesterol levels of leukemia group and AML were lower than that of controls. Mean plasma phosphate levels of leukemia and lymphoma cases (n=30), leukemia group (n=9) and lymphoma group (n=21) were 1.21±0.07 mmol/l, 1.33±0.17 mmol/l and 1.15±0.06 mmol/l, respectively. Mean phosphate level of controls was 0.94 mmol/l. Plasma phosphate levels of the whole cases and individual case groups were significantly higher than that of controls ($p<0.001$). Mean serum calcium levels of the whole cases, leukemia group and lymphoma group were 10.16±0.36 mg%, 10.03±0.75 mg% and 10.21±0.45 mg%, respectively. Mean serum calcium level of controls was 8.55±0.14 mg%. Serum calcium levels of the whole cases and individual case groups were found to be significantly higher than that of controls ($p<0.01$). The study showed that not only raised HbF but also hypocholesterolaemia might be the diagnostic clues in leukemia cases. Recognition of blood phosphate and calcium changes leads to appropriate therapy and a reduction of morbidity.

Keywords: Leukemia and lymphoma, Fetal hemoglobin, Cholesterol, Phosphate, Calcium levels

INTRODUCTION

Leukemia and lymphoma are not uncommon malignancies. Leukemia accounts for about 4% of all deaths from malignant disease although the proportion is greater in childhood.[1] Leukemia and lymphoma are equally frequent.[2] The adult level of fetal haemoglobin (HbF) is about 1%. Only 4% of normal adults have greater than 1% HbF.[3] HbF levels are abnormally high in some patients with leukemia. The highest levels, up to 76% HbF, are found in patients with the infantile form of chronic myelogenous leukemia and in patients with erythro-leukemia. Patients with all forms of hematologic malignancy may have modest elevations of HbF.[2] In a study in Romania, serum cholesterol and apoprotein B levels as well as serum cholinesterase activity were

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decreased in patients with acute leukemia, and the lowest levels being associated with the worst prognosis.[4]

Severe hypophosphatemia may occur during the blast crisis of malignant lymphoma. This has been attributed to excessive phosphate retention by cells, and is reversed with decline of the white cell count with treatment, when serum phosphate increases and cellular phosphate decreases. Low serum phosphate concentrations may occur in various forms of leukemia.[5]

When there is osteolysis as in NHL, adult T-Cell leukemia/lymphoma and plasma cell leukemia, hypercalcemia is very frequent. In CML, hypercalcemia usually associated with bone lesions, has been described occasionally. Hypercalcemia is seen in Hodgkin disease only when there is increased synthesis of 1, 25-dihydroxy vitamin D by Hodgkin disease tissue.[2] Study of blood cholesterol, phosphate and calcium levels in leukemia and lymphoma cases had not been done in Myanmar yet. This study aimed to determine fetal haemoglobin, total cholesterol, phosphate and calcium levels in patients with leukemia and lymphoma. The above biochemical parameters of the cases were compared with those of normal healthy subjects.

MATERIALS AND METHODS

An observational cross-sectional, comparative study was conducted. A total of 30 newly diagnosed and untreated cases of leukemia and lymphoma attended the Mandalay General Hospital and other hospitals as well as private clinics in Mandalay were studied. The diagnosis of the patients was done at Clinical Pathology Department, Mandalay General Hospital.

In this study, already treated cases, cases of leukemia and lymphoma within one week after blood transfusion and childhood (i.e under 14 years of age) leukemia and lymphoma cases were excluded. Equal number of control subjects of the comparable age and sex were also studied after taking informed consent. Five milliliters of blood were drawn from each case. Three milliliters were put in sterile plain bottle and 2 ml were put in the bottle containing ethylene diamine tetraacetic acid (EDTA) anticoagulant.

The plasma was promptly separated from the red cells and determination of plasma phosphate was done as quickly as possible to obtain actual values. After half an hour, when blood clot was retracted in sterile bottle, serum was decanted and centrifuged at 2000 rpm for 5 minutes to sediment the red blood cells. When serum was obtained, determination of cholesterol was done promptly.

HbF was determined by method of Singer, cited in Toro and Ackermann, 1975.[6] Principle of the method is based upon property of HbF to be more resistant to denaturation by alkali than other haemoglobins. The haemoglobin is subjected to the action of alkali for a timed period and then ammonium sulphate is added which precipitates the denatured haemoglobins not the HbF. The amount of haemoglobin in the filtrate as compared with the amount in the original sample represents the proportion of the HbF. Two milliliters of blood put in EDTA containing bottle were prepared to get haemolysate. The blood was centrifuged to get packed blood cells. Packed cells were washed three times with equal volume of normal saline by gently inversion and centrifugation. After final wash, water was added to packed cells and well shaken for 2 minutes. The resultant haemolysate was proceeded with Singer's method. Spectrophotometry was done at 540 nm.

Determination of serum total cholesterol was done by using direct method of Toro and Ackermann, 1975.[6] This method uses the Liebermann-Burchard reaction. Cholesterol reacts with strong concentrated acids as typical alcohol, and the products are colouredsubstances, chiefly cholestapolyenes and cholestapolyene carbonium ions. Acetic acid and acetic anhydride are used as solvents and dehydrating agents and

sulfuric acid is used as dehydrating and oxidizing reagent. Such reagent first moves a molecule of water, then oxidize the intermediate to produce 3, 5 cholestadiene. Sulfuric acid is converted to sulfurdioxide. The cholestadiene reacts further to form cholestapolyene carboniumions. The stability of these cations is dependent on the sulfuric acid concentration. The polyenes are the main chromophores and will obtain a green colour due to a cholestapolyene sulfonic acid. Spectrophotometry was done at 625 nm.

Determination of plasma phosphate was done by method of Delsal and Manhouri, cited in Wootton and Freeman, 1982.[6] This method is a variant of the phosphomolybdic acid reaction and is suitable for small batches of samples. A substituted phenol is used as a reducing agent and the pH is controlled by an acetate buffer. Copper in the buffer hastens colour development which is complete after 5 minutes. The blue colour is stable for at least 30 minutes. Spectrophotometry was done at 880 nm.

Determination of serum calcium was done by chloranilate method, cited in Toro and Ackermann.[7] Calcium was determined by precipitation as chloranilate. After washing of the precipitate to remove excess reagent, the precipitate was dissolved in EDTA solution and the colour of the chloranilate was determined. Spectrophotometry was done at 525 nm.

Results were presented as Mean±SEM. Differences in means between groups were analyzed by unpaired 't' test. Correlation statistics between variables were assessed by calculating the Pearson's coefficient. Level of significance was set at $p<0.05$.

Ethical consideration

Before collection of blood samples, explanation was given in detail to cases and controls. Written informed consents were obtained. Confidentiality was maintained. Approval was obtained from the Post-graduate Academic Board of Studies, University of Medicine, Mandalay.

RESULTS

A total of 30 patients with leukemia and lymphoma and 30 apparently normal subjects of same age and sex were studied in this study. Out of 30 patients, 9 were cases of leukemia and the remaining 21 were of lymphoma. Mean HbF levels of overall cases and controls were 1.95±0.19% and 1.39±0.25%, respectively. Mean HbF was higher than that of controls but it was not statistically significant (p=0.1).

HbF levels of lymphoma group and NHL sub-group were higher than that of controls. However, it was not statistically significant (p=0.4) (Table 1). HbF level of AML sub-group was significantly higher than that of controls ($p<0.05$).

Table 1. Comparison of mean HbF values of leukemia group and lymphoma group with that of controls

Subjects	Sample size	Mean±SEM (% of total Hb)	t value	p value
Leukemia group	9	2.72±0.47	2.48	<0.02*
Lymphoma group	21	1.63±0.14	0.72	0.4
Controls	30	1.39±0.25		

*=Statistically significant

Mean serum cholesterol levels of the whole cases and controls were 173.01±13.78 mg% and 177.18±7.68 mg%, respectively. Although serum total cholesterol level of the whole leukemia and lymphoma cases was lower than that of controls, it was not statistically significant.

Table 2. Comparison of mean serum total cholesterol values of leukemia group and lymphoma group with that of controls

Subjects	Sample size	Mean±SEM (mg%)	t value	p value
Leukemia group	9	126.5±17.38	-2.96	<0.01*
Lymphoma group	21	192.94±17.6	0.9	0.3
Controls	30	177.18±7.68		

*=Statistically significant

Serum total cholesterol of leukemia group was found to be significantly lower than that of controls ($p<0.02$). Serum total cholesterol levels of lymphoma group, AML subgroup and NHL subgroup were higher than that of controls but it was not statistically significant ($p>0.05$) (Table 2).

Mean plasma phosphate levels of the whole cases and controls were 1.21±0.07 mmol/l and 0.94±0.03 mmol/l, respectively. Mean plasma phosphate levels of each case group namely leukemia group, lymphoma group, AML subgroup and NHL subgroup were found to be significantly higher than that of controls ($p<0.001$) (Table 3).

Table 3. Comparison of mean plasma phosphate levels of leukemia group and lymphoma group with that of controls

Subjects	Sample size	Mean±SEM (mmol/l)	t value	p value
Leukemia group	9	1.33±0.17	3.66	<0.001**
Lymphoma group	21	1.15±0.06	3.35	<0.001**
Controls	30	0.94±0.03		

**=Highly significant

Mean serum calcium levels of the whole cases and controls were 10.16±0.36 mg% and 8.55±0.14 mg%, respectively. Mean calcium level of the whole cases was significantly higher than that of controls ($p<0.001$). All serum calcium levels of case groups were found to be significantly higher than that of controls ($p<0.01$) (Table 4).

Table 4. Comparison of mean serum calcium levels of leukemia group and lymphoma group with that of controls

Subjects	Sample size	Mean±SEM (mg%)	t value	p value
Leukemia group	9	10.03±0.75	3.14	<0.01*
Lymphoma group	21	I0.21±0.45	4.02	<0.001**
Controls	30	8.55±0.14		

*=Statistically significant, **=Highly significant

The HbF and serum total cholesterol value in the whole leukemia and lymphoma was not significantly correlated ($r=-0.21$, $p=0.2$). The HbF and serum total cholesterol value in leukemia group was not significantly correlated ($r=0.47$, $p=0.1$). The correlation between HbF and serum total cholesterol value in the AML subgroup was not significant ($r=0.33$, $p=0.3$).

DISCUSSION

In the present study, HbF levels of leukemia group and AML subgroup were significantly higher than that of controls. The HbF concentration of a subject diagnosed as erythroleukemia was 1.8%. The level was not an elevated one when compared with the results of other leukemias. In a study in Georgia,[8] it was stated that while slight to moderate elevation of HbF in leukemia was common, elevation of HbF to greater than 25% was rare except in erythroleukemia (French-American-British group, FAB classification-AML M6) and CML of childhood (juvenile chronic myelogenous leukemia).

In a study in Canada,[9] HbF and HbA_2 were estimated in 35 patients with malignant haematological disorders. The report of these authors was that in 9 out of 10 patients with AML, the HbF was greater than 2%. In the present study, the serum total cholesterol level of overall cases was not significantly different from that of controls. However, the level of leukemia group was significantly lower than that of controls. The serum cholesterol level of a patient with AML M1 (FAB classification-without maturation type) was found to be 38.3 mg% and it was marked hypocholesterolaemia. It agreed with result of a study done in Egypt.[10] Serum total cholesterol levels of two patients diagnosed as ALL were 123.1 mg% and 117.9 mg%, respectively. These results were noticeably lower than the mean value of normal subjects.

In this study, it was expected to show raised HbF and low cholesterol level as bad prognostic factors. Survival time could not be noted in this study since it was a cross-sectional study design. However, previous studies describes that acute leukemia and some subtypes of acute leukemia namely AML FAB M5, M6 type (FAB classification-monoblastic or monocytic type, erythroleukemia) and acute lymphoblastic leukemia FAB L3 (FAB classification-vacuolated blasts, basophilic cytoplasm) types can be regarded as having adverse prognosis. Findings in these subtypes could be compared with findings in other types of this study. HbF concentration of an erythroleukemia case (the only case) in this study was 1.8%. The level was not an elevated one when compared with the results of other types of leukemia. Any acute

lymphoblastic leukemia FAB L3 case was not investigated in this study. Limited sample size precluded this study getting enough case number for subtypes.

Although low plasma phosphate levels were expected to be found in this study, all plasma phosphate levels of the whole cases and subgroups were seen to be significantly higher than that of controls. The mechanism of elevation of plasma phosphate level in cases may be explained by one type of malignancy-associated hypercalcemia having hypercalcemia, normophosphataemia/hyperphosphataemia, and decreased nephrogenic AMP (local osteolysis).[11]

Treatment of malignancy may result in hyperphosphatemia due to cell death and release of intracellular phosphorus. Thus, phosphate therapy for hypophosphatemia occurring in the context of malignancy may lead to the enhancement of hyperphosphatemia after anticancer treatment.[12] In this regard, serum phosphorous concentrations should be closely monitored in patients with acute leukemia, the blast crisis of chronic myeloblastic leukemia and malignant lymphoma.

This study showed mean serum calcium levels of the overall cases and each case group were significantly higher than that of controls. The cause of elevated serum calcium level with concomitant elevated phosphate level in cases in this study may be invasion of bone by the tumor and release of phosphate as well as calcium.

In a study in United States,[13] it was stated that hypercalcemia was an ominous sign, because the median survival of acute leukemia patients was 3 months after hypercalcemia and chronic leukemia patients had a median survival of 15 days after hypercalcemia.

Conclusion

In conclusion, raised HbF might be a diagnostic clue in leukemia cases especially in AML. Hypocholesterolaemia might be a diagnostic clue in leukemia cases. Plasma phosphate changes should be recognized in leukemia and lymphoma cases as hypo- or hyperphosphataemia can occur at various conditions.

Recognition of hypercalcemia in leukemia and lymphoma cases is also needed and treatment of that condition may be advantageous in patient's wellbeing.

Competing interests

The authors declare that they have no competing interests.

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