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Research Article

Demographic and hematologic pattern of acute myeloid leukemia patients from lower Punjab area of Pakistan

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ABSTRACT

Purpose of this study was to evaluate the demographic and hematologic pattern of acute myeloid leukemia patients from lower Punjab area. Treatment of the acute myeloid leukemia patients is related with the unsatisfactory rates of complete responses that usually as short lived. At the relapse of leukemia patients the evaluation of investigational treatment strategies, therapeutic decision making by clinically useful prognostic index. All the hematological techniques were applied to increase the life expectancy of the leukemia patients. The study concluded that acute myeloid leukemia was prevalent in local population especially in males as compared to females. All the patients had varied complete blood count (CBC) profiles. The hematological tests are used to characterize the disease for its management and treatment to increase the life expectancy of the patients.

Keywords: Complete blood count, red blood cells, white blood cells, platelets, haematocrit, haemoglobin.

INTRODUCTION:

Leukemia is cancer of the blood cells and develops due to uncontrolled division of white blood cells. The white blood cells produced in bone marrow help the body to fight against infections. Leukemia disease starts with abnormal production of white blood cells called leukemia. These cells grow faster than normal cells but do not work like normal cells. Leukemia cells accumulation in the body and causes serious physiological problems like infections, bleeding, and anemia. These leukemia cells can spread to the body organs and cause swelling or pain. There are four main types of leukemia, acute lymphoblastic leukemia (ALL) acute myelogenous leukemia (AML) chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML). CLL and AML are the most common types of leukemia in adults while ALL is the most common leukemia in children (Colvin and Elfenbein, 2003).

ALL is more common in male as compared to the female. The people with down syndrome, bloom syndrome, X-linked agammaglobulinemia, anemia, ataxia telangiectasia and Severe combined immunodeficiency have high risk of this disease. Leukemia is also linked with pregnancy. Acute leukemia needs punctual and violent treatment, in spite of birth defects and large risks of pregnancy loss (Shapira and Pereg, 2008). High incidences of cancer are found in Khyber Pakhtoon Khwa province of Pakistan. This province has districts of low socioeconomic status such as those in Dir.

The human T-cell ALL support chromosomal translocation which involves NOTCHI, gene having transmembrane receptors that regulate the development of normal T cell. It is reported that 50% human T-ALL have activated mutations which have C-terminal PEST domain and extracellular heterodimerization domain of NOTCHI. In human T-ALL the activated NOCHI greatly expand its role in molecular pathogenesis (Andrew *et al.*, 2004). Acute lymphoblastic leukemia caused by chromosome

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translocation which causes uncontrolled growth of cells by increasing chemical signals and spread throughout the body. Damage can cause by the fusion of the genes and the deregulation of the promoter of another gene. This damage may be caused by environmental factors such as chemicals, drugs or radiation (Stiller *et al*., 1994; Stiller and Eatock, 1999).

Most of the acute lymphoblastic leukemia patients can have different types of treatments which depend upon the type of malignancy. Chemotherapy is the initial treatment of choice cytotoxic chemotherapy for acute lymphoblastic leukemia combines with various leukemic drugs. Chemotherapy for acute lymphoblastic leukemia consists of three phases: remission induction, intensification, and maintenance therapy. The chemotherapy regimens can be intensive and prolonged. Many patients have an intravenous catheter inserted into a large vein a cone-shaped port with a silicone nose that is surgically planted under the skin, usually near the collar bone, and the most effective product available, due to low infection risks (Messinger *et al*., 2012). For the better tolerance in pediatric patient's combination of prednisolone or dexamethasone, vincristine, asperginase, is used to induce remission. By irradiation of cytarabine and methotrexate central nervous system prophylaxis can achieved (Cortes *et al.,* 2010).

Radiation therapy (or radiotherapy) is used as a bone marrow transplant on painful bony areas, in case of serious disease. For the prevention of recurrence of leukemia in central nervous system prophylaxis radiations can be used for the treatment. Radiations therapy used as a common method in the treatment of whole brain prophylaxis of children with acute lymphoblastic leukemia. CNS chemotherapy provided favorable results with less developmental of side-effects. The whole-brain radiation has been more limited because most specialists in adult leukemia have been using intrathecal chemotherapy and have deserted the use of radiation therapy for CNS prophylaxis (Harrison, 2004).

In leukemia, chemotherapy mainly includes steroids, methotrexate and cranial irradiation are integrated within possible etiological factors for osteoporosis. Low levels of physical activity, pitiable nutritional eating and abridged vitamin D levels may have an impact on the development of bones in these children. When compared with the normal population the survivors of other malignancies increase of fat in body composition in survivors of ALL (Warner *et al*., 1997 and Nysom et al 1998). The effect of chemotherapy on bone health is much less clear. The children who have been treated with chemotherapy alone have shown normal backbone (Gilsanz *et al*., 1990) and reduced BMD femur and spine (Arikoski *et al*., 1999; Nysom *et al*., 1998).

Transfusion-related immune-modulation (TRIM) may affect the transfusions of acute lymphoblastic leukemia outcomes and most of the children with ALL receive blood transfusions. In renal transplantation studies reported the probable useful effect of TRIM to improve renal graft survival Opelz G. Then the unfavorable effects of TRIM were studied to show contribute to cancer growth, infections, and postoperative mortality (Landers, 1996).

Purpose of this study was to evaluate the demographic and hematologic pattern of acute myeloid leukemia patients from lower Punjab area of Pakistan.

METERIALS AND METHODS

The research work was conducted at Children's Hospital and Institute of Child Health, Multan. The blood samples of patients were collected from oncology department of The Children's Hospital & the Institute of Child Health, Multan facilitated by Dr. M. Zulfiqar Ali. The research work was conducted at Research laboratory, Department of applied chemistry and biochemistry, Government College University of Faisalabad.

Study Design: Study was designed to evaluate the effect of leukemia, chemotherapy and blood transfusion on biochemical profiles of the patients.

Inclusion Criteria: The Subjects fulfil the following criteria were included in the study age 2.5 to 14 years. The range of weight should be 11 to 56 kg. Physical examination, medical history and screening tests found to be normal by the investigator. The Subjects must give their written informed consent to participate in the study.

Physical examinations: Demographic data of the patients like age, body weight, body temperature, blood pressure, before the start of study.

SAMPLE COLLECTION AND HANDLING

Collection of blood samples: Five ml blood sample was collected from the 20 patients that were used for CBC report.

Complete blood count (CBC): Complete blood count of each patient including white blood cells (WBCs), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), red blood cells (RBCs), mean cell volume (MCV), platelets and hemoglobin (Hb) level was examined through hematologic analyser Sysmex XP-100 (Germany).

PROCEDURE

Haemoglobin in blood

The Drabkin Solution (5ml) was taken in test tube. 20ul of blood was added in this tube and wait for 10 mints. The haemoglobin and mate haemoglobin present in the blood was converted into synmate haemoglobin. The reading was noted with the help of spectrophotometer 546 nm against reagent blank. The concentration of haemoglobin was determined with the help of formula or graph.

$$Hb\% = \frac{Abs\ of\ sample - Abs\ of\ blank}{Abs\ of\ standard - Abs\ of\ blank} \times Conc.of\ standard \quad (1)$$

Red Blood Cells

The area of neubar's chambers was focus under the power of lens 10. The drop of blood was taken in test tube. The RBC pipette was taken and suck the blood up to point 0.5 by holding the pipette horizontally and touching the tip of pipette to the drop of blood. The blood was wiped from the tip of the pipette and sucks the haymes fluid to the point 101 and made the dilution up to 200. Solution was mixed by folding the rubber tube and rolling the pipette in the palm. Two or three drops from the pipette was discarded and allowed the next drop to appear at the top of the pipette. Then touch the drop at the edge of cover slip. Then Wait for few minutes for RBC settle down. Now count the number of RBC in the 4 corner chambers and 1 of the central chamber out of 25 chambers. Calculate the total number of RBCs with the help of following formula:

$$RBCs = \frac{No.of\ cells \times Dilution\ factor}{Volume} \quad (2)$$

White Blood Cells

The area of neubar's chambers was focus under the power of lens 10. The drop of blood was taken in test tube. The WBCs pipette was taken and the blood was sucked up to point 0.5 by holding the pipette horizontally and touching the tip of pipette to the drop of blood. The blood was wiped from the tip of the pipette and sucks the turck soln. to the point 101 and made the dilution up to 200. Solution was mixed by folding the rubber tube and rolling the pipette in the palm. Two or three drops from the pipette were discarded and allow the next drop to appear at the top of the pipette. Then touch the drop at the edge of cover slip. Now wait for few minutes for WBCs settle down. Now count the number of WBC in the 4 corner large chambers of haemocytometer. Calculate the total number of WBCs with the help of following formula:

$$WBCs = \frac{No.\ of\ cells \times Dilution\ factor}{Volume\ of\ blood} \quad (3)$$

Platelets

The area of neubar's chambers was focus under the power of lens 10. The drop of blood was taken in test tube. The PLTs pipette was took and suck the blood to point 0.5 by holding the pipette horizontally and touching the tip of pipette to the drop of blood. The blood was taken from the tip of the pipette and sucks the ammonium oxalate up to the point 101 and made the dilution 200. Solution was mixed by folding the rubber tube and rolling the pipette in the palm. Two or three drops from the pipette were discarded and allow the next drop to appear at the top of the pipette. Then touch the drop at the edge of cover slip. Wait for few minutes for PLTs settle down. Now count the number of PLTs was counted in all the chambers of neubar's chamber. Number of PLTs was counted with the help of following formula:

$$PLTs = \frac{No.of\ cells \times Dilution\ factor}{Volume} \quad (4)$$

Haematocrit: The blood was taken in the HCT test tube up to 100µl mark. HCT tube was

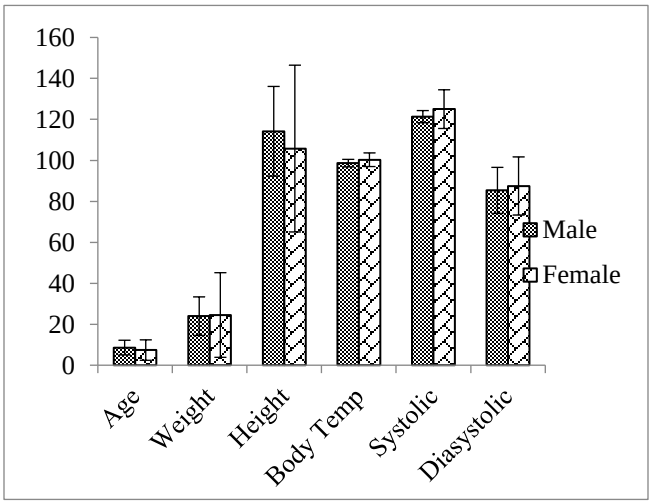

Figure 1: Demographic data of leukemia patients. The data is mean of 15 values for males and 4 for females.

centrifuged at 20,000 rpm for 10-15 mints. Then read the HCT with the help HCT tube grading.

Mean Cell Volume: The mean cell volume was calculated with the following formula:

$$MCV = \frac{hematocrit}{RBCs} \quad (5)$$

Mean Cell Haemoglobin: The mean cell haemoglobin was calculated with the following formula:

$$MCH = \frac{Haemoglobin}{RBCs} \quad (6)$$

Mean Cell Haemoglobin Concentration: The mean cell haemoglobin concentration was calculated with the following formula:

$$MCHC = \frac{Haemoglobin}{Hematocrit} \quad (7)$$

Differential Leukocyte Count: 10µl whole blood was taken on a slide, prepares blood film and dried it. Then fix the film with the help of methanol and dry it. The blood film was stained by using Giemsa stain. Then observed under microscope on oil emulsion lens (100X). Count the 100 WBCs and calculate percentage of each lymphocyte, monocyte and granulocyte. All the blood indices taken in duplicate and mean was calculated the data was tabulated and represented in the form of graph.

RESULTS

The study described effect of leukemia and commonly used drugs methotrexate and mercaptopurine on clinical profiles of leukemia patients. The data of hematological and biochemical parameters of patients was shown.

Demographic Data

The leukemic patients enrolled in the study were selected from different localities and populations. Fifteen male and four female patients belonged to Multan and different localities. Demographic data of each patient was recorded. The average age of leukemia patients was 8.34 years. Fifteen male and four female leukemic patients fell in range of 2.5 to 14 years. The patients were falling in childhood group. The average body weight of leukemia patients was 24.05 kg ranging from 14-56 kg. The average height of leukemia patients was 112.42 cm ranging from 60 to 162cm as shown in Figure 1.

Table 1: Socio-demographic characteristics of leukemia patients Parameters**.**

Parameters	No of patients.(n=19)
Residence	
Rural	12
Urban	7
Multan	7
Kotaddu	1
Rajanpur	1
D.G.khan	2
Muzaffar Ghhar	2
Liyyah	2
Socioeconomic status	
Upper	2
Middle	4
Lower	13
Education	
Literate	7
Illiterate	12
Awareness about disease	19
Behavioral problems	3
Physical symptoms	
Diarrhea	19
Fever	19
Nausea	19
Allopia	19
Mylo	19
Consanguinity	14
Blood group	
A+	6
A-	1
B+	2
B-	1
AB+	3
O+	6
Transfusion Twice a month	4
Thrice a month	2
Transfusion four time in month	7
No Transfusion	4

Table 1 descried the data of the leukemic patients. This table described about the patient from different localities either urban or ruler. The data also demonstrate the socioeconomic status, education, awareness about disease, behavioural problems. Physical symptoms, consanguinity, blood group of the patients and rate of transfusion in month.

Haematological Parameters

The blood parameters are important in the study of leukemia. Complete blood count (CBC) reflects the overall health status and development of leukemia in the patients and helps future plan of transfusion and chemotherapy. The overall haematological data of leukemia patients male and female are presented in Figures 2-6

White blood cells

The mean ± standard deviation (SD) value of white blood cells (WBCs) in 19 leukemic patients was $5.42\times10^3 \pm 3.062\times10^3/\mu L$. The mean ± SD value of WBCs in male was $5.35\times10^3 \pm 2.43\times10^3$ /μL. The mean ± SD value of WBCs in female was 5.67×10^3 ±5.34v/μL. The mean value of WBC was higher in leukemic patient than normal people (4-$11\times10^3/\mu L$). This may be due overall diseased condition and hyper-activity of immune system of the patients receiving blood from different donors. This is also evident from the prevalence of fever immediately after blood transfusion.

Haematocrit: Hematocrit is also known as packed cell volume (PCV). The mean ± SD value of hematocrit for all leukemia patients was 31.05 ± 5.71% ranging from 20.5 to 40.7%. The HCT value in leukemia patients was lower than reference value of normal people. HCT value of male leukemia patients was slightly higher than female leukemia patients. The mean ± SD value of hematocrit in male was 28.47 ± 5.09% ranging from 20.5 to 40.7% while in female was 34.53 ± 3.33% ranging from 21 to 39.4% as compared to 28.5 ± 2.2% reported by Jung *et al.* (2010).

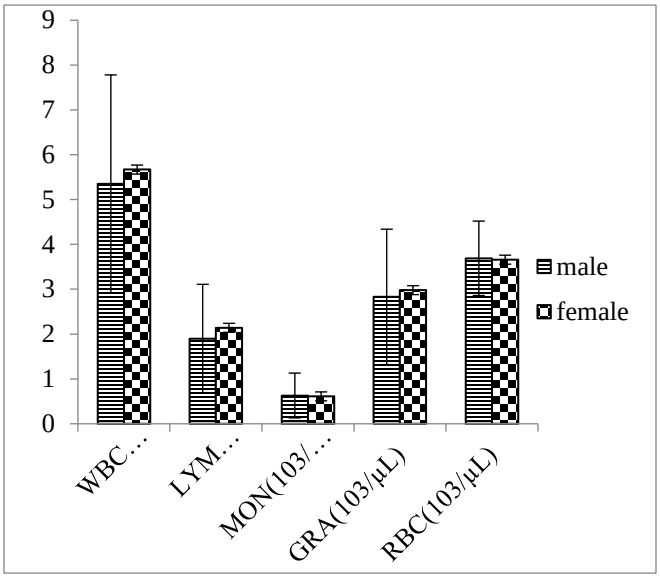

Figure 2: The comparative graph of complete blood count of the subjects. WBCs: White blood cells, LYM: Lymphocyte, MON: Monocyte, RBCs: Red blood cells.

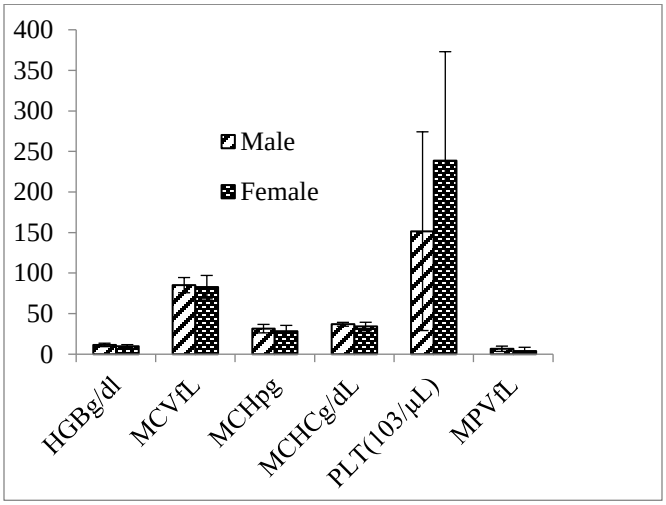

Figure 3: The graphical representation of complete blood count in male (n=15) and female (n=4) leukemic patients. HB: Hemoglobin MCV: Mean cell volume, MCHC: mean corpuscular hemoglobin concentration; PLT: Platelets; and MPV: Mean plate volume.

Mean corpuscular hemoglobin concentration: The mean corpuscular hemoglobin concentration (MCHC) of all leukemia patients was 36.37±3.08 g/dL. The mean corpuscular hemoglobin concentration of male patients was found 36.91±2.40 g/dL. The mean corpuscular hemoglobin concentration of female leukemia patients was 28.45±7.10 g/dL.

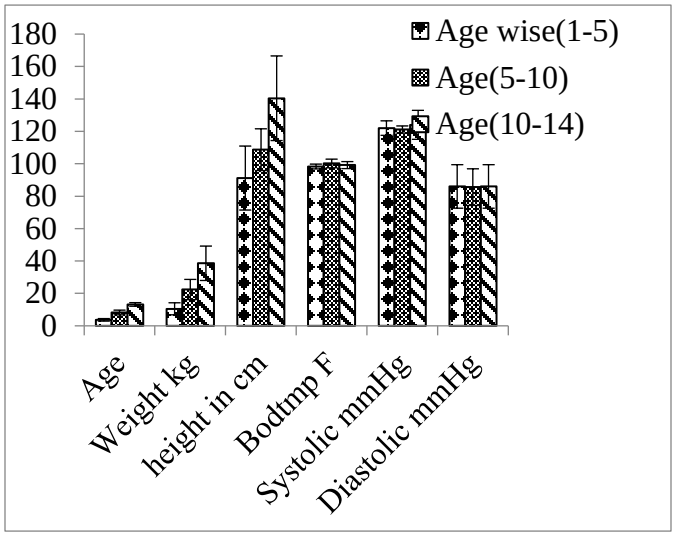

Figure 4: The graphical representations of the demographic data. The data was divided into three groups on the basis of age such as (1-5), (5-10), (10-14). On the basis of age, weight, height in cm, body temperature, systolic and diasystolic compared these three groups.

Mean corpuscular hemoglobin: The mean corpuscular hemoglobin level of 19 leukemia patients was 29± 5 pg which ranged from 30.87±5.26 pg. The mean corpuscular volume of male was found 31.52±5.24 pg. The mean corpuscular volume of female was 28.45±7.10 pg

Red blood cells: The average number of red blood cells (RBCs) in all of the leukemia patients was $3.69\times10^6\pm0.8\times10^6$1 µL. The mean value RBCs was lowers than normal ($4.2\text{-}6\times10^6$/µL). The lower level of red blood cells is due to their degradation as a result of abnormal globins molecule. The cells can't reach maturation. The amount of RBCs in male was $3.69\times10^6 \pm 0.83\times10^6$/µL, while in female; it was $3.66\times10^6 \pm 1. \times10^6$ /µL.

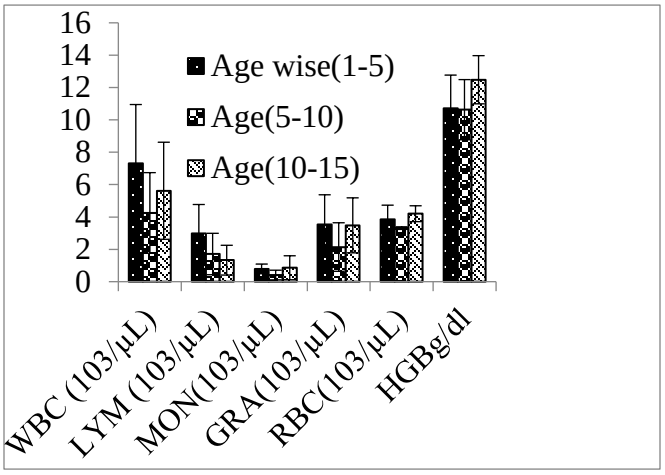

Figure 5: The comparative graph of complete blood count. The age group (1-5), (5-10), (10-15) years leukemic subjects WBCs: White blood cells, LYM: Lymphocyte, MON: Monocyte, RBCs: Red blood cells and HB: Hemoglobin.

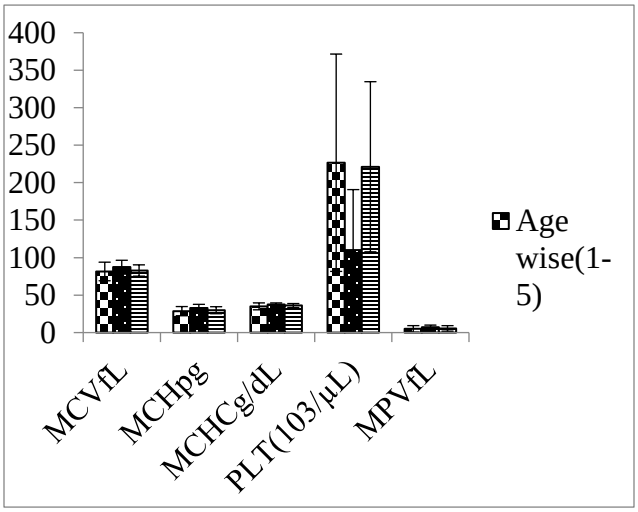

Figure 6: The comparative graph of complete blood count. The age group (1-5), (5-10), (10-15) year's leukemic subjects. MCV: mean corpuscular volume PLT: Platelets MCHC: Mean corpuscular hemoglobin concentration, MPV: Mean plate volume MCH: Mean corpuscular hemoglobin.

Hemoglobin level: The leukemia patients (n=19) had a large variation in hemoglobin values, amount of Hb in all the patients (n=19) was 11.14±1.91g/dl). The mean value of Hb in male (n=15) was 11.46±2.13 g/dl. The mean value of Hb in female (n=4) patients was 9.95±1.87 g/dl. The average value of Hb is much lower than normal value. The defects in hemoglobin synthesis are the major problems with all the patients of leukemia. The average value of Hb in female was lower than male leukemic patients

Platelets count: The platelets count of 19 leukemia patients was $170\times10^3 \pm 117.62\times10^3/\mu L$. The mean ± SD value of platelets count in male was $151\times10^3 \pm 122\times10^3/\mu L$. The mean ± SD value of platelet count in female was $238\times10^3 \pm 134\times10^3/\mu L$.

Mean corpuscular volume: The mean corpuscular volume (MCV) of 19 leukemia patients was found 84.58±9.57fL. The mean corpuscular volume of male was found 85.15±9.38fL. The mean corpuscular volume of female was 82.68±14.44fL.

Red blood cell distribution width: The red blood cell distribution width (RDW) of 19 leukemia patients was 18.11±3.96%. The mean ± SD value of red blood cell distribution width in male was 18.22±4.38%. The mean ± SD value of red blood cell distribution width in female was 17.7±5.18%. The average value of RDW in all leukemia patients was much higher than normal subject.

DISCUSSION

The study was conducted on 19 patients of acute myeloid leukemia and their CBC report was taken. The patient's data suggested that the disease is common in the local population. The mean age of the evaluable patients was 8.34 years range 2.5-14 and means WBC at the diagnosis was 5.42 ± 3.06 $10^3/\mu L$ ranges from 0.69-12.7 and their reference value was 4-11 $10^3/\mu L$ (Bushra *et al*., 2013). The amount of WBC in leukemia patient is very high. The amount of white blood cells increase in the body but these cells cannot defend against the disease. Number of cells increases uncontrolled division of cells which causes leukemia cancer. One female patient died at the end of study due to complications of leukemia. The mean age of the evaluable patients was reported 4.7 (range 0.0 to 18.0) years and the mean WBC at diagnosis was

10,100/pL (range 350 to 1, 05,000 /pL) (Lampert *et al.,* 1994).

The average haemoglobin level in the patients was 11.14±1.91 g/dl (range 6.7-14.1) g/dl and reference value 12-16 g/dl. The hemoglobin level in the leukemia patients is approximately adequate as compared to the reference value. The averages mean count volume in the patients was 84.58±9.57fl (range 61.1-102) and the reference value 76-96 fl. The mean count value in the leukemia patient was normal. The average platelets in the patient's body were 170±117.62 10^3/µL and (average 24-415) 10^3/µL and reference value of platelets 150-400 10^3/µL. The amount of platelets in leukemia patients is normal when compared to the reference value (Bushra *et al*., 2013). Zsofla *et al*., 2006 reported CBC of leukemia children. Of the 11 subjects with a markedly abnormal laboratory value (MCV 110m3, hemoglobin 9g/dL, platelets100, 000/m3).

It is suggested that the mean value of the RBCs in leukemia patients was 3.69±0.81 10^6/µL (range 2.11-5.29) and the reference value of the RBCs 4.2-6 10^6/µL. Uncontrolled division over burden the cells due to which many components cannot be synthesized in due to this reason this RBCs value in leukemia patients is low. Beverly and Katherine 2003 reported that the mean value of RBCs in leukemia patients was 4.2-6.2 million/ µL in male and 4.2-5.4 million/ µL in female patients (Bushra *et al*., 2013).

The mean corpuscular hemoglobin concentration (MCHC) in the ALL patient was 36.37±3.08g/dl (range 28.4-40.1) and the reference value was 32-36 g/dl. The Mean corpuscular hemoglobin (MCH) in the patients was 30.87±5.26 Pg (range 19.5-40.3) and the reference values 26-36 Pg (Bushra et al 2013). Beverly and Katherine 2003 reported that the mean value of the (MCHC) in the leukemia patients was 31-38%. This report also demonstrates that the comparison graphs on the basis of the age groups of male and female leukemia patients. The age group was (1-5) (5-10) (10-15), in these comparison graphs the values of the age, weight, height, body temperature and blood pressure. The comparison graphs also demonstrate about the CBC report of the leukemia. These graphs have different values of WBC, RBC, LYM, MON, PLT, HB, MCH, MCHC, on the basis of their age groups. Insomnia is considered as a symptom of difficulty in starting and maintaining sleep and usually associated with a day time efforts (Roth and Roehrs, *2003*). Among sleep-related complaint insomnia is believed, after pain, the most common overall complaint. Studies have indicated that if insomnia is not addressed appropriately it can cause significant morbidity. Moreover, the use of sleep inducer, benzodiazepines, in the control of insomnia had been shown associated with certain troubles including dependence and addiction, withdrawal symptoms and drug resistance (Hidderman, 1991).

Conclusion

The study concluded that acute myeloid leukemia was prevalent in local population especially in males as compared to females. All the patients had varied CBCs profiles. The haematological tests are used to characterize the disease for its management and treatment to increase the life expectancy of the patientsInsomnia is considered as a symptom of difficulty in starting and maintaining sleep and usually associated with a day time efforts.

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