

Prognosis of ALP, LDH, TP and Ca levels through the chemotherapy in Iraqi Patients with Multiple Myeloma

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Abstract:-

Twenty seven patients with multiple myeloma were included in this study and twenty seven a house hold relatives were taken as a control preferred age and sex match. Serum Alkaline phosphatase ALP, Lactate dehydrogenase LDH, total protein TP and calcium Ca levels were determined before and after the first course of chemotherapy treatment ,its about six weeks, using kits from Randox and Biomaghreb Companeis.

Serum ALP, LDH, TP and Ca levels before and after treatment were significantly higher than control groups, also their levels before treatment were significantly higher than after treatment.

There is a different change in the level of ALP, LDH, TP and Ca in serum of multiple myeloma patients before and after chemotherapy treatment as compared with control. Further investigation needed to estimate the accurate cases of these changes, assessing the disease severity, progression and to detect early relapse.

Introduction:

Multiple Myeloma is a malignant proliferation of plasma cells .Normal plasma cells are derived from B cells and produce immunoglobulins which contain heavy and light chains. In myeloma plasma cells produce immunoglobulins of single heavy and light chain a monoclonal protein commonly referred to as a para protein In some cases only light chain is produced, although a small number of malignant plasma cells are present in the circulation, the majority are present in the bone marrow ^[1].

Patients with myeloma which produces complete immunoglobulin molecules may also excrete increased amounts of light chain in their urine ^[2].

The disease is very uncommon under the age of 30, there after it becomes increasingly frequent, with a peak incidence between 60 and 70 years. Males are affected rather more frequently than females and black people of Central African origin three times more often than Caucasians^[3].

Alkaline phosphatase (ALP)(E.C 3.1.3.1) :-

This enzyme is present in high concentration in the liver, bone and intestinal epithelium. These tissues each contain specific isoenzymes of ALP ^[4].

Phosphatase transfer a phosphate moiety from one group to another, forming an alcohol and a second phosphate compound. The activity of ALP has been elevated in the bone and liver disease such as malignant disease and carcinoma with osteoblastic metastasis ^[5, 6]. Increase in ALP activity are the result of increased synthesis of the enzyme by cell lining the bile canaliculi ^[7].

Lactate Dehydrogenase (LDH) (E.C 1.1.2.4):-

Lactate dehydrogenase catalyzes the reversible interconversion of lactate and pyruvate in the presence of NADH. It is distributed generally in body cells and fluids ^[8].

Lactate dehydrogenase consists of 5 separable proteins, each made of tetramers of 2 types of polypeptide chains H and M, the isoenzymes correspondes to serum proteins α_1 , α_2 , β , γ_1 and γ_2 .

In myocardial infarction the α isoenzymes are elevated particularly LDH1- α yield a ratio of LDH1:LDH2 of greater than 1. The similar elevations occur with hemolytic anemia. LDH is elevated also in leukemia specially with acute myeloblastic leukemia (AML) and acute lymphoplastic leukemia (ALL) ^[9].

Total Protein (TP):-

Proteins are made from amino acids, they are important components of all cells and tissues. There are many different kinds of proteins in the body with many different functions. Examples of proteins are: enzymes, some hormones, hemoglobin, low density lipoprotein (LDL), fibrinogen, collagen, and immunoglobulins. Serum proteins are separated into albumin and globulins, normal values of total protein: 6.4 to 8.3 g/dl ^[10,11].

-Albumin is the highest concentration of protein in the serum. It also helps carry some medicines and other substances through the blood, it is important for tissue growth and healing and for keeping the osmotic pressure of the blood ^[12].

- Alpha-1 globulin. The alpha-1 portion (fraction) of globulins includes alpha-1 anti-trypsin and thyroxine binding globulin. High-density lipoprotein (HDL), the "good" type of cholesterol, is included in this fraction ^[12].

-Alpha-2 globulin. The alpha-2 fraction contains haptoglobin, ceruloplasmin, HDL, and alpha-2 macroglobulin. Alpha-2 globulin called haptoglobin, that binds with hemoglobin ^[12].

-Beta globulin. The beta fraction includes transferrin, plasminogen and beta-lipoproteins. Beta globulin proteins help carry substances, such as iron, through the bloodstream and help fight infection ^[12].

-Gamma globulin. These proteins are also called antibodies. They help prevent and fight infection. Gamma globulins bind to foreign substances, such as bacteria or viruses, causing them to be destroyed by the immune system. The gamma fraction includes different types of antibodies (immunoglobulins M, G, and A) ^[12].

All the plasma proteins are synthesized in liver except gamma globulins is synthesized by B-lymphocytes. 60% of plasma proteins are made up of the protein albumin, Globulins make up 35% of plasma proteins ,4% is fibrinogen. Regulatory proteins which make up less than 1% of plasma proteins are proteins such as enzymes, proenzymes and hormones ^[13] .

Calcium (Ca): -

Calcium is the most abundant mineral in the human body. The average adult body contains in total approximately 1 kg, 99% in the skeleton in the form of calcium phosphate salts. The extracellular fluid (ECF) contains approximately 22.5 mmol, of which about 9 mmol is in the serum. Approximately 500 mmol of calcium is exchanged between bone and the ECF over a period of twenty-four hours. The serum level of calcium is closely regulated with a normal total calcium of 2.2-2.6 mmol/L (9-10.5 mg/dL) and a normal ionized calcium is calcium that is freely flowing in your blood and not attached to proteins. It is also called free calcium of 1.1-1.4 mmol/L (4.5-5.6 mg/dL) ^[14] .The amount of total calcium varies with the level of serum albumin, a protein to which calcium is bound.

All cells need calcium in order to work. Calcium helps build strong bones and teeth. It is important for heart function, and helps with muscle contraction, nerve signaling, and blood clotting. Maintenance of normal calcium balance and serum calcium levels depend on integrated regulation of calcium absorption and secretion by the intestinal tract, the excretion of calcium by the kidney. Both processes are stimulated by parathyroid hormone. Although calcium flow to and from the bone is neutral, about 5 mmol is turned over a day. Bone serves as an important storage point for calcium, as it contains 99% of the total body calcium. Calcium is released from bone by parathyroid hormone. The parathyroid glands are located behind the thyroid, and produce parathyroid hormone in response to low calcium levels. The parafollicular cells of the thyroid produce calcitonin in response to high calcium levels. Calcitonin stimulates incorporation of calcium in bone, although this process is largely independent of calcitonin. Primarily calcium is regulated by the actions of Vitamin D, parathyroid hormone and calcitonin ^[15,16,17] .

Subjects and methods:-

Patients:-

Blood sampling were drawn from (27) patients with multiple myeloma 12 males with age range (47-62) years, and (15) females with age range (40-60) years. Patients referred to the (National center of hematology-Baghdad).

The criteria of inclusion in this study are:-

1-All patients were newly diagnosed.

2-The samples were collected before any chemotherapy treatment and after the first course of chemotherapy treatment, it was about 6 weeks.

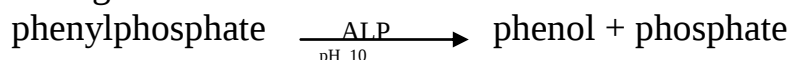
3-Twenty seven house hold relatives were taken as a control 12 males with age range (43-55) years, and (15) females with age range (32-57) years.

Venous blood samples (5 ml) from the control and each patient before and after chemotherapy treatment, transferred into plain tube then the blood was left to clot and serum was obtained by centrifugation at 3000 rpm for 10 min then serum was removed and kept at (-20°C) till analysis.

Methods:-

1-Determination of Alkaline phosphatase activity:-

Colorimetric determination of ALP activity was obtained according to the following reaction:-



The phenol liberated was measured in the presence of amino-4-antipyrine and potassium ferricyanide. The presences of sodium arsenate in the reagent stop the enzymatic reaction^[18].

2-Determination of Lactate Dehydrogenase activity:-

This method is based on the reduction of pyruvate to lactate in the presence of NADH by the action of lactate dehydrogenase.



The pyruvate that remains unchanged reacts with 2,4-dinitrophenyl hydrazine to give the corresponding phenylhydrazone, which was determined colorimetrically in an alkaline medium^[19].

3-Determination of serum total protein:-

Cupric ions in an alkaline medium, interact with protein peptide bonds resulting in the formation of a colored complex^[20].

4-Determination of serum calcium:-

Calcium ions form a violet complex with O-Cresolphthalein complex in an alkaline medium^[21].

Statistical methods:-

The results were analyzed statistically, and values were expressed as (mean ± standard deviation). The level of significance was determined by employing (student t-test). Only when the (P value) was less than 0.05 was the difference between two groups is considered statistically significant.

Results and discussion:-

Table (1) and figure (1) showed the mean serum ALP activity of patients before and after treatment compared to control. It was found that mean of serum ALP activity in patients before and after treatment was statistically significant higher than of control groups (P< 0.05). Serum ALP activity in patients before treatment was statistically significant higher than serum ALP activity of patients after treatment (P<0.05). Elevation of serum ALP occurs in a wide variety of clinical situation. The

highest level was found in patients with cancer disease^[22]. The ALP variant produced by tumor appeared in the serum^[23]. This enzyme which is produced by tumor was called [Regan isoenzyme]. Regan isoenzyme is an isoenzyme of ALP, which is biochemically and immunologically indistinguishable from that of placenta one . It is found in serum and tumor tissue of patients having variety of malignant cell involving different organs^[24,25]. In some cases the enzyme may contains different isoenzymes which differs in their effective rate toward the active center and the change in the steriostructure of the enzyme during the development of the disease ,this explained the raising of ALP activity in this study before treatment and patients than control^[26,27].

Table (1) and figure (1) showed the mean serum LDH activity of patients before and after treatment and control .It was found that mean serum LDH activity in patients before and after treatment was statistically significant higher than of control groups ($P < 0.05$) .Serum LDH activity in patients before treatment was statistically significant higher than serum LDH activity of patients after treatment ($P < 0.05$). Nearly every type of cancer can cause LDH levels to be elevated. LDH levels can be used to monitor treatment of some cancer, including Ewing's Sarcoma, non-Hodgkin's lymphoma, and some types of leukemia. Elevated LDH levels can be caused by a number of noncancerous conditions, including heart failure, anemia and lung or liver disease. The decrease or return to normal in the level of a tumor marker may indicate that the cancer has responded favorably to treatment ,therefore LDH activity in the study lowered after treatment^[22].

Table (1) and figure (1) showed the mean serum TP concentration of patients before and after treatment compared to the control .It was found that mean serum TP concentration in patients before treatment was statistically significant higher than of control groups ($P < 0.05$), mean serum TP concentration in patients after treatment was statistically significant higher than of control groups ($P < 0.05$).Serum TP concentration in patients before treatment was statistically significant higher than serum TP concentration of patients after treatment ($P < 0.05$).The increased total protein levels in cancer patients may be an evidence for the uncontrolled behavior of the cancerous cells, for instance, the elevation of total protein levels in multiple myeloma reflects the excessive production of paraproteins by cancerous plasma cells^[28].

Table (1) and figure (1) showed the mean serum Ca concentration of patients before and after treatment and control .It was found that mean serum Ca concentration in patients before and after treatment was statistically significant higher than of control groups ($P < 0.05$) .Serum Ca concentration in patients before treatment was statistically significant higher than serum Ca concentration of patients after treatment ($P < 0.05$). Serum Ca level is closely associated with bone lesions in multiple myeloma. Serum Ca levels are high in patients with large tumor masses and bone lesions. Moreover, hypercalcemia may lead to death in multiple myeloma^[29].

Table (1):-Mean, standard deviation (SD), P value of serum (ALP,LDH,TP and Ca) in multiple myeloma patients before and after treatment.

	Control Mean±SD	Before Mean±SD	After Mean±SD	P value C-B	P value C-A	P value A-B
ALP U/L	57.60±3.92	155.3±9.99	97.42593±9.51	P<0.05	P<0.05	P<0.05
LDH U/L	98.35±10.92	335.53±29.90	211.96±15.75	P<0.05	P<0.05	P<0.05
TP g/L	76.33±2.18	97.87±10.35	87.98±7.54	P<0.05	P<0.05	P<0.05
Ca mg/L	91.40±1.95	115.58±3.21	105.19±4.36	P<0.05	P<0.05	P<0.05

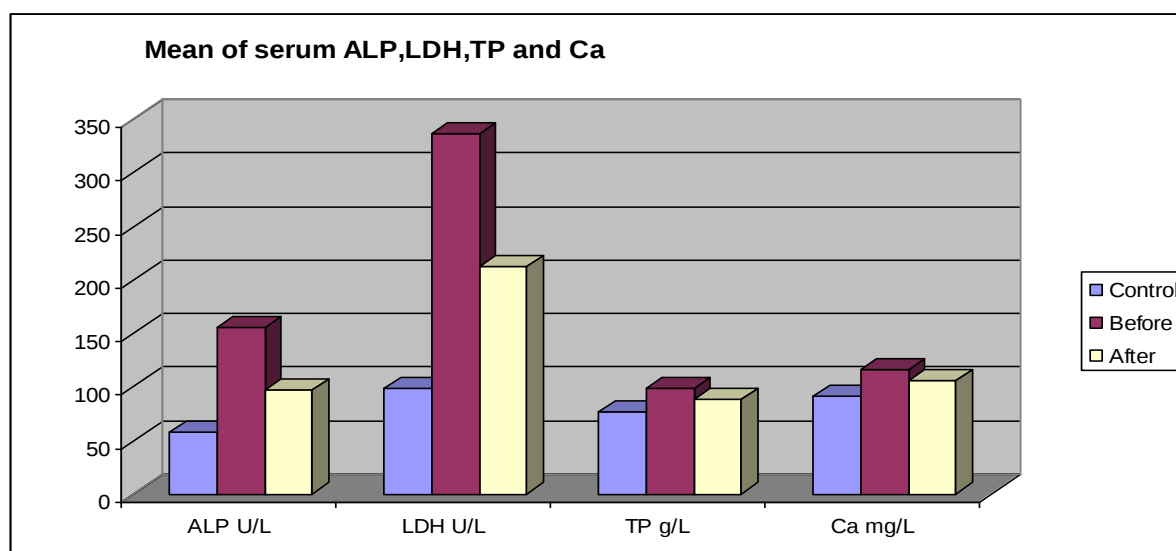


Figure (1):-Mean, standard deviation (SD), P value of serum (ALP, LDH, TP and Ca) in control and multiple myeloma patients before and after treatment.

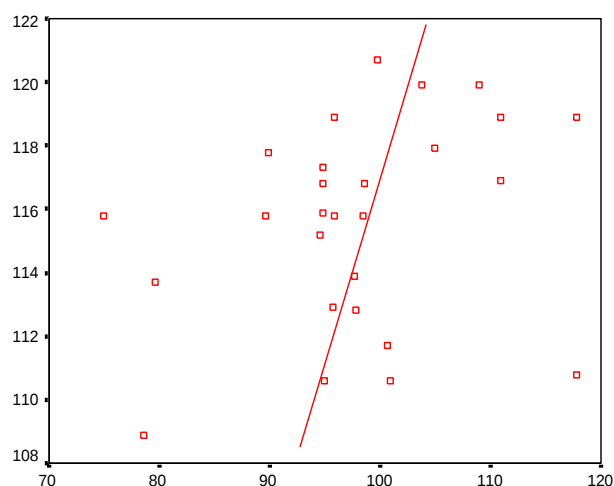
Correlation coefficient

1- Positive correlation was found between Ca and TP before treatment in patients with multiple myeloma ($r=0.288$). There is insignificant relation found between Ca and TP, ($P> 0.05$) as indicated in figure (2). Negative correlation was found between Ca and TP after treatment in those patients ($r=-0.037$), there is insignificant relation found between Ca and TP, $P> 0.05$ as indicated in figure (3).

2-Negative correlation was found between ALP and Ca before treatment in patients with multiple myeloma ($r=-0.233$). There is insignificant relation found between ALP and Ca, ($P> 0.05$) as indicated in figure (4). Negative correlation was found between ALP and Ca after treatment in those patients ($r=-0.234$), there is insignificant relation found between ALP and Ca, $P> 0.05$ as indicated in figure (5).

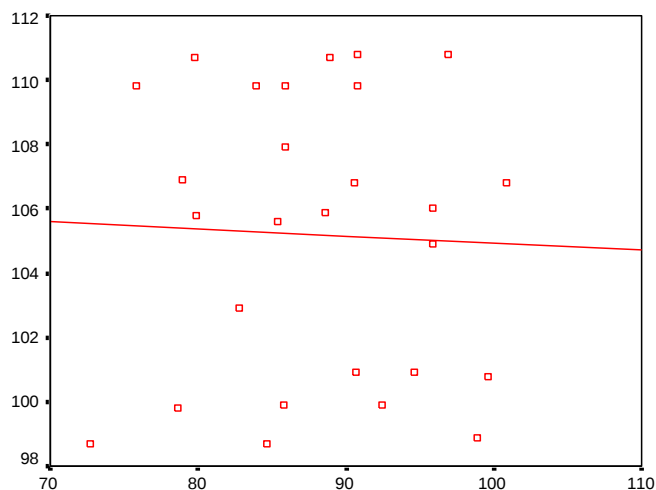
3-Positive correlation was found between LDH and Ca before treatment in patients with multiple myeloma ($r=0.173$). There is insignificant relation found between LDH and

Ca, ($P>0.05$) as indicated in figure (6). Negative correlation was found between LDH and Ca after treatment in those patients ($r=-0.346$). There is insignificant relation found between them, ($P>0.05$) as indicated in figure(7).



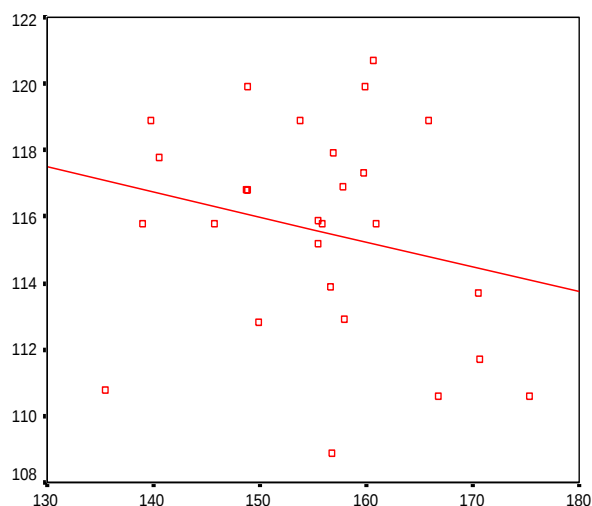
Serum TP before treatment

Figure(2):-Correlation between Ca and TP before treatment



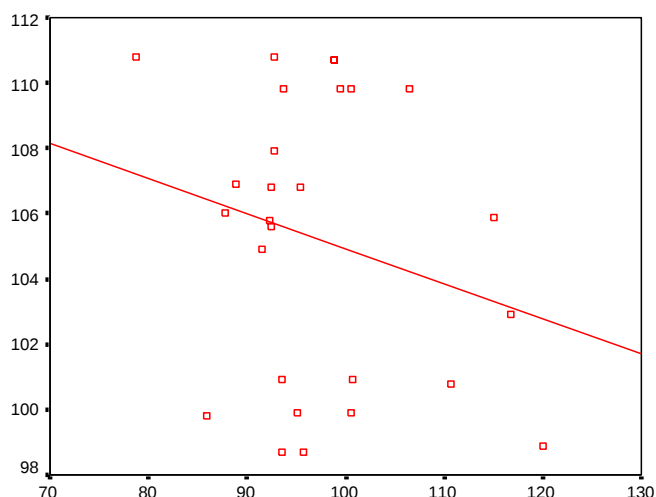
Serum TP after treatment

Figure(3):-Correlation between Ca and TP after treatment



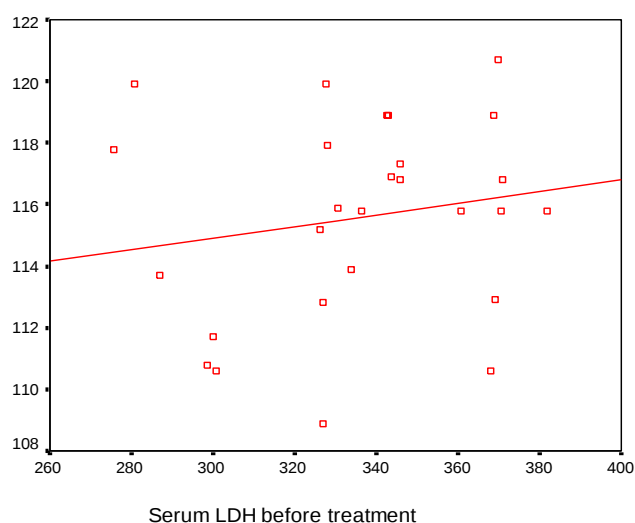
Serum ALP before treatment

Figure(4):-Correlation between Ca and ALP before treatment

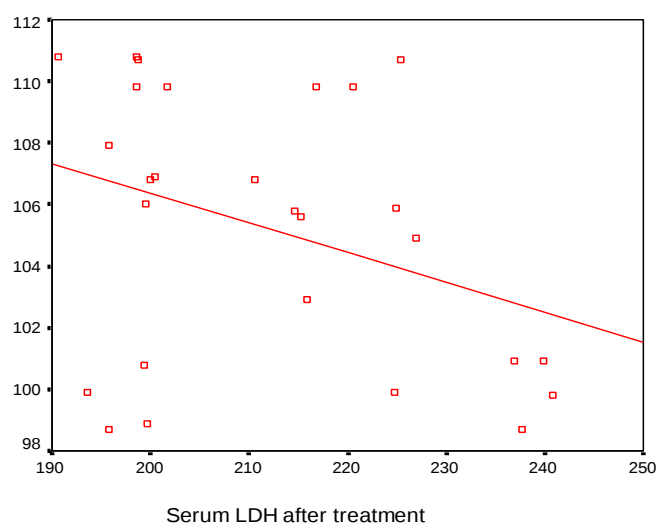


Serum ALP after treatment

Figure(5):-Correlation between Ca and ALP after treatment



Figure(6):-Correlation between Ca and LDH before treatment



Figure(7):-Correlation between Ca and LDH after treatment

References:-

- 1-Christophe H.,Edwin R.Joh A. “Davidson’s principles and practice of medicine” Churchill livingstone ,18th ed. (1999).
- 2- Enitaza D. GEORGE, M.D., and Richard Sadovsky “Multiple Myeloma: Recognition and Management”. State University of New York Health Science Center , Brooklyn, New York, by the American Academy of Family Physicians, 1999.
- 3-Christopher R.W.Edwards and Ina,A.D.Bouchier,Davidson’s Principles and practice of medicine” Churchill livingstone ,16th ed. (1992).
- 4-William J. Marshall:Clinical chemistry, London,UK.4th ed 2000.
- 5-Whitby C. Smith F. and Beckett J.: Lectyren notes on clinical chemistry,Black well scientific publications 4th ed,1988.
- 6-Kaplan A. “Clinical chemistry theory analysis and correlation, 2nd ed.The C.V.Mosby Co. 1989.
- 7-Allan Gaw;Robert A. Cowan: Clinical biochemistry 2nd ed,1999.
- 8-Zilva J.F.and P.R.Pannall:Clinical chemistry in dignosis and treatment,Lloyd-Luke –London,1984.
- 9-Al-Modaphar S.,Riad Rashed :The biochemistry Eshbilia office ,Baghdad,1985.
- 10-Adkins JN et al (2002). Molecular and Cellular Proteomics 1: 947-955.
- 11-Jacobs JM et al (2005). Journal of Proteome Research 4: 1073-1085.
- 12- J. Jerry Kaneko, John W. Harvey, Michael L. Bruss.;Clinical biochemistry of domestic animals ; 5th ed. San Diego, Calif. : Academic Press, 1997
- 13- Theodore X. O’Connell, M.D., Timothy J. Horita, M.D., and Barsam Ka sravi , “Understanding and Interpreting Serum Protein Electrophoresis”, by the American Academy of Family Physicians, California, 2005
- 14- Fukagawa M, Kurokawa K, Papadakis MA. Fluid & electrolyte disorders. In: McPhee SJ, Papadakis MA, Tierney LM Jr. Current Medical Diagnosis and Treatment , New York, NY: McGraw Hill; 2007

- 15-Marshall, W. J. 1995. Clinical Chemistry, 3rd ed. Mosby, London.
- 16-Heaney, R. P., et al. J. Am. Coll. Nutr. 2000 Apr;19(2 Suppl):83S-99S.
- 17- Baron J, Beach M, Mandel J, van Stolk R, Haile R, Sandler R, Rothstein R, Summers R, Snover D, Beck G, Bond J, Greenberg E. N. Engl J Med 340 (2): 101-7(1999)
- 18-Pesce A.J.;Kapan L.A. "Methods in clinical chemistry" Mosby company, 527;543-596,1987.
- 19-Wroble Wski F.,Ladue J.,Proc. Soc. Exp. Biol., 90:210,1955.
- 20-Peter T.J.Clin. Chem.14,1147,1968.
- 21-Prince et al, Radiology;227:639-646, 2003 .
- 22-Cancer Net,NCI :Tumor Marker 1998.
- 23-Fishman W.H. :Ann. N.Y.Act.Sc,116 :745 1969.
- 24-Stikbach L.,Krant M.J. ;and Fishman W.H. :N.Eng.J.,Med ;281 :757 1969.
- 25-Nathanson and Fishman W.H. :Cancer 27 :1388,1971.
- 26-Thomas E.D.:Kahan L.Douglass C.L.;Frank A.;and Neilcarey R.:J.Cancer Res;41:1110;1981.
- 27-Koss B.;and Greengard O.;J.Cancer; 42:2164 ;1982.
- 28-Hoffbrand, A.V.,and Pettit ,J.E.,Essential haematology ,2nd ed. ;Blackwell;Scientific publication 1989.
- 29- Shimura Hiroki(Tohodai I Naikagakudaiichi); Journal of the Medical Society of Toho University;49;6;353-362(2002).

الخلاصة

تم في هذا البحث جمع مصل الدم الى ٢٧ مريض مصاب بتعدد الدم النقائي و ٢٧ الى اشخاص غير مصابين من الاقرباء والقريين من المريض من حيث العمر والجنس (المجموعة الضابطة). تم تقدير مستويات المتغيرات التالية: (ALP,LDH,TP and Ca) الموجودة في مصل الدم قبل وبعد اخذ الجرعة الاولى من العلاج الكيميائي وكانت حوالي ستة اسابيع .

وجد ان مستويات ALP, LDH, TP and Ca قبل وبعد العلاج له قيمة معنوية احصائية يعتد بها اعلى من المجموعة الضابطة.

هناك تغيرات مختلفة ظهرت في مستويات المتغيرات السابقة للمرضى المصابين قبل وبعد العلاج الكيميائي مقارنة بالمجموعة الضابطة. لذلك فان هذه التحاليل مطلوبة لتعيين ومتابعة التغيرات لحالة المرض، ومتابعة شدة وتطور المرض والكشف المبكر للانتكاسات التي قد تحدث .