

**Estimation of Immunoglobulins and complement component C3 and C4 in
some patients with Hodgkin and Non-Hodgkin Lymphoma in Basrah
Governorate**

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Abstract

The humoral immune response including serum Immunoglobulins (IgG, IgM and IgA) and complement components (C3 and C4) level in Hodgkin (HL) and Non-Hodgkin(NHL) Lymphoma patients were studied. Forty five samples were taken (20 NHL ,10 HL , 15 control) , examined by single Radial Immunodiffusion assay (SRID).The results showed a non significant elevation of IgG level in the sera of HL & NHL patients 1097.9 mg/dl and 1088.3 mg/dl , respectively , in comparison with control group (950.7 mg/dl) ; Non significant elevation of IgM level also had been reported in the sera of HL & NHL patients 232.71 mg/dl and 228.6 mg/dl , respectively , while it reached 166.78 mg/dl in control group. The results revealed a non significant decrease in IgA level in the sera of HL patients (227.2 mg/dl) while NHL patients showed non significant elevation of it (324.1 mg/dl) while it reached 304.3 mg/dl in control group. There was a non significant decrease in complement component C4 level in the sera of HL patients 21.331 mg/dl while NHL patients showed non significant and slightly increase of it (25.565 mg/dl) in comparison with the mean value of the control group which reached 24.95 mg/dl. complement component C3 level was non significantly increase in the sera of HL patients 141.98 mg/dl while NHL patients showed non significant decrease in complement component C3 level 116.66 mg/dl in comparison with the mean value of the control group which reached 124.01 mg/dl.

Introduction:

Lymphomas are tumors of immune cells; they are solid tumors within lymphoid tissue such as bone marrow and lymph nodes. Hodgkin's and non-Hodgkin's lymphomas are examples (Doan *et al.*,2008), which separated from each other on the basis of the presence or absence of a unique type of cells known as the Reed-Sternberg cell (RS). These RS cells are present in the various forms of Hodgkin's disease, but are not found in the other malignant lymphomas (Hean,1995), they are characterized by their large size and classic binucleated structure with large eosinophilic nucleoli. Two antigenic markers are thought to be provide diagnostic information : CD30 and CD15 (Yahalom and Straus,2008). Lymphomas such as other hematological malignancies can be associated with dysregulation of the immune system (Mashhadi *et al.*,2009). Different studies have evaluated qualitative and quantitative immunological variables including serum immunoglobulin's level in malignancies (Welch and Lilleyman,1995).In the other hand, Immunological and cytological studies provided additional diagnostic information in patients with malignant lymphoma and a number of monoclonal antibodies have been described which react with antigens on Reed – Sternberg cell and may help in diagnosis (Hoffbrand and Pettit,1988)

Most of mortal cases happened as a result of immune deficiency or

dysregularity of patient's immune system and chemotherapy that associated with the disease so the aim of this study is to estimate the humoral immune response in sera of Hodgkin and Non-Hodgkin lymphoma patients in Basrah including immunoglobulins (IgG , IgM and IgA) and complement component C3 and C4.

Materials and methods:

Blood samples were collected from 45 healthy people and patients with Hodgkin and non-Hodgkin lymphoma from Basrah Oncology Center undergone chemotherapy as it described in table (1) . Samples were taken before chemotherapy, they transferred immediately to the laboratory and the sera were separated by centrifugation and saved at 0 C° until use. For the detection of immunoglobulin's and complement component's level, 5µl were taken from the serum and added to each pore of the single radial immunodiffusion plates of IgG , IgM , IgA , C₃ , C₄ (LTA s.r.l./Milano/Italy) the plates were placed in a moist chamber and incubated at its suitable incubation period as shown in table (2). The diameter of the precipitation ring was measured with a slide rule (immunoviewer) and compared with the reference values in the given reference table (Mancini *et al.*,1965 ; Fahey and McKelvey,1965) and the results were statistically analyzed by ANOVA test under probability (p<0.05) using minitab program .

Table (1): Characterization of Samples

DISEASE	NO. SAMPLES	AGE (YEARS)	MALE	FEMALE
Hodgkin lymphoma (HL)	10	(17 – 43)	3	7
Non-Hodgkin lymphoma (NHL)	20	(31 – 86)	12	8
Control (healthy)	15	(20 - 35)	9	6
Total	45		24	21

Table (2): Suitable Incubation Period and Normal references values of different SRID Plates

PLATS TYPES	IGG	IGM	IGA	C3	C4
Incubation period*	72h	96h	72h	72h	72h
Normal Reference Values (mg/dl)	800-1800	60-280	90-450	91-156	20-50

* The incubation period represent the maximum time of incubation and it can be read in shorter time (36 h.) according to the kit that had been used.

Results:

Patients characterization

We notes from table (1) and even if it's in a small group selected randomly the fact that NHL incidence is higher in males than females (60% male , 40% female) and it appear in elderly age (more than 31) while HL incidence in females more than males (70% females , 30% males) and appear in a younger age (more than17).

Immunoglobulin G level

The results showed a non significant elevation of IgG level in the sera of both

HL and NHL patients and its means reached about 1097.9 mg/dl and 1088.3 mg/dl , respectively , in comparison with control group 950.7 mg/dl . More interesting 5% of the NHL patients had a higher level than the highest normal reference value (1800 mg/dl) while 35% of them showed low values in comparison with the lowest normal reference value (800 mg/dl). Figure (1) and picture (1).

Immunoglobulin M level

As it had been notes there is a non significant elevation of IgM level in the

sera of both HL and NHL patients and its means reached about 232.71 mg/dl and 228.6 mg/dl , respectively , in comparison with control group 166.78 mg/dl. There were about 30% of HL patients showed higher values than the normal reference values given at the reference table (280 mg/dl) while only 10% showed that in patients with NHL. Also 10% of NHL patients showed values higher than the highest value given in the reference table (532.5 mg/dl). Figure (2) and picture (2).

Immunoglobulin A level

The results demonstrate a non significant decrease in IgA level in the sera of HL patients and the mean value was 227.2 mg/dl in comparison with the mean value of the control group 304.3 mg/dl in spite of that 5% of them showed values higher than the highest normal reference values (450 mg/dl). NHL patients showed non significant elevation in immunoglobulin A level 324.1 mg/dl in comparison with the mean value of the control group 304.3 mg/dl, 5% of them showed values higher than the highest normal reference values (450 mg/dl) and more interesting 15% of them reveal values lower than the lowest reference value in the reference table (90 mg/dl). Figure (3) and picture (3).

Complement component C4 level

The present data showed a non significant decrease in C4 level in the sera of HL patients and the mean value was 21.331 mg/dl in comparison with the mean value of the control group 24.95 mg/dl, while NHL patients showed non significant and slightly increase in C4 level 25.565 mg/dl in comparison with the mean value of the control group 24.95 mg/dl , with 10% of the patients who reveal values lower than the lowest value in the reference table (4.8 mg/dl). Figure (4) and picture (4).

Complement component C3 level

A non significant increase shown in C3 level in the sera of HL patients and the mean value was 141.98 mg/dl in comparison with the mean value of the control group 124.01 mg/dl, with 40% of them revealed a higher values than the highest normal reference values (156 mg/dl), while NHL patients showed non significant decrease in C3 level 116.66 mg/dl in comparison with the mean value of the control group 124.01 mg/dl, in spite of this result 15% Of them revealed values higher than the highest normal reference values (156 mg/dl). Figure (5) and picture (5).

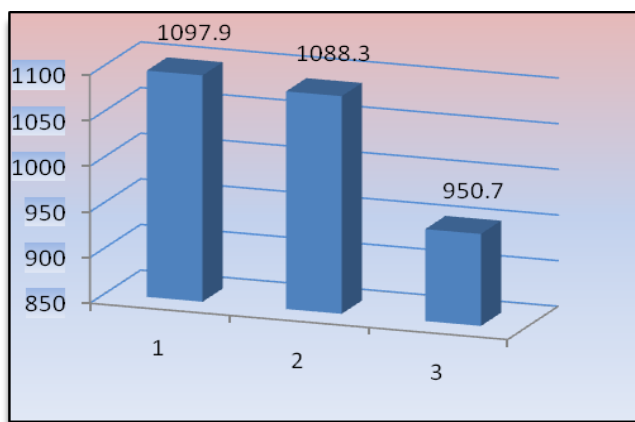


Figure (1): mean values of IgG level in patients with HL and NHL compared with control

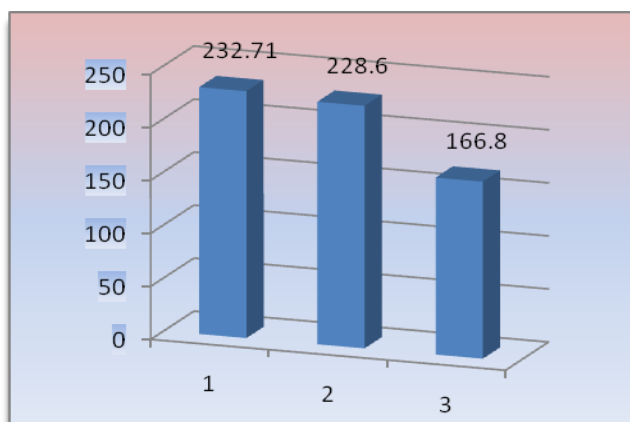


Figure (2): mean values of IgM level in patients with HL and NHL compared with control

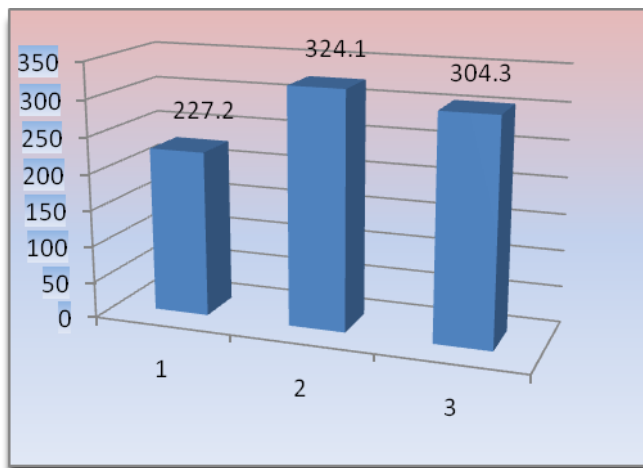


Figure (3): mean values of IgA level in patients with HL and NHL compared with control

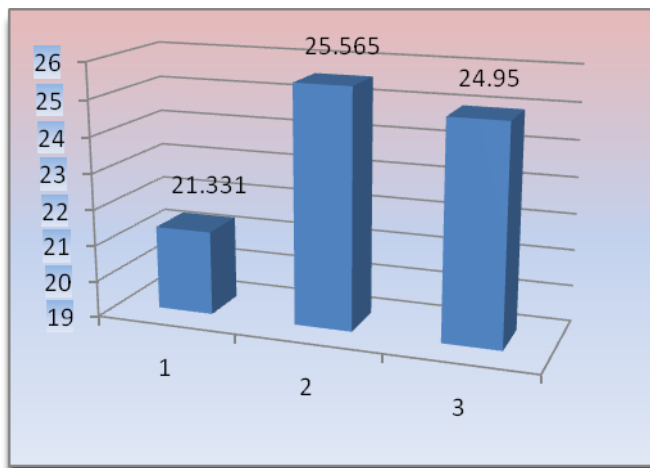


Figure (4): mean values of C4 level in patients with HL and NHL compared with control

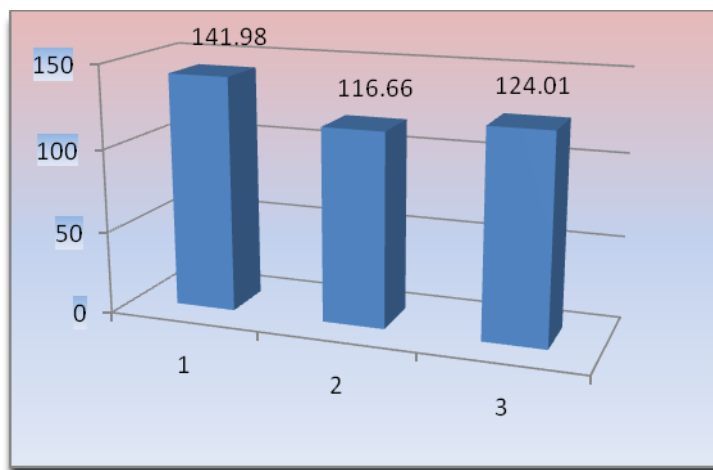
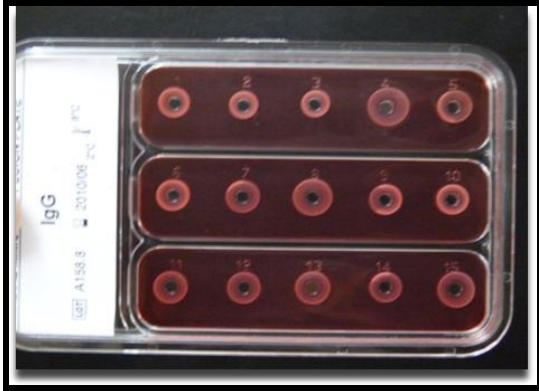


Figure (5): mean values of C3 level in patients with HL and NHL compared with control

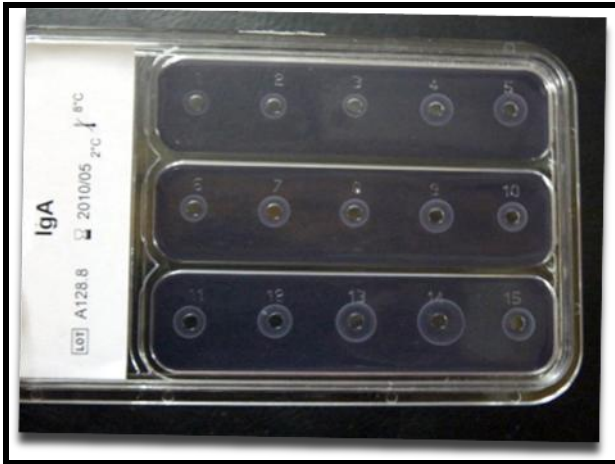
1= Hodgkin lymphoma (HL), 2= Non-Hodgkin lymphomas (NHL), 3= Control group



Picture (1): precipitation ring of IgG



Picture (2): precipitation ring of IgM



Picture (3): precipitation ring of IgA



Picture (4): precipitation ring of C4



Picture (5): precipitation ring of C3

Discussion:

It had been concluded from the characteristic table that non-Hodgkin lymphomas incidence is higher in males than females and appear in elderly age (more than 31) this matches the study in which was reported that NHL incidence increase's with aging and it summarized that in persons older than 65 the incidence of NHL is 87.2 per 100.000 (Rosen *et al.*,2008) while Hodgkin lymphomas incidence in females higher than males ,other reported studies showed that male to female ratio is 1.3 : 1.0 , the present results include the age of patients showed that HL incidence is in younger ages (more than17) agreed with study reports that the greatest peak of HL is in the third decade of life (Yahalom and Straus, 2008).

The recent results which showed a non significant changes in the levels of the immunological parameters agreed with the non significant changes found by a study of immunoglobulins levels in patients with hematological malignancies in Iran this non significant results may be resulted from the fluctuation and variation in the levels of immunoglobulins and complement in these malignancies (Mashhadi *et al.* ,2009 ; Carli *et al.*, 1979) . An elevation in the levels of IgG and IgM in both Hodgkin and non-Hodgkin lymphoma patients and the elevation of IgA in non-Hodgkin lymphoma patients which may be explained by the presence of tumor antigens that may stimulate the humoral immune response (Eales,1996), IgG and IgM activate the classic pathway of complement , leading to lysis of cellular immunogens and stimulation of phagocytosis to all immunogens (Smith and Wood,1996) also many studies showed that the identification of tumor – reactive antibodies in cancer patients is

possible and this suggests that antibodies may play an important role in the immune response to tumor cells, and may cause lyses of tumor cell either by fixing the complement to the tumor cell membrane or by antibody – dependent cell- mediated cytotoxicity (ADCC) (Eales,1996).

The complement which is the major effector of the humoral branch of the immune system consist of 20 chemically distinct serum proteins and glycoproteins (Kuby, 1992) C3 associated with the activation of the three pathways of the complement and mostly with the alternative pathway (Eales,1996) while C4 associated with the classical pathway (Benjamin *et al.*,2000) the elevation of C4 in NHL may represent that the classical pathway is domain pathway of activation while the elevation of C3 in HL may represent that the alternative pathway is domain pathway of activation, the elevation of C3 and C4 has been reported in other types of lymphoma such as Burkitt's lymphoma (Umukoro and Onyesom,2005). This elevation may compensate the deficiency in cellular immune response which defends the host against the tumor (Carli *et al.*, 1979) and may also be caused by the continuous presence of tumor's antigens and the presence of antigen – antibody complexes which stimulate the activation of the complement (Verhaegen *et al.*,1976)

The decrease of IgA and C4 in HL and of C3 in NHL may caused by chemotherapy which contained an immunocompromised drugs such as Prednidon which effect the entire immune system but it appear as an inhibitor in these parameters .

The result demonstrate a fluctuation in the level of the given parameters some are more higher than the reference

values which may represent a dysregularity of the patient's immune system (Welch and Lilleyman,1995) and some are lower than the reference values which may represent an immunological deficiency, generally, this fluctuation may be caused by several interpenetrated reasons such as the age, nutrition (Weimer *et al.*,1964) and number of chemotherapy doses the patient receives. These abnormalities and fluctuation in the present study agreed with many studies included various types of malignances (Carli *et al.*, 1979) and hematological malignancies (Mashhadi *et al.*, 2009)

These recent data make it necessary to recommend an immunological test for each cancer patients in order to pay more attention to the their immunological status which is effected by both tumor and the therapy and to provide an intensive care for them.

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تقييم الاميونوكلوبيولينات و مكونات المتمم C3 و C4 في بعض المرضى المصابين بسرطان الدم اللمفاوي الهوجكين و غير الهوجكين في البصرة

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الخلاصة

تضمنت الدراسة الحالية دراسة المناعة الخلطية و المتضمنة مستويات الكلوبولينات المناعية (IgG و IgA و IgM) و مكونات المتمم (C4 و C3) في مصول المرضى المصابين بسرطان اللمفاوي نوع الهوجكين (HL) و غير الهوجكين (NHL) ، ٤٥ عينة دم اخذت من المرضى و من الاشخاص الاصحاء كمجموعة سيطرة (٢٠ بسرطان اللمفاوي غير الهوجكين و ١٠ بسرطان اللمفاوي الهوجكين و ١٥ سيطرة) و فحصت باختبار الانتشار المناعي الشعاعي البسيط (SRID) . اظهرت النتائج ارتفاع غير معنوي في مستوى الاميونوكلوبيولين G في مصول كل من مرضى الـ HL و NHL (١٠٩٧,٩ ملغم / دل و ١٠٨٨,٣ ملغم / دل ، على التوالي) بالمقارنة مع مجموعة السيطرة (٩٥٠,٧ ملغم / دل) كما اظهرت النتائج ارتفاع غير معنوي في مستوى الاميونوكلوبيولين M في مصول كل من مرضى الـ HL و NHL (٢٣٢,٧١ ملغم / دل و ٢٢٨,٦ ملغم / دل ، على التوالي) بالمقارنة مع مجموعة السيطرة (١٦٦,٧٨ ملغم / دل) ، بالنسبة للاميونوكلوبيولين A فقد ظهر انخفاض غير معنوي في مستواه في مرضى HL (٢٢٧,٢ ملغم / دل) في حين اظهر ارتفاع غير معنوي في مرضى NHL (٣٢٤,١ ملغم / دل) بالمقارنة مع مجموعة السيطرة (٣٠٤,٣ ملغم / دل) و كان هناك انخفاض غير معنوي في مستوى مكون المتمم C4 في مرضى HL (٢١,٣٣١ ملغم / دل) في حين كان هناك ارتفاع قليل و غير معنوي في مرضى NHL (٢٥,٥٦٥ ملغم / دل) بالمقارنة مع مجموعة السيطرة (٢٤,٩٥ ملغم / دل) ، مكون المتمم C3 اظهر ارتفاع غير معنوي في مرضى HL (١٤١,٩٨ ملغم / دل) في حين ظهر انخفاض غير معنوي له في مرضى NHL (١١٦,٦٦ ملغم / دل) بالمقارنة مع مجموعة السيطرة (١٢٤,٠١ ملغم / دل) .