# Is Th-1/Th-2 paradigm a prognostic indicator on childhood Non-Hodgkin lymphoma

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**الخلاصة**

تمتاز اورام الغدد اللمفاويه (اللاهوجكن) باختلاف سلوكها البايولوجي و فعالية المرض ، كما ان مستويات البروتينات في بلازما الدم ترتبط مع فعاليات المرض المختلفه لذا فمن الممكن استخدامها كعوامل لمتابعة مال المريض.

اجريت هذه الدراسه لتقييم امكانية استخدام مستويات (الانترلوكين – الرابع و العاشر و الانترفيرون كاما) كعوامل متابعه لمال المرضى و مقدار فائدتها في تنبأ مصير الاطفال المصابين باورام الغدد اللمفاويه (اللاهوجكن).

الاشخاص و طريقة العمل: ضمت هذه الدراسه 99 شخصا ، 66 طفل مصاب باورام الغدد اللمفاويه و 33 طفل سليم ظاهريا كمجموعه ضابطه مطابقه من حيث الجنس و العمر. عينة دم وريدي اخذت من كل شخص و عزل منها المصل لاستخدامه في قياس الانترلوكين الرابع و العاشر و الانترفيرون-كاما بطريقة الروز المناعي المرتبط بالانزيم (الاليزا).

لوحظ ارتفاع نسبة جميع المدورات المناعيه في مصول المرضى عند مقارنتها مع مستوياتها في مصول المجموعه الضابطه. و عند دراسة العلاقه بين مستوى الانترلوكين العاشر و عوامل المال السيء (تمت دراستها في دراسات سابقه) مثل (الجنس: ذكر ، العمر: اقل من عشرة اعوام ، ضراوة المرض ، انتشار المرض ، عدم الاستجابه للعلاج ، موت المريض اثناء فترة العلاج) و قد وجدت علاقه ايجابيه بين مستوى الانترلوكين العاشر و عوامل المال السيء. و عند دراسة العلاقه بين مستوى الانترفيرون – كاما و عوامل المال الحسن و جدت علاقه ايجابيه بينهما و خاصة مع (العمر و الاستجابه للعلاج و حياة المريض). لم نلاحظ اي علاقه واضحه بين الانترلوكين الرابع و عوامل المال السيء او الحسن.

استنتجنا من هذه النتائج انه من الممكن استخدام نسبة الانترلوكين العاشر / الانترفيرون – كاما ، كعلامه مصليه لتوقع فعالية المرض و مال المريض تضاف الى العوامل الاخرى .

**Abstract**

Non-Hodgkin lymphomas (NHLs) characterized by variable biologic behavior. The levels of some plasma protein may correlate with disease activity, so they could behave as prognostic factors.

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This study was conducted to evaluate the possible prognostic value of the (IL-10, IL-4 and IFN-gamma) and to assess their usefulness in the prediction of the outcome in childhood NHL patients.

Sixty-six patients with NHL and 33, age and sex matched, apparently healthy children, were included. Blood sample were taken from each subject, and sera were used in measurement of IL-10, IL-4, and IFN-γ.

The sera of all patients showed higher levels of IL-10, IL-4, and IFN-g than did the control group. The serum level of IL-10 revealed positive correlation with bad prognostic factors (male sex, less than 10 years old, high grad, high stage, non-responding to chemotherapy, and non-surviving). Whereas, IFN- γ showed a negative correlation with bad prognostic factors (male sex, high grade and stage) and a positive with good prognostic factors (age, response to chemotherapy and the survival). IL-4 revealed no clear cut correlation, neither with good nor with bad prognostic factors.

We conclude the existence of IL-10/IFN-g as serum marker for disease severity and predictor of response to chemotherapy in NHL, independent of and additive to the other prognostic factors.

Key words: NHL, serum marker of disease severity, IL-10/INF-g.

## Introduction

Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of malignant diseasesthat are result from a clonal proliferation of lymphocytes at various stages of maturation, and are characterized by variable biologic behavior, ranging from well differentiated to very aggressive forms of the disease (1). In Iraq, malignant lymphoma constitute about 25.7% of all childhood cancers, being second in relative frequency after lymphatic leukemia (2). Several studies have previously reported a different clinical and biological features

of the disease to be used as reliable prognostic factors like age, sex, stage, grade, histopathological types, response to chemotherapy and the survival (1, 2, 3). The levels of some plasma protein may reflect processes specific to a particular cancer and correlate with disease activity, so they could behave as prognostic factors and changes in their plasma level may associate with prognosis and disease outcome (3). The presence of too many prognostic factors and unfortunately, some of these parameters are difficult or inconvenient to evaluate serially in individual patients or routinely in some medical centers and therefore, cannot be widely applied and it will be more convenient to find a limited useful prognostic factors for such evaluation. Thus, the present study aimed to evaluate the role of serum levels of IL-10 and IL-4 (Th-2 cytokines) and IFN- γ ( Th-1 cytokine) as one of the possible prognostic factors that may help in follow up the disease.

## Subjects, materials and methods:

***Subjects:*** Sixty-six patients with NHL, 38 males and 28 females, their age range from 1–15 years old were included in this study. They were attendants of oncology clinic at central pediatric hospital in Baghdad from 20$^{th}$ of the December 1999 to 20$^{th}$ of December 2002. The diagnosis in each case was established by clinical and histopathological examination. In addition, Thirty-three, age and sex matched, apparently healthy children, were included as a control group.

**Base-line data:** For each patient the following information was obtained: age, sex, histopathological type, the grade and data about the primary site, tumor dimension and the stage of the disease. Moreover, the patients were followed-up with respect to their response to chemotherapy and survival.

**Samples:** Two ml venous blood was aspirated from each subject. The blood samples were collected in plain tubes and left to clot, and then centrifuged at 2000 rpm for 5 minutes at room temperature to separate the sera, which were

dispensed into sterile tightly closed Eppendorf tubes (0.1ml aliquots) and stored at –20C until assayed.

**Method**

The measurement of cytokines by a solid-phase, sandwich, enzyme-linked immunosorbent assay (ELISA) was performed as described previously by Hsu, et al (4) with some modification, and was as the following: microtiter plates were prepared by coating each well with 100ul of capture mAb diluted to (5ug/ml) in coating buffer (0.1 M carbonate/bicarbonate buffer, Ph 9.6). After overnight incubation at 4C, plates were emptied and washed with washing buffer (PBS containing 0.05% Tween-20). Uncoated site were blocked with 100 ul/well blocking buffer (0.1% bovine serum albumin in an adequate coating buffer) for one hour at 37C, incubation was carried out in a shaking incubator. 100ul of (1/40 diluted) patient's serum in dilution buffer (PBS pH 7.4 containing 0.5% BSA and 0.5% Tween-20) were added to each well and 100ul of dilution buffer alone was run on each plate as a negative control. Patients and control samples were allowed to incubate 2 hours at room temperature. After washing of the plates, 100ul of diluted biotinylated detection mAbs (1 ug/ml) was added to each well. Following another 2 hours incubation, plates were washed and 100ul of (1/500) diluted streptavidin-HRP was added to each well. After 30 minutes incubation, plates were washed and 100ul of a substrate/chromogen (H2O2/OPD) freshly prepared working solution was added. After 10 minute, 25ul of stopping buffer (4N H2SO4) was added to each well to terminate the reaction. Plates were read at 492 nm with an automated microplate reader to determine the absorbency (OD-values).

## Results

### Serum levels of the cytokines:

The sera of all patients with NHL showed a significantly higher levels of IL-10, IL-4 and IFN- γ than did the control sera (P<0.001, 0.05, and 0.01, respectively) (Table-1).

**Table (1): The mean ± SE of the (O.D. value) of the serum IL-10, IL-4 and IFN-g in each NHL-patients groups and in control group.**

| | SNCL | LB | LC | MC | control |
|---|---|---|---|---|---|
| IL-10 | 0.56± 0.01 a | 0.52±0.01 b | 0.52±0.02 b | 0.59±0.02 c | 0.29 ±0.003 d |
| IL-4 | 0.46±0.01 a | 0.39±0.03 b | 0.45±0.01 a | 0.45±0.01 a | 0.28 ±0.001 c |
| IFN-γ | 0.34 ±0.01a | 0.29 ±0.01 b | 0.36 ±0.02 c | 0.37 ±0.02 c | 0.30 ±0.001 d |

Note: SNCL (small noncleaved cells malignant lymphoma), LB (lymphoblastic malignant lymphoma), LC (large cells malignant lymphoma), MC (Mixed cells malignant lymphomas). Means carry different letters (within the rows) are significantly different at 5% level.

The increased level of IL-10 was more pronounced the following subgroups of patients: males, less than 10 years old, advanced stages (III&IV), high grade, small noncleaved cells (SNCL), non-responder to chemotherapy progressive and stand still (PD&St.D), and dead patients (P<0.01) (Table-2). Whereas, The increased level of IFN- γ was more pronounced in the following subgroups of patients: females, more than 10 years old, stage II, intermediate grade, lymphoblastic malignant lymphoma (LB), responder to chemotherapy complete and partial respond (CR&PR), and survivals (P<0.05, 0.05, 0.01, 0.01, 0.05, 0.01 and 0.01, respectively). (Table-2).

**Table (2): The mean±SE of IL-10, IL-4 and IFN-g in NHL patient.**

| | | IL-10 | IL-4 | IFN- γ |
|---|---|---|---|---|
| Sex | Male | 0.59 ± 0.02 | 0.43 ± 0.014 | 0.32 ± 0.01 |
| | Female | 0.52 ± 0.01 | 0.44 ± 0.014 | 0.36 ± 0.01 |
| Age | > 10 years | 0.49 ± 0.014 | 0.44 ± 0.01 | o.33 ±0.01 |
| | < 10 years | 0.61 ± 0.012 | 0.44 ± 0.014 | 0.35 ±0.011 |
| Grade | Intermediate | 0.48 ± 0.013 | 0.46 ± 0.012 | 0.30 ±0.02 |
| | High | 0.62 ± 0.013 | 0.42 ± 0.011 | 0.36 ±0.012 |
| Stage | II | 0.51 ± 0.01 | 0.41 ± 0.01 | 0.40 ±0.02 |
| | III | 0.56 ± 0.015 | 0.48 ± 0.012 | 0.33 ±0.01 |
| | IV | 0.58 ± 0.011 | 0.40 ± 0.011 | 0.29 ±0.013 |
| Histopathology | SNCL | 0.55 ± 0.012 | 0.46 ± 0.013 | 0.34 ±0.01 |
| | LB | 0.50 ± 0.015 | 0.39 ± 0.014 | 0.32 ±0.01 |
| | LC | 0.50 ± 0.012 | 0.45 ± 0.014 | 0.35 ±0.01 |
| | MC | 0.54 ± 0.013 | 0.45 ± 0.011 | 0.34 ±0.02 |
| Response to chemotherapy | CR | 0.36 ± 0.012 | 0.45 ± 0.011 | 0.37 ± 0.01 |
| | PR | 0.55 ± 0.01 | 0.43 ± 0.013 | 0.36 ± 0.01 |
| | St.D | 0.58 ± 0.014 | 0.48 ± 0.011 | 0.34 ± 0.01 |
| | PD | 0.60 ± 0.016 | 0.39 ± 0.012 | 0.29 ± 0.01 |
| Survival | Survive | 0.47 ± 0.013 | 0.45 ± 0.014 | 0.44 ± 0.011 |
| | Death | 0.63 ± 0.012 | 0.43 ± 0.013 | 0.23 ± 0.01 |

**Note: SNCL (small noncleaved cells malignant lymphoma), LB (lymphoblastic malignant lymphoma), LC (large cells malignant lymphoma), MC (Mixed cells malignant lymphomas). CR (complete**

**response), PR (partial response), St.D (stand still disease), PD (progressive disease)**

The results of the control group were used to determine the cut-off value for the OD of the serum levels of IL-10, IL-4 and IFN-g. The cut-off value was calculated as the upper limit of the 99% confidence interval (CI), which is (mean of OD-value + 2.57(STDxSEM) (5). The upper limit was calculated using the t-distribution of the OD-values to take into consideration the small number of observation and to reduce the size of error in passing a decision for evaluation of which of these is elevated and which of these is normal. On the lights of these values the results of the patients were presented as elevated and normal (Table-3).

**Table (3): The results of the IL-10, IL-4, and IFN- γ in patients' sera in comparison to the cut-off value.**

| | | SNCL | LB | LC | MC | Total patients |
|---|---|---|---|---|---|---|
| IL-10 | Elevated | 27 | 6 | 4 | 4 | 41 |
| | Normal | 10 | 5 | 5 | 5 | 25 |
| IL-4 | Elevated | 15 | 3 | 3 | 3 | 24 |
| | Normal | 22 | 8 | 6 | 6 | 42 |
| IFN- γ | Elevated | 2 | 0 | 3 | 4 | 9 |
| | Normal | 35 | 11 | 6 | 5 | 57 |

Elevated serum level of IL-10, IL-4, IFN- γ detected in 62%, 36.4%, 13.6% of the patients respectively. Such observation was significant in IL-10 ($P< 0.05$), especially SNCL patients.

## Relationship among different cytokines in patients' sera

Table (4) summarized the correlation among IL-10, IL-4, and IFN- γ in all patients' sera. The serum level of IL-10 correlates positively with IL-4 ($r=0.360$; $P<0.05$) and correlates negatively with IFN- γ ($r= -0.632$; $P<0.01$). IL-4 serum level has a weak negative correlation with INF- γ ($r= -0.233$; $P<0.05$).

**Table (4): the correlation among different studied cytokines.**

| Cytokines | IL-10 | IL-4 | IFN- γ |
|---|---|---|---|
| IL-10 | …… | + 0.360 | - 0.632 |
| IL-4 | | …… | - 0.233 |
| IFN- γ | | | …… |

## The relationship among the serum levels of each cytokines with different clinical and demographic finding

Serum level of IL-10 revealed a positive correlation with male sex (r= 0.226; P<0.05), stage (r= 0.473; P, 0.05), Grade (r= 0.533; P<0.01), and negative correlation with age (r= -0.358; P<0.05), response to chemotherapy (r= -0.891; P<0.01) and with survival (r= -0.790; P<0.01). IL-4 revealed no clear correlation neither with good nor with bad prognostic signs. Except a weak negative correlation with response to chemotherapy and survival (r= -0.296 and r= -0.226; P< 0.05 in both cases). Whereas, IFN- γ serum level showed a positive correlation with age (r= 0.430; P<0.05), response to chemotherapy (r= 0.788; P<0.01), survival (r= 0.441; P<0.05) and a negative correlation with male sex (r= -0.164;P<0.05), Grade (r= -0.430, P<0.05), stage (r= -0.318, P<0.05). (Table-5).

**Table (5): The correlation of different cytokines with other prognostic factors.**

| | IL-10 | IL-4 | IFN- γ |
|---|---|---|---|
| Male sex | + 0.226 | + 0.120 | - 0.164 |
| Age | - 0.358 | - 0.083 | + 0.430 |
| Grade | + 0.533 | + 0.164 | - 0.476 |
| Stage | + 0.473 | + 0.092 | - 0.318 |
| Histopathology | + 0.079 | + 0.053 | - 0.070 |
| Response to chemotherapy | - 0.891 | - 0.296 | + 0.788 |
| Survival | - 0.793 | - 0.226 | + 0.441 |

The results of IL-10, IL-4 and IFN- γ in sera of NHL patients in comparison to the cut-off value were summarized in (Table-6). The results showed the frequency of patients in each prognostic group.

**Table (6): The NHL patients' sera levels of IL-10, IL-4 and IFN-g in comparison to the Cut-Off value.**

| | | IL-10 | | IL-4 | | IFN-g | |
|---|---|---|---|---|---|---|---|
| | | Normal | Elevated | Normal | Elevated | Normal | Elevated |
| Sex | Male | 14 | 23 | 21 | 16 | 33 | 4 |
| | Female | 11 | 18 | 21 | 8 | 24 | 5 |
| Age | >10 years | 9 | 19 | 18 | 10 | 26 | 2 |
| | <10 years | 16 | 22 | 24 | 14 | 31 | 7 |
| Stage | II | 8 | 2 | 4 | 6 | 5 | 5 |
| | III | 13 | 30 | 31 | 12 | 40 | 3 |
| | IV | 4 | 9 | 7 | 6 | 12 | 1 |
| Grade | Inter-mediate | 15 | 39 | 35 | 19 | 52 | 2 |
| | High | 10 | 2 | 7 | 5 | 5 | 7 |
| Histopath-ology | SNCL | 10 | 27 | 22 | 15 | 35 | 2 |
| | LB | 5 | 6 | 8 | 3 | 11 | 0 |
| | LC | 5 | 4 | 6 | 3 | 6 | 3 |
| | MC | 5 | 4 | 6 | 3 | 5 | 4 |
| Response to therapy | CR | 5 | 4 | 6 | 3 | 2 | 7 |
| | PR | 15 | 7 | 19 | 11 | 20 | 2 |
| | St.D | 2 | 9 | 4 | 5 | 11 | 0 |
| | PD | 2 | 9 | 5 | 5 | 11 | 0 |
| Survival | Live | 24 | 29 | 34 | 19 | 44 | 9 |
| | Dead | 1 | 12 | 8 | 5 | 13 | 0 |

## Discussion

Although, the start points in the use of Th-1/Th-2 paradigm was directed against its role in the manipulation of immune response (6), the latter discovery that T-cells may be polarized during an ongoing disease or immune response into the so called Th-1 and Th-2 subsets (7) encourage study their role in pathogenesis of different diseases. An additional impetus for such investigation in later years has been provided by the discovery that Th-1 and Th-2 subsets display distinct cytokines

profile and effectors function and the Th-1 secrete IL-2, IFN- γ and TNF-β, then provide prediction by their ability to promote cell-mediated immune responses (43) encourage clinical studies to investigate the therapeutic implication of such paradigm manipulation in different diseases (8) and to study the possible utility of this paradigm in follow-up of the patient and possible role in prediction of the prognosis and outcome of malignant diseases (11, 12, 13).

While the research on the prognosis of NHL is still far from completion, a few common interesting paradigms emerge, as prognostic factors in different diseases and have received prominent attention; one of these paradigms is the Th-1/Th-2 ratio.

We conducted this study to examine the correlation of Th-1/Th-2 cytokines profile (IFN-g/IL-10 and IL4) in patient sera with previously reported prognostic factors, aiming to investigate any possible role of this paradigm in the prediction of prognosis and outcome of childhood NHL patients.

The results of this study may help in answering three possible sets of question. The first set regards the possible role of these cytokines in the prognosis and prediction of the disease outcome.

Our results revealed a positive correlation of IL-10 with bad prognostic factors and a negative correlation of IFN-g with bad prognostic factors, these data may suggest a possible role for the circulating levels of IL-10 and IFN-g in prediction of increased disease severity and mortality in childhood NHL patients. These data supported by the following observation in this study:

- The levels of IL-10 were significantly higher in males than in females. Whereas, IFN- γ levels higher in female sex group.
- The levels of IL-10 were significantly higher in children with less than 10 years old than those with more than 10 years old. Whereas, IFN-g levels were higher in children with more than 10 years old.

- The levels of IL-10 were significantly higher in high grade than in intermediate grade. Whereas, IFN-g levels were higher in intermediate grade when compared with high grade.
- The levels of IL-10 were significantly higher in stage IV than in stage III and II. Whereas IFN-g levels were higher in stage II.
- The levels of IL-10 were significantly higher in poor responding patients to chemotherapy (St.D and PD groups). Whereas, IFN-g levels were higher in good responding patients (i.e. CR and PR patient groups).
- The levels of IL-10 were significantly higher in the non-survivors (dead) than in the survivors. Whereas, IFN-g levels were higher in survivors patient group.

These data may indicate a possible role of any of these cytokine (IL-10/IFN-g) levels alone or as a paradigm in prediction of bad or good prognosis and existence of IL-10 alone or with IFN-g as serum marker(s) for disease severity in NHL independent of and additive to the other factors. IL-4 showed no clear cut correlation neither with good nor with bad prognostic factors and this may indicate that this cytokine is not obligatory participant in the disease prognosis or out come.

These findings are consistent with previous studies done by( 9, 10)whom detect a high serum level of IL-10 in about 50% of NHL patient, and we differ from this study in the percentage of the patients with high serum level of IL-10, we detect a high serum levels in about (62%). IL-10 production has been reported as negative prognostic factors by(11), in patients with Melanoma and other solid tumors particularly with lung, gastrointestinal, and renal cell cancers. High level of IL-10 in non-responder patients after chemotherapy was observed by (11, 12).

The second set of questions regards the source of IL-10 in NHL patients. Normally the major source of IL-10 in vivo seems to be macrophages (14). In addition to T-cell subsets (Th-2, Tc-2, Tr-1) (15) and monocytes (16). Other cells like B-

cells (17), eosinophils (18), Mast cells (19) and probably Keratinocyte (20). The proposed elevated level of IL-10 in NHL, in T-cell NHL most probably Th-2 cells since they associated with elevated IL-4 also. But most of our patients were B-cell NHL and not associated with coincidental increase in IL-4. There are two possible source of IL-10, the first is the tumor cells (malignant B-cells) since the tumor cells from B, T, and NK-cell lymphoma are able to produce biologically active IL-10 as reported by (21, 22). The second source could be the viruses, since certain viruses have the ability to induce production of host IL-10 by macrophages such as respiratory syncytial viruses, human rhinoviruses-14, and parainfluenza viruses-3, whereas other viruses encode their own viral IL-10 homologs such as EBV, CMV (23, 24, 25), so the source of IL-10 in our patient sera could be of viral origin (since some of our patients were found to be positive for EBV, and CMV as previously reported by (42).

The stress mediators seem to play an important role in regulating IL-10 production (12), baby with NHL expose to different kind of stress, including the stress of repeated injection (trauma), and stress of pain, and in some patient the stress of the major operation (surgery). All kind of the stress in our patients could be another source for production of IL-10.

A third possible set of question regarding How can IL-10 predict the worse prognosis? Our data (negative correlation between IL-10 and IFN-g) may indicate that IL-10 could mediate its action at least in part via decrease IFN-g production, thus decrease the cell mediated immune responses which is important in control tumor establishment, progress and metastasis. Other possible mechanisms could be: In humans, a Th-cell phenotype in which high level of IL-10 appears to favor deactivation of an inflammatory response (26). The potent of IL-10 as anti-inflammatory agent derives from its capability to inhibit the monocytes/macrophage. IL-10 has been shown in vitro to potently down regulate LPS induced production of TNF-alpha, IL-1, IL-6, IL-8, G-CSF, and GM_CSF (16). It also

inhibits LPS induced and hyaluronan induced synthesis of macrophage inflammatory protein-1 alpha (MIP-1 alpha) and MIP-1 beta, two members of the C-C family of chemokines (27, 28). IL-10 also suppresses macrophage synthesis of reactive oxygen intermediate (29) and nitric oxide (30) and blocks cyclo-oxygenase-2 dependent synthesis of interstitial collagenase and gelatinase B (31). Moreover, in monocyte cultures, IL-10 has been shown to diminish cell surface expression of P75 TNF-receptors (32) and soluble IL-1ra (33). Taken together, IL-10 produces diverse anti-inflammatory effects.

Other studies reported an association between IL-10 release, immunodepression, and decreased resistance to infections (34,35). Moreover, IL-10 suppresses proinflammatory cytokines production and the Ag-presenting capability of monocyte/macrophage and DCs (36); therefore IL-10 represents a substantial suppressor of the cellular immunity (14). In addition IL-10 inhibits the LPS-induced synthesis of proinflammatory mediators in eosinophils and mast cells ( 37, 38 ). IL-10 effects seem to inhibit both the Th-1type and Th-2 type responses, although the effect on Th-1 cell appears to be stronger (39). Furthermore, there are several lines of evidence that IL-10 over expression in different malignancies might contribute to tumor development, in particular, by suppressing the anti-tumor immune responses (as we mention before) and IL-10 might even be a tumor cell growth factor in certain tumor, such as B-cell lymphoma and melanoma (40). Finally, (41) reported that IL-10 increasing Fas/Fas-L mediated apoptosis and the activated lymphocyte could be removed by this mechanism during the course of malignancy.

## Conclusions

our study may indicate the existence of IL-10/IFN-g as serum markers for disease severity and predictor of response to chemotherapy in NHL-patients, independent of and additive to the other factors. However, the clinical significance of this

study is uncertain for three reasons: 1- It was performed in small number of patients. 2- It was performed in a single center (Central Pediatric Hospital), and although we have no referral system at all, still referral bias unavoidable. 3- No previous study has had sufficient size to address a number of important issues relating to the elaboration of cytokines in NHL, including the influence of the age, sex, and the histopathological type of NHL on cytokine level.

## References

1- Sirpa, J.,Jalkanen, Heikki, J., Pekka, K., (1990). Prognostic value of lymphocyte homing receptors and S-phase fraction in NHL. Blood ;75(7): 1544-56.
2- Iraqi cancer research center (1999). Ministry of health, Iraqi cancer board: results of Iraqi cancer research 1995-1997 Baghdad, Iraq.
3- Jalkanen, S., Joensuu, H., Soderston, K., (1991). Lymphocyte homing and clinical behavior of NHL. J Clin Invest; 87:1835-40.
4- Hsu, S., Raine, E., Fanger, H., (1981): Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled Ab (PAP) procedure. J Histochem Cytochem; 29 : 577-80.
5- Al-Murani, W., Al-Shummari, A., Al-Obaidi, A.,(2000): New approach for the calculation of Cut-off value in immunological and diagnostic tests. Iraqi J Microbiol. 12(1):1-9.
6- Mosmann, T., Cherwinski, H., Bond, M., et al.(1986). Two types of murine T-cells clone. I-Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136: 2348-57.
7- Romagnani, S., (1997). The Th-1/Th-2 paradigm. Immunol Today 18:263-66.
8- Parronchi, P., Maggi, E., Romagnani, S., (1999). Redirecting Th-2 responses in allergy. Curr Topics Microbiol Immunol 238:27-56.
9- Blay, J., Burdin, N., Rousset, F., et al. (1993). Serum IL-10 in NHL: a prognostic factor. Blood 82: 2169-74.

10-Stasi, R., Zinzani, L., Galieni, P., et al (1994). Prognostic value of serum IL-10 and soluble IL-2R levels in aggressive NHL. Br J Haematol 88: 770-77.
11-Ordemann, J., Jacobi, C., Barumann, C., et al (2002). Immunomodulatory changes in patients with colorectal cancer. Int J Colorect. Dis. 17:37-41.
12-Wojciechowska, C., Asudullah, K., Nestler, D., et al (1998). Sympathetic activation triggers systemic IL-10 release in immunodepression induced by brain injury. Nat Med 4:808-13.
13-Nemunaitis, J., Fong, T., Shabe, P., et al (2001). Comparison of serum IL-10 levels between normal volunteers and patients with advanced melanoma. Cancer Invest. 19: 239-47.
14-Spits, H., Malefyte, R., (1992). Functional characterization of human IL-10. Int Arch Allergy Appl Immunol 99:8-15.
15-Fiorentino, D., Bond, M., Mosmann, T.,(1989). Two types of mouse T-helper cell. IV-Th-2 clone secrete a factor that inhibits cytokin production by TH-1 clones. J Exp Med 170:2081-95.
16-Malefyte, R., Abrams, J., Bennett, B., et al (1991): IL-10 inhibits cytokin synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 174: 1209-20.
17-Pistoia, V., (1997). Production of cytokin by human B-cells in health and disease. Immunol Today 18:343-50.
18-Nakajima, H., Gleich, G., Kita, H., (1996). Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. J Immunol 156: 4859-66.
19-Lin, T., Befus, A., (1997). Differential regulation of mast cell function by IL-10 and stem cell factors. J Immunol 159: 4015-23.
20-Teunissen, M., Koomen, C., Jansen, J., et al (1997). In contrast to their murine counterparts, normal human keratinocyte and human epidermoid cell lines A431 and HaCat fail to express mRNA and protein. Clin Exp Immunol 107:213-23.
21-Boulland, P., Gaulard, P., (1998). Human IL-10 expression in T/natural killer-cell lymphomas: association with anaplastic

large cell lymphomas and nasal NK-cells lymphomas. Am J Pathol 153:1229-37.
22-Jones, K., Aoki, Y., Chang, Y., et al (1999): Involvement of IL-10 and viral IL-6 in the spontaneous growth of Kaposi's sarcoma herpesvirus-associated infected primary effusion lymphoma cells. Blood 94:2871-79.
23-Kotenko, S., saccani, S., Izotova, L., et al (2000). Human CMV-harbors its own unique IL-10 homology (CMV-IL-10). Proc Natl Acad Sci USA 97: 1695-700.
24-Moore, K., Malefyte, R., Coffman, R., et al (2001). IL-10 and the IL-10 receptor. Annu Rev Immunol 19: 683-765.
25-Spenscer, J., Lockridge, K., Barry, P., et al (2002). Potent immunosuppressive activities of CMV-encoded IL-10. J Virol 76: 1285-92.
26-Katsikis, P., Cohen, S., Londei, M., et al (1995). Are CD4 positive Th-1 cells proinflammatory or anti-inflammatory? The ratio of IL-1 to INF-g or IL-12 determines their function. Int Immunol 7: 1287-94.
27-Horton, M., Burdick, M., Strieter, R., et al (1998). Regulation of hyaluonan induced chemokine gene expression by IL-10 and IFN-g in mouse macrophages. J Immunol 160: 3023-30.
28-Sherry, B., Espinoza, M., Manogue, K., et al (1998): induction of the chemokine B peptides, MIP-Ia and MIP-1b, by lipopolysaccharide is differentially regulated by immunomodulatory cytokines IFN-g, IL-10, IL-4 and TGF-B. Mol Med 4: 648-57.
29-Bogdan, C., Vodovotz, Y., Nathan, C., (1991). macrophage deactivation by IL-10. J Exp Med 174:1549-55.
30-Cenci, E., Romani, L., Mencacci, A., et al (1993). IL-4 and IL-10 inhibit nitric oxide dependent macrophage killing of candida albicans. Eur J Immunol 23: 1034-38.
31-Meriz, P., DeWitt, D., Stevenson, W., Wahl, L., (1994). IL-10 suppression of monocyte prostaglandin H synthase-2. J Biol Chem 269: 21322-29.
32-Joye, D., Gibbons, D., Green, P., et al (1994). Two inhibitors of pro-inflammatory cytokines release, IL-10 and IL-4, have

contrasting effects on release of soluble P75 TNF-R by cultured monocytes. Eur J Immunol 24:2699-705.

33-Jenkins, J., Malyak, M., Arend, W., (1994). The effects of IL-10 on IL-1R antagonist and IL-1 beta production in human monocyte and neutrophiles. Lymphokine and cytokine Res 13:47-54.

34-Volk, H., Reinke, P., Docke, W., (2000). Clinical aspects from systemic inflammation to "immunoparalysis". Chem Immunol 74: 162-77.

35-Spies, C., Kern, H., Schroder, T., et al (2002). Myocardial ischemia and cytokine response are associated with subsequent onset of infections after noncardiac surgery. Anesth Analg 95:9-18.

36-Romagnani, S., (1995). Biology of human Th-1 and Th-2 cells. J Clin Immunol 15:121-29.

37-Takanaski, S., Nonaka, R., Xing, Z., et al (2004). IL-10 inhibits LPS-induced survival and cytokine production by human peripheral blood eosinophils. J Exp Med 180:711-715.

38-Arock, M., Zuang, A., Singer, M., et al (1996). IL-10 inhibits cytokine generation from mast cells. Eur J Immunol 26: 166-70.

39-Asudullah, K., Sterry, W., Stephane, K., et al (1998). IL-10 is a key cytokine in Psoriasis: proof of principle by IL-10 therapy- a new therapeutic approach. J Clin Investig 101: 783-94.

40-Matsuda, M., Salazar, F., Petersson, M., et al (1994). IL-10 pretreatment protects target cells from tumor- and allo-specific cytotoxic T-cells and down-regulates HLA-class I expression. J Exp Med 180: 2371-76.

41-Georgescu, L., Vakkalanka, R., Elkon, K., Crow, M., (1997). IL-10 promotes activation-induced cell death of SLE lymphocytres mediated by Fas-ligand. J Clin Investig 100: 2622-33.

42-Abdul-Razak, H., Ahmed, Nidhal, Abdul-Muhymen,M. Ibraheem, J., Al-Naser, Rajii, M., Al-Hadythi, (2001). Immunophenotyping of childhood NHL in Iraq. Ph.D thesis submitted to Al-Nahrin Medical College.

43-Abbas, A., Murphy, K., Sher, A., : Functional diversity of helper T-lymphocytes. Nature 1996; 383: 787-93.