**IJH Iraqi Journal of Hematology**

**i**n a Peer Reviewed Scientific biannual Medical Journal published by the National Center of Hematology Al-Mustansiriya University, Baghdad-Iraq.

**November 2015**

**Volume 4, Issue 2**

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First issue published in 2011
First editor-in-chief Dr. Nabil Salman Murad
First editor director Dr. Adeeb Alshami
Dear doctors and colleagues

We would like to congratulate all the colleagues and specially those who work in the hematology field, clinical and laboratory on publishing the volume 4 issue 2 of the Iraqi Journal of hematology. This issue contains 4 original research in addition to case report that has been accepted to be presented by author during the 5th annual meeting of the national center of hematology that arranged to be held in Baghdad, cristal grand Ishtar hotel in 7 November 2015. We sincerely hope from the authors to continue their support and cooperation through sending original articles, case reports, scientific comments and criticism to the editors in order to keep the journal going on and to keep raising its standards. This issue contains the following: ten original scientific papers,

Kindest regards

Editorial Board
The Iraqi Journal of Hematology is a periodic peer-reviewed journal published biannually by the National Center of Hematology with the cooperation of the Iraqi Society of Hematology. The journal welcomes original articles, case reports and letters to editor in all fields relevant to Hematology. Review articles are also welcomed. However, review articles of high standards will be considered. Arabic or English languages could be used.

Papers are accepted on the understanding that the subject matter has not and will not be submitted simultaneously to another journal. The following notes are expected to be considered carefully in writing manuscripts.


2- Three clear and complete copies (including figures and tables) should be submitted. Manuscripts and figures will not be returned to the authors irrespective of the editorial decision to accept, revise or reject them.

3- Manuscripts must be accompanied by a covering letter signed by all authors that the paper has not been published and will not be submitted to another journal if accepted in the Iraqi Medical Journal.

4- The title page should include:
   - Titles of the paper in Arabic and English.
   - Correct first name, middle name and family name of all authors in Arabic and English as well as a maximum of two highest academic degrees for each author.
5- Abstracts for original articles should contain a structured abstract of no more than 250 words in Arabic and English. Abstract headings include: Background, Objectives, Methods, Results and Conclusions.

Abstracts in English of the review articles and case reports should be unstructured and of not more than 150 words.

6- The main text of the original article should be divided into sections; each section should be started on a new page after the title page:

A. Introduction: should state clearly the purpose and rationale of the study.

B. Methods: should include selection of subjects, identifications of the methods, apparatus and chemicals used and include statistical analysis.

C. Results: presented in a logical sequence preferably with tables and illustrations emphasizing in the text only the important observations.

D. Discussion: emphasizes new findings of the study, implications and reference to other relevant studies.

E. Acknowledgements: only to persons who have made substantive contribution to the study.

F. References: should be in the Vancouver style. They should appear in the text by numbers in the order. List all authors when six or less; when seven or more, list only first six and add et al. Journal titles should be abbreviated in accordance with index Medicus. Examples of correct reference forms are given as follows:


7- Illustrations: photographs unmounted on glossy paper should be provided with magnification scale if appropriate. Lettering should be in either letraset or stencil of comparable size. Illustrations should be marked on the back with the figure number, title of the paper
and name(s) of the author(s) with soft pencil. All photographs, graphs and diagrams should be referred to as figures and should be numbered consecutively in the text in Arabic numerals. The legends to illustrations should be typed on a separate sheet. Tables should be numbered consecutively in the text in Arabic numerals and each typed on a separate sheet. Vertical lines normally will not be printed.

8- Measurement is preferably expressed in SI units.
9- Use only standard abbreviations in the title and abstract. The full term for which the abbreviations stand should precede its first use in the text.
10- Page proof will be sent to the corresponding author for proof correction. Major alterations from the text cannot be accepted.

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LEARNING OBJECTIVES

- Define Liposomes; review their discovery and their clinical applications.
- Describe Liposomal Drug Delivery System and its use in various drug products in Clinical Use.
- Describe in details indications, efficacy and safety of old and current liposomal drug products used in benign and malignant hematology.
- Describe clinically relevant pharmacokinetic and efficacy differences between conventional drugs and liposomal drugs used in hematology.

INTRODUCTION TO LIPOSOMES AND THE LIPOSOMAL DRUG DELIVERY SYSTEM

DEFINITION

Liposomes derived by the combination of two Greek words, “lipos” meaning fat and “soma” meaning body. They are synthetic spherical molecules 20nm-20 μm in diameter formed from self-assembly of lipids. ¹ They are composed of self-assembled spherical vesicles consisting of one or multiple lipid bilayers surrounding an internal aqueous core. ² Since their discovery, liposomes have become one of the most highly investigated nanostructures used in nanomedicine and Bionanotechnology. ²

HISTORICAL PERSPECTIVES

In their 1965 citation classic, the late Alec Bangham and colleagues published the first description of swollen phospholipid systems that established the basis for model membrane systems. ³ Within a few years, a variety of enclosed phospholipid bilayer structures

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consisting of single bilayers, initially termed ‘bangosomes’ and then ‘liposomes’, were described, and the early pioneers such as Gregory Gregoriadis, established the concept that liposomes could entrap drugs and be used as drug delivery systems.  

STRUCTURE OF LIPOSOMES

Figure 1 showed the structure of the liposomes. As seen in Figure 1A, liposomes are composed of self-assembled spherical vesicles consisting of one or multiple lipid bilayers surrounding an internal aqueous core. Bilayer thickness is 5 nm thick composed of hydrophobic acyl lipid tail region and a hydrophilic headgroup region. Figure 1 B- Electron Microscopy photo of the liposome structure.
CLASSIFICATION OF LIPOSOMES

According to the number and size of lipid bilayers, liposomes can be classified according to their diameter, small (<100 nm), large (100-1000 nm) or giant (>1000 nm), and number of bilayer, single (unilamellar) or multiple (multilamellar) as seen in Table 1.

LIPOSOMES AS A DRUG CARRIER (THE LIPOSOMAL DRUG DELIVERY SYSTEM):

Liposomes are well-established vehicles for the administration of therapeutic and diagnostic agents. Liposomes are reliable drug delivery systems because they are non-toxic, biocompatible, and capable of prolonging bioavailability of the encapsulated agent by reducing or preventing drug degradation and enhancing solubility and stability. Liposomes also open the therapeutic window, reducing adverse effects by altering the pharmacokinetic and pharmacodynamics characteristics of the encapsulated agent.

<table>
<thead>
<tr>
<th>Suffix</th>
<th>Name</th>
<th>Size (Nanometer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV</td>
<td>Small Unilamellar</td>
<td>&lt;40</td>
</tr>
<tr>
<td>LUV</td>
<td>Large Unilamellar</td>
<td>100-1000</td>
</tr>
<tr>
<td>MLV</td>
<td>Multilamellar</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

FDA-APPROVED LIPOSOMAL AND LIPID-BASED PRODUCTS USED IN HAEMATOLOGY

Table 2 – FDA-Approved liposomal and lipid-based products used in Hematology

<table>
<thead>
<tr>
<th>Drug Product</th>
<th>Generic Name</th>
<th>Indication</th>
<th>Year of Approval</th>
</tr>
</thead>
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<tr>
<td>Doxil®/Caelyx®</td>
<td>Doxorubicin</td>
<td>Multiple Myeloma</td>
<td>1995</td>
</tr>
<tr>
<td>DaunoXome®</td>
<td>Daunorubicin</td>
<td>Acute Myeloid Leukemia</td>
<td>1996</td>
</tr>
<tr>
<td>AmBisome®</td>
<td>Amphotericin</td>
<td>Invasive aspergilosis</td>
<td>1997</td>
</tr>
<tr>
<td>Abelcet®</td>
<td>Amphotericin</td>
<td>Invasive aspergilosis</td>
<td>1995</td>
</tr>
<tr>
<td>Amphotec®</td>
<td>Amphotericin</td>
<td>Invasive aspergilosis</td>
<td>1996</td>
</tr>
<tr>
<td>DepoCyt®</td>
<td>Cytosine Arabinoside</td>
<td>Lymphomatous and Neoplastic Meningitis</td>
<td>1999</td>
</tr>
<tr>
<td>Marqibo®</td>
<td>Vincristine</td>
<td>Acute lymphoblastic leukemia</td>
<td>2012</td>
</tr>
</tbody>
</table>

Adapted from: Theresa TM. Allen, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications. Advanced Drug Delivery Reviews 2013; 65: 36–48

ANTIFUNGAL DRUGS

Lipid formulations of amphotericin (Abelcet®, Amphotec and AmBisome®) are polyene antifungals used for the treatment of aspergillosis and yeasts and are significantly less toxic and are recommended when the
conventional formulation of amphotericin is contra-indicated because of toxicity, especially nephrotoxicity or when response to conventional amphotericin is inadequate; and are more expensive.  

Liposomal amphotericin B is as effective as conventional amphotericin B for empirical antifungal therapy in patients with fever and neutropenia, and it is associated with fewer breakthrough fungal infections, less infusion-related toxicity, and less nephrotoxicity.  

**ANTICANCER DRUGS**

Nanomedicine is an attractive option to palliate the shortcomings of chemotherapy, including severe adverse side effects and multidrug resistance.  

Most of the therapeutic agents encapsulated in liposomes are anticancer drugs. Nevertheless, albeit the increasing number of liposomal formulations of anticancer agents entered into clinical trials, few of them have been granted approval for cancer treatment.  

Liposomes have been by far the most used nanovectors for drug delivery, with liposomal doxorubicin receiving US FDA approval as early as 1995.  

**Liposomal Doxorubicin (Doxil®/Caelyx®)**

Doxorubicin is an anthracycline widely used to treat solid and hematological tumors, but its major drawback is its related cardiotoxicity. In cardiotoxicity, positively charged doxorubicin’s affinity for negatively charged cardiolipin, a lipid abundant in heart tissue, is thought to be involved in drug localization in the heart tissue. Polyethylene glycol (PEG)-Liposomal doxorubicin (PLD) (Figure 2) is a formulation of the anthracycline doxorubicin in which the drug is encapsulated in PEG-coated liposomes. This alters the pharmacokinetic properties of doxorubicin, prolong circulation time and enhancing localization to the tumor and avoiding opsonization and destruction by reticuloendothelial system (RES) agents (hepatocytes and Kupffer cells). It is associated with significantly reduced cardiotoxicity.  

Doxil is the first FDA-approved nano-drug and has the most extensive clinical
use in the treatment of solid and AIDS-Related Kaposi’s Sarcoma.\textsuperscript{14}
Haematological Malignancies and

Table 3 showed the toxicity differences between PLD and conventional doxorubicin.\textsuperscript{15}

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Doxorubicin</th>
<th>Caelyx/Doxil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesicant effect</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>Infusion reaction</td>
<td>-</td>
<td>+*</td>
</tr>
<tr>
<td>Nausea/Vomiting</td>
<td>++</td>
<td>+/-</td>
</tr>
<tr>
<td>Myelo-suppression</td>
<td>+++</td>
<td>+ (no gr. 4)</td>
</tr>
<tr>
<td>Stomatitis/Mucositis</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Hand-Foot (HPS)</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Cardiotoxicity</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Alopecia</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Max. Tolerated Dose</td>
<td>60 mg/m\textsuperscript{2}</td>
<td>50 mg/m\textsuperscript{2}</td>
</tr>
<tr>
<td>Dose Intensity</td>
<td>20 mg/m\textsuperscript{2}/wk</td>
<td>12.5 mg/m\textsuperscript{2}/wk.</td>
</tr>
<tr>
<td>Max. Cum. Dose</td>
<td>450 mg/m\textsuperscript{2}</td>
<td>&gt;1000 mg/m\textsuperscript{2}</td>
</tr>
</tbody>
</table>

Palmo-Plantar Erythrodysaesthesia (PPE), also known as hand-foot syndrome is a unique adverse effect of liposomal doxorubicin. It is a cutaneous reaction to the liposomal formulation of doxorubicin due to leakage of small amounts of Caelyx into the capillaries in the palms of hands and soles of feet. It may result in redness, tenderness, and peeling skin that generally seen after 2–3 treatment cycles and can be managed with pyridoxine and corticosteroids and may requires an altered dosing pattern for Caelyx administration. \(^5,16\)

**Indications of Doxil/Caelyx in Hematology:** PLD is FDA-approved for the treatment of multiple myeloma in 2007 in combination with bortezomib in patients who have received at least one prior therapy and who have already undergone or are unsuitable for bone marrow transplant... The dose is 30 mg/m\(^2\) on day 4 of the bortezomib 3-week regimen as a 1-h infusion given immediately after the bortezomib infusion. The bortezomib regimen consists of 1.3 mg/m\(^2\) on days 1, 4, 8, and 11 every 3 weeks. Caelyx dosing should be repeated as long as patients respond satisfactorily and tolerate treatment. \(^16\)

Non-FDA Approved off-label indications of Doxil/Caelyx include:

1. **Aggressive Non-Hodgkin’s lymphoma** such as diffuse large B-cell lymphoma where it replaced the doxorubicin in R-CHOP (DRCOP regimen). The dose of PLD is 40 mg/m\(^2\) (maximum 90 mg) IV infusions over 1 hour. \(^17\)

2. **Cutaneous T-cell Lymphoma:** PLD dose is 20 mg/m\(^2\) days 1 and 15 every 4 weeks for 6 cycles. \(^18\)

3. **Relapsed /refractory Hodgkin’s lymphoma:** PLD was incorporates in the salvage GVD regimen. The dose is 10 mg/m\(^2\) (post-transplant patients) or 15 mg/m\(^2\) (transplant-naïve patients) days 1 and 8 every 3 weeks (in combination with gemcitabine and vinorelbine) for 2-6 cycles.

**Liposomal Daunorubicin (DaunoXome)**

DaunoXome® is a commercial liposomal formulation of daunorubicin.
in which the drug is entrapped into small unilamellar vesicles. It is FDA approved in the treatment of AIDS–related Kaposi’s sarcoma and does not yet gain FDA approval for hematological malignancies.

DaunoXome® has been tested as a single agent or in combination with arabinosyl cytosine in the treatment of patients with acute myeloid leukemia (AML) in relapse or in patients with newly diagnosed AML or with disease failing initial remission-induction therapy. The results have indicated that DaunoXome® can be used at high doses, up to 150 mg/m² for 3 days, safely with acceptable toxicity. The anti-leukemia activity has been reported to be at least equal or superior to that of free daunorubicin. Mucositis appeared more frequently than cardiotoxicity and high complete remission rates have been reported in patients with AML in first relapse.

Latagliata et al. explored the efficacy of liposomal daunorubicin versus daunorubicin in acute myeloid leukemia patients aged older than sixty years. Liposomal Daunorubicin seemed to improve overall survival and disease-free survival in the long-term follow-up, because of a reduction on late relapses.

**Liposomal Cytarabine (Depocyt)®**

DepoCyt (e)® (cytarabine liposome injection) is a sustained-release liposomal formulation of the chemotherapeutic agent cytarabine. DepoCyt(e) is indicated for the intrathecal treatment of lymphomatous meningitis. and is the only liposomal drug administered for intrathecal administration. The drug was granted accelerated approval by the FDA in 1999 and full approval in 2007.

As opposed to conventional cytarabine, which is administered in the hospital twice weekly by spinal injection, DepoCyt(e)® extends the duration of cytarabine efficacy to allow for injection once every two weeks in an outpatient setting.

A randomized Phase III study has shown that liposomal cytarabine injected once every two weeks produced a high response rate (71% versus 15%, P = 0.006) and a better quality of life as measured by Karnofsky score (P = 0.041) relative to that upon treatment.
with free cytarabine injected twice a week. \(^{24}\)

In a phase II European trial of DepoCyt\(\text{e}^{\circ}\) in central nervous system relapse of acute lymphoblastic leukemia or Burkitt's lymphoma/leukemia, the use of Liposomal cytarabine (50 mg) given intrathecally together with systemic or intrathecal dexamethasone once every 2 weeks, liposomal cytarabine showed excellent antileukemic activity. \(^{25}\)

**Liposomal Vincristine (Morqibo)\(^\circ\)**

Morqibo\(^\circ\) is a liposome-encapsulated form of vincristine sulfate, FDA-approved in 2012 and is indicated for the treatment of adult patients with Philadelphia negative acute lymphoblastic leukemia in second or greater relapse or whose disease has progressed following two or more anti-leukemic therapies. The recommended dose is 2.25 mg/m\(^2\) weekly over 1 hour. \(^{26}\), Compared to vincristine sulfate injection, the risk of teratogenicity in pregnancy, death after intrathecal administration and neuropathy seems comparable to vincristine sulfate while the risk of myelosuppression including grade 3-4 cytopenia and tumor lysis syndrome occur with increasing frequency with Morqibo\(^\circ\). Liposomal vincristine may also not be immediately bioavailable compared to vincristine sulfate injection but can be used in relapsed patients as monotherapy resulting in a meaningful clinical outcome such as the ability to bridge to transplantation. \(^{26}\), \(^{27}\)

LIPOSOMAL PRODUCTS USED IN HAEMATOLOGY IN CLINICAL TRIALS

Table 4 showed liposomal drugs used in malignant hematology that are non-FDA approved and still in clinical trials.

<table>
<thead>
<tr>
<th>Drug Product</th>
<th>Generic Name</th>
<th>Indication</th>
<th>Trial Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atragen®</td>
<td>Tretinoin</td>
<td>Acute promyelocytic leukemia</td>
<td>Phase II</td>
</tr>
<tr>
<td>L-Annamycin®</td>
<td>Doxorubicin</td>
<td>Pediatric and Adult Relapsed ALL and AML</td>
<td>Phase I /II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adult Relapsed ALL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doxorubicin-resistant blood cancer</td>
<td></td>
</tr>
<tr>
<td>CPX-351®</td>
<td>Cytarabine: daunorubicin</td>
<td>Acute myeloid leukemia</td>
<td>Phase II</td>
</tr>
<tr>
<td>LEM-ETU</td>
<td>Mitoxantrone</td>
<td>Acute Leukemia</td>
<td>Phase I</td>
</tr>
<tr>
<td>Sideral® Forte</td>
<td>Ferric diphosphate</td>
<td>Iron deficiency anemia, anemia of chronic Kidney disease</td>
<td>Phase II/III</td>
</tr>
</tbody>
</table>
Adapted from: Theresa TM. Allen, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications. Advanced Drug Delivery Reviews 2013; 65: 36–48

**Liposomal Iron (Sideral Forte)**

For the treatment of all anemia’s responsive to oral iron therapy, such as hypochromic anemia associated with pregnancy, chronic or acute blood loss, dietary restriction, metabolic disease and post-surgical convalescence. Useful in treating iron deficiency anemia or increased requirements of Iron and Vitamin C. The iron Included in SIDERAL FORTE®, is uniquely coated with liposomal technology that allows the molecule to pass through the stomach, avoiding any gastrointestinal irritation, to be directly through the lining of the gastrointestinal tract. Oral Liposomal iron allows protecting gastrointestinal mucosal tissue from pro-oxidant effect of iron and guarantees the absolute absence of any side effects, such as gastralgia, nausea, constipation and stain of faeces. Clinical studies performed confirm the better tolerability of liposomal iron.

*Indications of Sideral Forte ® in Hematology :*

1. Iron deficiency anemia
2. Chemotherapy –related anemia
3. Refractory anemia treated with Epo alpha
4. Inflammatory bowel disease
5. Coeliac disease– liposomal iron is gluten-free and is useful in the treatment of coeliac disease patients with iron deficiency anemia
6. Dialysed and Pre-dialysed patients with chronic kidney disease CKD.

In a recent study, 99 patients with chronic kidney disease CKD (stage 3–5, not on dialysis) and iron deficiency anemia [hemoglobin (Hb) ≤12 g/dL, ferritin ≤100 ng/mL, transferrin saturation ≤25%] were assigned (2:1) to receive oral liposomal iron (30 mg/day or a total dose of 1000 mg of IV iron gluconate for 3 months. Oral liposomal iron is a safe and efficacious alternative to IV iron gluconate to correct anemia in ND-CKD patients, although its effects on repletion of iron stores and on stability of Hb after drug discontinuation are lower.
**Liposomal All-Trans Retinoic Acid (Lipo-ATRA) (Atragan) ®**

Liposomal ATRA (Atragan) ® is an intravenous liposomal formulation of ATRA used in the treatment of acute promyelocytic leukemia. It can cure acute promyelocytic leukemia when used as monotherapy and is of value in patients who cannot swallow or absorb capsules, patients with a nasogastric tube, or small children and in unconscious and intubated patients but does not gained approval from the FDA.

In a study aimed to investigate single-agent liposomal all-trans retinoic acid (Lipo-ATRA) in untreated acute promyelocytic leukemia (APL), Induction therapy consisted of Lipo-ATRA 90 mg/m² i.v. every other day. Patients in complete remission (CR) continued to receive Lipo-ATRA 90 mg/m² i.v. three times a week for 9 months. The results were compared with those of a historical control group treated with oral ATRA and idarubicin. Lipo-ATRA induced CR in 79% of patients; CR rates were 92% and 38% in patients with white blood cell (WBC) counts <10 x 10⁹/L and >10 x 10⁹/L, respectively.

**Liposomal Annamycin**

Annamycin is a highly lipophilic form of the anthracycline doxorubicin with the ability to bypass multidrug resistance mechanisms of cellular drug resistance. Clinical trials on this drug include its use in pediatric and Adult relapsed ALL and AML.

In a phase I/II multicenter, open-label, study to determine the maximally tolerated dose (MTD) of nanomolecular liposomal annamycin in adult patients with refractory ALL, Single-agent nanomolecular liposomal annamycin appears to be well tolerated, and shows evidence of clinical activity as a single agent in refractory adult ALL.

**Liposomal Cytarabine Daunorubicin (CPX-351) ®**

(CPX-351) ® is a liposomal cytotoxic combination of Cytarabine: Daunorubicin in a fixed 5:1 molar ratio for the treatment of relapsed and refractory acute myeloid leukemia. In the first Phase II study conducted in man, CPX-351 induction was administered on days 1, 3, and 5 by 90-minute infusion to 48 relapsed or
refractory patients with acute myeloid leukemia (AML) or high-risk myelodysplasia. CPX-351 appears to be well-tolerated and capable of inducing CRs in patients with relapsed or refractory AML. The recommended dose and schedule for phase II study (MTD) is 101 units/m² administered on days 1, 3, and 5 of each induction course.  

CONCLUSION

Liposomal drugs are effective and relatively safe drugs and showed promise in the treatment of difficult –to-treat blood diseases, both benign and malignant. Although the cost and remote toxicity concerns are an issue, extensive preclinical knowledge and clinical expertise is being accumulated and it is quite likely that liposomes will replace many drugs used in the hematology in the future.

References


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Association of human herpesvirus 6 with lymphoid malignancies in Iraqi patients

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2. Professor of clinical hematology/The National center of hematology/ Al-Mustansiriyyah University
3. Dept Med. virology /College of Health and Medical Technology/ Foundation of Technical Education

ABSTRACT:

Background: Human herpesvirus type 6 (HHV-6) is associated with roseola infantum during childhood followed by life-long latency that periodically reactivated in immunocompromised individuals. In spite of several studies to establish the pathogenic role of HHV-6 in lymphoid malignancies, the issue is still controversial.

Objectives: This study was arranged to explore the association of HHV-6 infection in lymphoid malignancies using different serological and molecular techniques and to quantify the plasma viral load.

Patients and methods: This cross-sectional case control study was conducted in National Center for Hematological Diseases (NCHD) at Al-Mustansiriyyah University and Baghdad Teaching Hospital (BTH) in Baghdad-Iraq from September 2013 till April 2015. The patient group consists of 11 patients with Hodgkin lymphoma and 39 Non-Hodgkin's lymphoma of both sexes. The age range was between 15-80 years. The diagnosis of lymphomas was based on hematological and histopathological criteria. 59 apparently healthy individuals were enrolled as control group. They were chosen from unpaid blood donors. The age range was between 18-59 years. Human privacy was respected by taken participant's oral consensus. The seropositivity rate of anti-HHV-6 IgG and IgM antibodies were detected by enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescent test (IFAT). The molecular detection and determination of plasma viral DNA load was achieved by quantitative polymerase chain reaction (qPCR). All data were statistically analyzed, and P values < 0.05 were considered significant.

Results: The anti-HHV-6 IgG positivity rate by IFAT was insignificantly higher in HL (81.8% vs 61.0% p=0.186) and NHL (64.1% vs 61.0%, p =0.758) compared to control group. The anti-HHV-6 IgG positivity rate by ELISA was 81.8% in HL, 84.6% in NHL versus 72.9 % in controls which were insignificant in both groups (p=0.534 and p=0.173) respectively. The anti-HHV-6 IgM positivity rate by ELISA technique among patients with HL was significantly higher compared to controls (27.2% vs 6.8%, p= 0.038), but not significant in NHL (17.9% vs 6.8%, p= 0.086). HHV-6 DNA was detected in (27.3%) patients with HL by PCR technique, but none of the controls or NHL patients was positive. The plasma viral DNA load of the patient with HL was 1.4± 0.3 x10^5 copies/milliliter.

Conclusion: Although a higher anti-HHV-6 antibodies positivity rate among patients with HL and NHL, the pathogenic role of the virus in the development of these malignancies was difficult to be ascertain.

Keywords: Human herpesvirus-6, lymphoid malignancies, Hodgkin's lymphoma
Introduction:
The subfamily Beta herpesvirinae contain lymphotropic viruses that have a lesser confined cell tropism including HHV-6, which belong to the Roseolovirus genus (1,2). The HHV-6 was wide prevalent virus as the transmission occurs easily via saliva and air droplets. The primary infection of HHV-6 established latency, most expected in macrophages and/or monocytes (3,4). Viral reactivation may lead to severe secondary complications particularly in immunocompromised patients as those of bone marrow transplantation (5,6). HHV-6 was divided into 2 subtypes; HHV-6A and HHV-6B (7). HHV-6A involves many strains derived from adult and many researchers thought that the virus more neuroinvasive (8,9). HHV-6B is etiologic agent of roseola infantum, in children; despite the fact that the two viruses have 95% homology in their sequence (10).

Reactivation initiate periodically in immunocompetent carrying the virus in latent stage and the reactivation was asymptomatic, but serious complication can be occurred in immunocompromised individuals (11). The HHV-6 may have a role in malignancies directly by the ability of HHV-6 to infect CD4+ T-cells and induce apoptosis and indirectly may contribute to cancer by immune suppression (12). Currently it became known that HHV-6 can also infect hematopoietic stem cells (HSCs), epithelial cells of the thymus and natural killer cells (NK) and the later have great magnitude for immune maturation as well as protection against cancer and viral infections. Therefore, the active infection of HHV-6 can promote pathologic property of other viral infections (13,14,15).

Previous reports concerning HHV-6 positivity of HL have resulted in conflicting findings. However, an association between the virus and nodular sclerosis subtype of Hodgkin’s lymphoma (NSHL) has been documented by many investigators by different laboratory techniques (16). It was reported that a higher rate of HHV-6 DNA in series of angioimmunoblastic T cell lymphoma (AITL), which is a subtype of T-cell non-Hodgkin’s lymphoma was well characterized, in contrasted with other subtypes of lymphoma and controls (17). Furthermore, a clear association between histological progression of AITL and the detectable copy number of both EBV and HHV-6 B in the AITL tissue specimens was confirmed (18).

Patients and methods:
This prospective cross-sectional study was carried out at the NCH at Al-Mustansyria University and BTH in Baghdad-Iraq from September 2013 till April 2015. The patients group consists of 11 patients (3 male and 8 female) with HL and 39 NHL (21 male and 18 female) and the control group include 59 apparently healthy individuals, randomly selected from unpaid blood donors attending the NBB in Baghdad. The age range of patients with HL was 17-70 years, 20-80 years in NHL and 18-59 in healthy controls. Diagnosis of these malignancies was based on hematological, bone marrow and histopathological criteria. Ten milliliter of venous blood samples were withdrawn aseptically from patients and controls and the blood sample was divided in two parts first with EDTA tube for plasma separation and the second in plane tube for serum separation.

Both serum and plasma sample were stored in aliquots at -80°C. Detection of anti-HHV-
Association of human herpesvirus 6 with lymphoid

6 IgG antibodies was done by ELISA (Abnova company - Taiwan) and by IFAT (VIDIA company, Czech Republic) while the anti-HHV-6 IgM was detected by ELISA (Abnova company - Taiwan). The HHV-6 A-B genome was quantified using the real-time PCR for the (Genetic PCR Solutions TM, Spain). Another PCR kit was used for titration HHV-6 dtec-qPCR DIA.PRO (Diagnostic Bioprobes, Italy). Human privacy was respected by taking participant’s oral consent. Data were analyzed using the SPSS-22 (Statistical Packages for Social Sciences- version 22).

Results:
The anti-HHV-6 IgG positivity rate by IFAT among HL patients and controls. 9 (81.8%) patients and 36 (61.0) controls. The difference between the two groups was statistically insignificant (p=0.186), Table (1).

On the other hand, the anti-HHV-6 IgG positivity rate among HL patients using ELISA was 81.8%, while that of the controls was 72.9 %, the difference between the two groups was statistically insignificant (p=0.534), table (2).

The anti-HHV-6 IgM positivity rate as detected by ELISA technique among patients with HL was significantly higher compared to controls (27.2% versus 6.8%), (p= 0.038), Table (3).

Table (1): Number and percentage of anti-HHV-6 IgG in HL patients compared to control group by IFAT test.

<table>
<thead>
<tr>
<th>IFAT</th>
<th>HL</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
<td>81.8</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>18.2</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>100</td>
</tr>
</tbody>
</table>

P=0.186 (No significant difference between proportions using Pearson Chi-square test at 0.05 level)

Table (2): Number and percentage of anti-HHV-6 IgG in HL patients compared to control group by ELISA.

<table>
<thead>
<tr>
<th>ELISA</th>
<th>HL</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent %</td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
<td>81.8</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>18.2</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>100</td>
</tr>
</tbody>
</table>

P=0.534 (No significant difference between proportions using Pearson Chi-square test at 0.05 level.
Table (3): Number and percentage of anti-HHV-6 IgM in HL patients compared to control group by ELISA.

<table>
<thead>
<tr>
<th>ELISA-IgM</th>
<th>HL</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>27.2</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>72.7</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>100</td>
</tr>
</tbody>
</table>

P=0.038 (Significant difference between proportions using Pearson Chi-square at 0.05 levels.

The HHV-6 DNA was detected in 3 (27.3%) patients with HL, while none of the controls showed positive result. The plasma viral DNA load of the patient with HL was 1.4±0.3 x10^5 copies/mL.

Table (5) showed that the anti-HHV-6 IgG positivity rate among NHL patients using IFAT was 64.1%, and that of the controls was 61.0%. The difference between the two groups was statistically insignificant (p=0.758).

Using the ELISA, the anti-HHV-6 IgG positivity rate among NHL patients was 84.6% versus 72.9% in the controls, (Table 6). Again the difference between the two groups was statistically insignificant (p=0.173).

Table (7) revealed that the anti-HHV-6 IgM positivity rate as detected by ELISA technique was higher among patients with NHL (17.9%) compared to controls (6.8%). However, the difference was failed to reach the levels of statistical significance (p=0.086).

Table (4): Number and percentage of HHV-6 DNA in HL patients compared to control group by PCR.

<table>
<thead>
<tr>
<th>PCR</th>
<th>HL</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>27.3</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>72.7</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>100</td>
</tr>
</tbody>
</table>

Table (5): Number and percentage of anti-HHV-6 IgG in NHL patients compared to control group by IFAT test.

<table>
<thead>
<tr>
<th>IFAT</th>
<th>NHL</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>64.1</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>35.9</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100</td>
</tr>
</tbody>
</table>

P=0.758 (No significant difference between proportions using Pearson Chi-square test at 0.05 level.)
Table (6): Number and percentage of anti-HHV-6 IgG in NHL patients compared to control group by ELISA.

<table>
<thead>
<tr>
<th>ELISA</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>33</td>
<td>84.6</td>
<td>43</td>
<td>72.9</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>15.4</td>
<td>16</td>
<td>27.1</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100</td>
<td>59</td>
<td>100</td>
</tr>
</tbody>
</table>

P=0.173 (No significant difference between proportions using Pearson Chi-square test at 0.05 level)

Table (7): Number and percentage of anti-HHV-6 IgM in NHL patients compared to control group by ELISA.

<table>
<thead>
<tr>
<th>ELISA-IgM</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>17.9</td>
<td>4</td>
<td>6.8</td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>82.1</td>
<td>55</td>
<td>93.2</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100</td>
<td>59</td>
<td>100</td>
</tr>
</tbody>
</table>

P=0.086 (not Significant difference between proportions using Pearson Chi-square at 0.05 levels.

**Discussion:**

In spite of higher positivity rate of HHV-6 IgG detected by IFAT in our HL patients versus healthy controls, there was insignificant difference between the two groups. Similar results were previously reported in HL patients using IFAT (19). In another study, the HHV-6 IgG antibody titer was found to be elevated in relapsed HD patients post therapy in comparison with patients who did not (20). Further analyses by (21) found that increased HHV-6 seropositivity is associated with ratio of geometric mean titer in HD young adults lacking social contact in the family group that may refer to late exposure to HHV-6 in those patients, suggesting that HHV-6 must be incorporated in additional explorations of the etiology of HD.

Using the ELISA technique, again our results showed higher but insignificant positivity rate of anti-HHV-6 IgG among HL patients versus healthy controls. These results are versus the results of previous study which found significant differences in HHV-6 seropositivity rate and titer of antibodies between patients with HD and low-grade NHL in compared to normal controls (21). In the UK, a study investigated case clustering searching for EBV- Reed–Sternberg cell status by detection of EBV and HHV-6 serologic results, found that higher anti-HHV-6 antibody titers was primarily in patients with Reed–Sternberg cells negative for EBV, suggesting an etiologic exposure for HD independent from EBV (22). The high prevalence of antiHHV-6 IgG in the general population clearly documents the wide circulation of this lymphotropic virus that may indirectly contribute to the pathogenesis of the lymphoproliferative disorder (23).

Our results show that there was significant increase in the levels of anti-HHV-6 IgM among patient with Hodgkin's
lymphoma compared to healthy subjects. In this context, our findings are in agreement with previous report in Belem, Brazil which examined a total of 323 patients with lymphadenopathy who were selected and screened for the presence of HHV-6 IgM by ELISA and the results found that 25% of lymphadenopathy cases were positive for HHV-6 IgM antibodies \(^{(24)}\). Although most viral lymphadenopathy is caused by EBV infection, CMV and HHV-6 are rare causes of mononucleosis in approximately 5% of cases \(^{(25,26)}\). Moreover, it has been reported that 3 patients with cervical lymphadenopathy were exhibited an IgM response or a high IgG titer to HHV-6 \(^{(27)}\).

On another hand, two studies by \(^{(28)}\) showed that 5% of normal adults may have detectable IgM.

The HHV-6 DNA detection rate was significantly higher among HL patients in our results. Similar results were reported by previous studies; in one of these the HHV-6 DNA was recorded in 13/45 (28. 8%) biopsies tissue samples from HD by nested PCR, even though no positive cases were discovered by blot. In another study, 12% of HD patients were positive for HHV-6 DNA by PCR \(^{(29,30)}\) investigated both plasma samples and WBC's from patients with HL or NHL for detection of HHV-6 DNA and CMV DNA by PCR technique beside determination of the serum CMV antibody titer, 46% were positive for herpesvirus DNA (HHV6 or CMV) in WBC's or plasma which was significantly higher compared to pediatric control group, and of these 43% had active CMV infection, concluded that the presence of HHV-6 can be considered as a predicting indicator of cellular immunosuppression preceding the onset of CMV infection which may result in a severe outcome among pediatric lymphoma patients. Detection of HHV-6 DNA in lymphoid cells was another line of researches, and in this regard several other studies had yielded variable results; \(^{(31)}\) used PCR for detection of HHV-6 DNA in lymph node specimens of 52 patients with HL, and found that 73 % were positive versus 68.4% positive in the control group. Related results were found that the HHV-6 DNA was integrated into host DNA of lymphoma cells \(^{(32)}\). In another study, the HHV-6 DNA was found in 16.6% in lymphocytes and histiocytes and occasionally in Hodgkin and Reed-Sternberg cells \(^{(33)}\). Additionally, using qPCR in lymph node specimens of 86 patient with HL found that 79.1% were positive for HHV-6 genome, and the positive result was observed most often in the nodular sclerosis group (83.6%) of positive cases \(^{(34)}\).

Keeping in the same line, \(^{(35)}\) used PCR in lymph node specimens, and found that 13 % of patient with HL was positive for HHV6 DNA. While another study used quantitative PCR in lymph node biopsy found that 35.1% were positive for HHV-6 DNA, with all Hodgkin's lymphoma patients infected with HHV-6 presented with the nodular sclerosis subtype \(^{(36)}\). The later finding was supported by a study which found that 86% of nodular sclerosis HL (NSHL) had positive HHV-6 DNA \(^{(16)}\). Obviously, these studies and others offer arguments in favor of an implication of HHV6 in NSHL \(^{(37)}\). On the contrary, in minority of studies, HHV6 DNA was failed to be detected in HL patients \(^{(38)}\).

Taken together, the common feature of these studies and ours is the high detection rate of HHV6 in HL patients; however, discrepancies in the detection rates may be attributed to many factors including
the type of specimens used, type of PCR employed and sample size. Of note, in our study, the real-time PCR was applied on plasma samples. Undoubtedly, beside the opportunity to measure the viral load, the recognition of HHV6 genome in plasma samples was considered as a right indicator of active viral infection and better correlates with clinical outcomes particularly when the plasma viral load was high (30,39).

Although the HHV-6 IgG positivity rate was elevated among NHL patients compared to healthy group; the difference between the two groups was statistically insignificant. These results are consistent with a previous results of a study documented that higher IgG positivity rate among NHL by IFAT (19). Nevertheless, the current results are inconsistent with that reported a significantly higher HHV-6 IgG among lymphoma/myeloma versus healthy controls (23). This discrepancy may be related to the fact that more than one type of blood malignancies was included in that study. Using the ELISA technique for detection of HHV-6 IgG, again our results revealed an insignificantly increase in positivity rate among NHL versus healthy control. These results are agree with prior studies documented a higher but insignificant increase of HHV-6 IgG in NHL compared to normal subjects (19,20).

The positivity rate of HHV-6 IgM in our results as detected by ELISA technique was found to be non-significantly higher in NHL patients versus healthy controls. Unfortunately, studies concerning the HHV-6 IgM in NHL were scares in the literature. However, our results are inconsistent with previous studies stated that 3 instances of patients with integrated HHV-6 into PBMC DNA have been illustrated, the results showed that the HHV6– IgM titer was negative and the IgG titer was either negative or at borderline level in each of three cases. (29,40). Additionally, the present results are also in concordant with the study of (23), who assessed the HHV-6 IgM among lymphoma/myeloma patients; they were unable to detect IgM antibody among any serum sample.

The current results revealed that the HHV-6 DNA was undetected in 39 patients with NHL by PCR. Nevertheless, the possibility that the HHV-6 might be involved in the genesis of some B cell tumors began with the first hint that the HHV6 sequences associated with B cell tumors in a limited number of cases (41), who found that the viral sequence was detected in 4 out of 40 patients with NHL. Similarly results were reported by another study in the same year in which only 2 out of 117 patients with NHL were positive for HHV-6 DNA as detected by blot hybridization (42). Furthermore, two other studies reported a total of three NHL patients were positive for HHV-6 DNA out of 113 patients examined, suggesting that HHV-6 is not likely to have a big etiologic effect in the developing of B-cell NHL (43,44). Moreover, our results are also inconsistent with the study by (45) on 76 patients with NHL; he found that 59% of patients have detectable HHV-6 DNA in biopsy specimens by PCR assay.

Undoubtedly, when we talking about the detection of viral DNA, the type of specimen included and the type of detection tool are critical, and both are largely determine the study outcomes. For instance, application of conventional PCR in peripheral mononuclear cells of children patients with NHL, 33% and 10% of patients and control had detectable HHV-6
DNA respectively \(^{(38)}\). While utilization of immunohistochemistry and Southern blot techniques was failed to detect HHV6 DNA in lymph node biopsy samples \(^{(45,46)}\). Likewise, 45\% of NHL patients were HHV-6 DNA positive by conventional PCR in formalin-fixed and paraffin-embedded lymph node tissues \(^{(18)}\). Similarly, 27 \% of patients with different types of NHL were positive for HHV-6 DNA by PCR \(^{(47)}\).

The presence of co-viral infection may also affect the detection rate. Since accumulated evidences on the role of certain viruses in causing NHL had been previously documented \(^{(48,49)}\). In this context, in a study on detection of herpes virus DNA (HHV6 or CMV) in patients with HL or NHL, the results showed that 46\% were DNA positive in sample of WBC’s or plasma by means of PCR assay and the same study found that 56\% CMV infection were clustered among NHL cases \(^{(30)}\). HHV-6 DNA was identified in lymph node specimens of 53\% from patients with AIDS-associated NHL versus 35\% in HIV-seronegative patients with NHL \(^{(50)}\). It is important to mention that a quantitative real-time PCR for detection of HHV6 A and B genome in plasma or blood samples (DIA-PRO, Diagnostic Bioprobes, Melano-Italy) was employed in our study which is highly sensitive and precise assay. However, the inconsistency of our results with others is largely attributed to the use of plasma instead of whole blood specimens. These findings revealed that there was an increased positivity rate of anti HHV-6 among patients with HL and NHL; however, the pathogenic role of the virus in the development these malignancies was difficult to be ascertain.

**Conclusion:** Although a higher anti-HHV-6 antibodies positivity rate among patients with HL and NHL, the pathogenic role of the virus in the development of these malignancies was difficult to be ascertain.

**References:**


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Microalbuminuria in hemoglobinopathy patients who are taking Deferasirox

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Anwer Sh. Jaafar, C.A.B.M.S (Int. Med)³

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Abstract

Background: Chelation therapy which is needed to prevent or reverse iron overload may also affect renal function in patients with hemoglobinopathy. The early indicator as well as predictor of nephropathy and glomerular damage among patients with sickle cell disease and thalassemia is microalbuminuria (MA).

Objective: This study was aimed to estimate the frequency of MA in patients with thalassemia and sickle cell syndromes who were taking deferasirox and to find if any relationship between the level of MA and other parameters like age, gender, type of hemoglobinopathy, serum creatinine and ferritin levels.

Materials and methods: This is a clinical study in hemoglobinopathy patients that taking deferasirox (oral iron chelator) in Center For Hereditary Blood Diseases (HBDC) in Basrah during the period between April 2013 and February 2014. The informations were took from patients by a questionnaire form and urine samples were collected from each patient for measurement of microalbuminuria by Enzyme-linked immunosorbent assay method (ELISA) and blood samples for biochemical tests including serum creatinine and ferritin.

Results: In this study, 100 patients were 38 males and 62 females with mean of age was 25.74±10.59 years. MA detected in 31 patients and it was more among males (36.8%, 37.0 mg/ml) as compared to (27.4%, 26.0 mg/ml) of female and more in sickle cell syndrome (35%, 35.0 mg/ml) as compared to (25%, 24.0 mg/ml) of thalassemia patients and more in patients with age <30 years (35.5%, 35.5 mg/ml) as compared to patients with age ≥ 30 years (16.7%, 22.0 mg/ml). There was no significant relationship between MA and serum creatinin or serum ferritin levels but significant relationship was found between MA level and age.
Conclusion:
In conclusion, the study might reflect the relatively low prevalence rate of MA among non-proteinuria, deferasirox-taking patients with hemoglobinopathies in Basrah. Microalbuminuria affected by many factors including age, gender, diagnosis and other factors.

Key words: Microalbuminuria, Hemoglobinopathies

Introduction

Hemoglobinopathies are recognized as one of the most common inherited disease worldwide and it is causing a major health burden in Basrah city. The term hemoglobinopathies includes all genetic globin disorders but the main two groups are β-thalassemia disease due to inherited defect in the beta globin chain synthesis and sickle cell disease due to structural defect in hemoglobin SS (Hb SS) molecule (patient has inherited two hemoglobin S genes, one from each parent).\(^1\,^2\)

Hemoglobinopathies were originally characteristic of the tropic and subtropics but are common worldwide due to migration.\(^3\) The estimated prevalence of carriers of any hemoglobin gene variant is higher in South-east Asian region 45.5%, followed by African region 44.4%, Eastern Mediterranean region 21.7% and is lowest in European region 3.3% of population.\(^4\) Although there is a difference in the worldwide prevalence according to the type of hemoglobin disorder, but both sickle cell and thalassemia syndromes are widely spread throughout Mediterranean Basin and Arab Peninsula from Yemen through Saudi Arabia to Iraq.\(^5\) Accurate Information about hemoglobinopathy prevalence in Iraq is lacking with frequency variation in different geographical areas but Basrah is endemic in both thalassemia and sickle cell syndrome (SCS) and first report by Alkasab et al (1981) showed an overall HbS prevalence was 13.3% of the studied cases.\(^6,^7\)

Renal complications and nephropathy are known complications in patients with thalassemia and sickle cell disease. Chronic anemia and iron overload are common mechanisms for renal complications in hemoglobinopathy but other pathologic changes could result in tubular and glomerular function disturbances.\(^8\,^12\) Chelation therapy which is needed to prevent or reverse iron overload may also affect renal function in patients with hemoglobinopathy. Deferasirox, the newer oral iron chelator, can cause increase in serum creatinine, proteinuria, and even renal failure.\(^13,^14\) The presence of chronic kidney disease is significantly shorten the survival of patients with hemoglobinopathy because of association with very high mortality and accelerated cardiovascular disease.\(^15,^16\) Furthermore, several studies suggested that the
Microalbuminuria in hemoglobinopathy patients risk for death is increased independently in patients even with less severe renal dysfunction.\(^{17,18}\)

The early indicator as well as predictor of nephropathy and glomerular damage among patients with sickle cell disease and thalassemia is microalbuminuria (MA).\(^{19,20}\) The aim of study to estimate the frequency of MA in patients with thalassemia and sickle cell syndromes who were taking deferasirox and to find if any relationship between the level of MA and other parameters like age, gender, type of hemoglobinopathy, serum creatinine and ferritin levels.

Subjects, materials and methods:

The study was conducted at Center For Hereditary Blood Diseases HBDC during the period between April 2013 and February 2014, Basrah is southern governorate in Iraq and endemic in Hemoglobinopathy.\(^{6,7}\) The Study subjects consisted of 100 adult patients (38 male and 62 female) (60 with sickle cell disease and 40 with thalassemia disease) who were taking deferasirox (Exjade\(^{®}\), Novartis company, tablets 125, 250 and 500 mg) (dose 20-40 mg/kg, orally) during different intervals of treatment (chronic disease) and having no frank proteinuria on general urine examination (GUE). They were apparently healthy and were recruited as they attended to the outpatient clinic.

The patients had been excluded with age less than 16 years, patients had proteinuria (frank nephropathy), and patients that were took hydroxyurea treatment.

Urine samples were collected from each patient for measurement of microalbumin by ELISA method\(^{21}\) and blood samples for biochemical tests including serum creatinine (creatinine kit)\(^{22}\) and serum ferritin (ferritin kit)\(^{38}\).

Measurement of Microalbumin in urin by ELISA method:\(^{21}\)

Procedure:Preparation of reagents:

- Wash Buffer (NaN3 <0.1%).
- Sample Buffer (NaN3 <0.1%).
- Conjugated enzyme solution (polyclonal rabbit anti-human albumin, 15 ml and Proclin 300 <0.5%).
- Substrate solution TMB (3,3´,5,5´-Tetramethyl-benzidine, 15 ml).
- Stop Solution (1 M sulfuric acid).

-Preparation of samples

- Undiluted urine sample.
- If the concentration of samples are very high. We can be diluted of samples with buffer and dilutions concentration taken during calculation.

-Steps of procedure:

1-Put 20 MI of calibrators, undiluted samples and controls in to the wells.
2-Put to each well 100 MI of conjugated enzyme solution.
3-Wait for 30 min at room temperature.
4. Remove the contents of wells and wash with 300 mL of wash sol. 3 times.

5. Add 100 mL of substrate solution TMB into each well.

6. Wait for 15 min at room temperature.

7. Put to each well 100 mL of stop solution and leave it for 5 min.

8. Read the results at the optical density (450 nm).

**Calculation:**
- For quantitative results, We plotted the calibrator optical density against the calibrator concentration to find a calibration curve. The concentration of samples may then be measured from the calibration curve. (Cut-off (0 - 25 μg/mL))

**Measurement of Creatinine by creatinine kit in serum:**
Creatinine in alkaline medium produces a yellow-orange color solution with picric acid.  

**Procedure:**
- R1: Sodium hydroxide (150 mmol/L) and Disodium phosphate (6.4 mmol/L).
- R2: Picric acid (4 mmol/L) and Sodium dodecyl sulfate (0.75 mmol/L).

<table>
<thead>
<tr>
<th>Pipette in 1 ml pathlength cuvette</th>
<th>Blank (optional)</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent R1</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>D.W</td>
<td>100 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td>100 mL</td>
<td></td>
</tr>
<tr>
<td>Specimen (Note 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Incubate the samples at the room temperature, then add:**

Mix well. Wait 30 sec, measure absorbance A1 at 490 nm versus D.W or blank. Exactly 2 min. after the first reading measure absorbance A2.

**Calculation:** Serum: result = \(
\frac{(A2-A1) \text{Assay}}{(A2-A1) \text{Standard}} \times \text{Standard concentration}
\)
Measurement of Ferritin by ferritin kit in serum: 38

Procedure: Preparation of reagents:

1- Conjugate enzyme reagent (monoclonal antiferritin)

2- Standard reagent (human spleen or liver ferritin in serum of bovine with preservatives).

3- Tetramethylbenzidine (TMB) reagent.

4- Diluted hydrochloric acid (stop solution).

Preparation of samples: serum should be obtained from a whole blood specimen without lipemic (milky), hemolytic (bright red) or turbid samples.

Steps of procedure:

1- Put 20μL of samples and standards into appropriate wells.

2- Add 100μL of Conjugate Enzyme Reagent into each well.

3- Mix gently for 30 seconds for complete mixing.

4- Incubate for 45 minutes at room temperature (18-25°C).

5- Wash the wells 5 times with deionized or distilled water.

6- Put 100 μL TMB Reagent into each well with gently mix.

7- In the dark Incubate at room temperature for 20 minutes.

8- Add of Stop Solution (100Ml) into each well.

9- Read optical density at 450nm within 15 minutes.

Calculation:

A standard curve was plotted between the absorbance for each standard against its concentration in ng/mL on graph paper, where the concentrations on the horizontal axis (x) and the absorbance on the vertical axis(y). We measured the corresponding ferritin concentration in ng/mL from the standard curve.

Statistical analysis:

The Mann-Whitney U test of SPSS (version 18) is used to find the relation between two different subjects in the experiment, when the assumptions of the t-test have been violated (the data is not normal distribution). The Mann-Whitney U test (independent two sample test) used to find the relation between MA levels and age, gender and diagnosis, there is only one significant relationship between MA levels and age. Also this test used to find correlation coefficient and linear regression between MA levels with age, serum creatinine and
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serum ferritin, there is only one significant correlation and linear regression between MA levels and age.

Results:

Of the 100 studied patients, 38 were males (14 with MA (36.8%)) and 62 were females (16 with MA(27.4%)) and the mean of age for total patients was 25.74±10.59 years and the mean of age for patients with MA was 22.9±8.23 years. Microalbuminuria detected in 31 patients and it was more among males 36.8% as compared to 27.4% of female and more in SCS 35% as compared to 25% of thalassemia patients and more in patients with age <30 years (35.5%) as compared to patients with age ≥ 30 years (16.7%) as shown in table 1.

Microalbuminuria was detected significantly at higher levels 35.5 mg/ml among patients younger than 30 years as compared to 22.0 mg/ml among patients older than 30 years as shown in table 2 and figure 1.

Although MA was detected at higher level 37.0 mg/ml among males than 26.0 mg/ml among females, but it was statistically not significant as shown in table 3.

Also there was no significant difference in the level of MA, but it was more among patients with sickle cell syndrome (35.0 mg/ml) as compared to (24.0 mg/ml) among patients with thalassemia as shown in table 4.

On Further statistical analysis using log microalbuminuria level in relation with age, serum creatinine and serum ferritin levels, table 5. There was no significant relationship between log MA level and each of serum creatinine and serum ferritin levels with the only significant relationship was found between log MA level and age.
Table 1: General characteristics of patients

<table>
<thead>
<tr>
<th>Characteristics of patients</th>
<th>Total patients</th>
<th>Patients with MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>38</td>
<td>14 (36.8%)</td>
</tr>
<tr>
<td>Female</td>
<td>62</td>
<td>17 (27.4%)</td>
</tr>
<tr>
<td>Mean of ages (years)</td>
<td>25.74±10.59</td>
<td>22.9±8.23</td>
</tr>
<tr>
<td>&lt; 30 years</td>
<td>76</td>
<td>27 (35.5%)</td>
</tr>
<tr>
<td>≥ 30 years</td>
<td>24</td>
<td>4 (16.7%)</td>
</tr>
<tr>
<td>Types of hemoglobinopathies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalassemias</td>
<td>40 (13 male &amp; 27 female)</td>
<td>10 (25%) (4 male &amp; 6 female)</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>60 (25 male &amp; 35 female)</td>
<td>21 (35%) (10 male &amp; 11 female)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>31 (31%)</td>
</tr>
</tbody>
</table>
Table 2. Microalbuminuria levels (mg/ml) according to the age group of patients.

<table>
<thead>
<tr>
<th>Age, median (IQR)</th>
<th>Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30 year</td>
<td>≥ 30 year</td>
</tr>
<tr>
<td>35.5 (21.0 – 90.0) mg/ml</td>
<td>22.0 (12.0 – 40.0) mg/ml</td>
</tr>
</tbody>
</table>

Figure 1. The relationship between Log microalbumin (mg/ml) and age of patients
Table 3. Microalbuminuria levels (mg/ml) according to gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Median (IQR)</th>
<th>Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>37.0 (21.7 – 153.5) mg/ml</td>
<td>Z value 1.46, P value 0.142</td>
</tr>
<tr>
<td>Female</td>
<td>26.0 (18.2 – 73.6) mg/ml</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Microalbumin levels (mg/ml) in patients’ urine with thalassemia and sickle cell syndrome.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Median (IQR)</th>
<th>Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalasemia</td>
<td>24.0 (16.1 – 62.8) mg/ml</td>
<td>Z value 1.41, P value 0.157</td>
</tr>
<tr>
<td>Sickle cell syndrome</td>
<td>35.0 (21.0 – 107.6) mg/ml</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. The relationship between log microalbuminuria levels and age, serum creatinine and ferritin in patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log microalbuminuria</td>
<td>- 0.203</td>
<td>0.043</td>
</tr>
<tr>
<td>Age</td>
<td>- 0.090</td>
<td>0.373</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>- 0.030</td>
<td>0.565</td>
</tr>
</tbody>
</table>

Discussion:

Microalbuminuria which is common finding in hemoglobinopathies and it is of a particular importance in patients taking the newer iron chelator (deferasirox) which can
be a nephrotoxic\textsuperscript{23,24,25} and the MA was measured as an early predictor of nephropathy.\textsuperscript{19,20,26} 100 patients (60 sickle cell disease and 40 thalassemia disease) were took in this study to estimate the frequency of occurrence of MA among patients.

In this study the MA was detected in 31\% of the studied patients, and it was more occurring among patients with SCD 35\% as compared to 25\% of patients with thalassemia disease as table 1. Up to the knowledge based on reviewing with other studies and previous reports. There don’t find a comparative figures in the same study but generally the MA and renal dysfunction were relatively more prevalent and more severe among sickle population with HbSS than in non HbSS hemoglobinopathies, probably because the protective effect of the major genetic modifier which is fetal hemoglobin (HbF) level in non HbSS hemoglobinopathies\textsuperscript{27} due to HbF is protective gene that reduce incidence of vasoocclusion, one factor that increased prevalence of nephropathy associated with sickle cell disease.\textsuperscript{37}

Microalbuminuria may be affected by many factors including age, gender, diagnosis and other factors.\textsuperscript{28} Anyhow, in most cases of sickle nephropathy starting insidiously in the very young age with glomerular hyperfiltration and leading to MA in late childhood or early adulthood and progressed slowly over time.\textsuperscript{27} Therefore there was a strong correlation between the prevalence of MA and age as shown by other studies.\textsuperscript{28,29,30} On the other hand, MA was commoner finding in female as compared to male gender.\textsuperscript{26,31} In contrast, this study showed that the MA was significantly more prevalent in patients younger than 30 years as compared to older age group. There is not a true correlation and might be explained by the small sample size and that the majority of the studied patients were younger than 30 years. The results also showed that MA was more among male gender and probably because SCS more among males (25 out of 38 males with SCD (65.8\%) than thalassemia syndrome (13 out of 38 males with thalassemia (34.2\%)) and nephrotoxicity more associated with SCS.\textsuperscript{29, 32}

The present study failed to show any significant relation between the occurrence of MA and the levels of serum creatinine and serum ferritin both in patients with SCS and patients with thalassemia syndromes. Some studies appeared that MA (preclinical marker of renal damage) was significant increased with renal damage in sickle cell
disease while serum creatinine levels without significant differences (33,34) but other studies showed that sickle nephropathy correlated with both MA and serum creatinine. (35, 36)

Although the present study had some limitations; for example the small sample size, no control group and no creatinine clearance could be measured at time of the study. However, The conclusion of this study could reflect the relatively low prevalence rate of MA among non-proteinuria, deferasirox-taking patients with hemoglobinopathies in Basrah. Microalbuminuria may be affected by many factors including age, gender and diagnosis.

The recommendation to treat the patients with MA with antiproteinuric agents to protect against progression to frank proteinuria, and also we recommend for a more comprehensive case-control study to evaluate the nephrotoxic effect and other safety profile of deferasirox among our patients.

Acknowledgment

We would like to thank Dr. Huder Kadem, Ph. Montaser Yaqub and all members of Center For Hereditary Blood Diseases in Basrah for their cooperation and help.

Conclusion:

The study might reflect the relatively low prevalence rate of MA among non-proteinuria, deferasirox-taking patients with hemoglobinopathies in Basrah. Microalbuminuria affected by many factors including age, gender, diagnosis and other factors.
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Asaad A. Kh, Zainab H. A, Anwer Sh. J.

References:


12. Jung Hyun Kang, Bo Rha Park, Kyung Soo Kim, Do Yeun Kim, Hee Jin Huh, Seok Lae Chae, and Sung Joon Shin. Beta-Thalassemia Minor Is Associated with IgA
Microalbuminuria in hemoglobinopathy patients


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E-mail: zha_23980@yahoo.com
The use of D-dimer in exclusion of diagnosis of suspected Deep Vein Thrombosis

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ABSTRACT

Background: Deep venous thrombosis is a common disorder associated with significant morbidity, chronic venous insufficiency as well as fatal pulmonary embolism. Venography has been the gold standard of diagnosis, however it has been replaced in most areas by duplex ultrasound which is generally very good method. An interesting new approach to the diagnosis of DVT is D-dimer testing. D-dimer levels reflect the amount of lysed, cross-linked fibrin and may be useful diagnostic marker in the clinically suspected DVT. D-dimer can be measured either quantitatively by ELISA or qualitatively by latex agglutination.

Objectives: The aim of the study was to evaluate the use of D-dimer in exclusion of the diagnosis of DVT.

Patient and Methods: A total of 50 patients presented to vascular outpatient department with clinical suspicion of DVT have been studied. Patients with old DVT, patients on anticoagulant, and patient with severe infection or inflammation were excluded. Venous duplex ultrasonography of the affected limb or limbs was done and citrated blood sample was analyzed for D-dimer by a VIDAS method for all patients blindly to the results of venous duplex. Sensitivity, specificity, negative and positive predictive values were calculated. ROC curve then was generated from sensitivity and 1 - specificity values at a continuum of D-dimer level to determine the optimal cut-off level of VIDAS D-dimer for exclusion of DVT.

Results: The mean age of DVT group was 43 year. DVT was confirmed in 37 patients (74%), and excluded in 13 patients (26%) by venous duplex. The mean D-dimer level in the DVT group was 5498.021ng/dl while in non DVT group was 1906.384ng/dl this difference was statistically significant (P=0.0003). The sensitivity, specificity, negative and positive predictive values of VIDAS method at cut-off points (500 and 900) ng/dl were (100%, 33%, 100%, 82%) respectively, and at 3000ng/dl (71%, 75%, 47%, 90%) respectively.
Conclusions: VIDAS D-dimer method is a sensitive method that can be used in the initial management of deep vein thrombosis if a level of 900ng/dl is used as a cut-off point for exclusion of deep vein thrombosis. VIDAS D-dimer method is not a specific test so it cannot be used for the diagnosis of deep vein thrombosis.

Key words: D-Dimer, Diagnosis, DVT

Introduction:

It has been accepted that an objective diagnosis of deep vein thrombosis is mandatory because clinical evaluation is inaccurate. This is unfortunately because clinical features can be used to classify patients with symptoms suggesting DVT and to improve diagnosis strategies. Studies have demonstrated that by categorizing the patients' pretest probability of DVT into low, moderate, or high likelihood, diagnostic precision can be improved.

Investigators demonstrated that the use of model of pretest clinical probability of DVT combined with common femoral and popliteal vein compression ultrasound decreased the number of false-positive and negative diagnoses, using ascending venography as the definitive diagnostic test.

The clinical features in an extensive venous thrombosis are more reliable since majority of the patients usually present with severe pain in the calf, thigh, or rapid swelling of the leg. On examination the affected limb appear pale or cyanosed and often cold with poor capillary return. There is marked tenderness along the course of thrombosed vein in the calf muscle. Femoral vein thrombosis is usually associated with swelling of the foot and calf but because the thrombi are rarely completely obstructive and the veins are paired, swelling is not universal. Iliofemoral vein thrombosis represents the most extensive form of DVT and usually associated with tenderness in the groin and swelling of entire leg.

D-dimer has been extensively investigated during the recent years and has been consistently found to be of value in the diagnostic approach of venous thromboembolism. D-dimer is a neoantigen formed when thrombin initiates the transition of fibrinogen to fibrin and activates factor XIII to cross link the fibrin formed.

The D-dimer is a fragment of fibrin that contains one intermolecular cross-link between the gamma chains of two fibrin monomers. This cross-linkage occurs in fibrin but not fibrinogen. It is thus specific for fibrin. Fibrin D-dimer derivatives were not detected in either citrate or EDTA anticoagulated plasma from healthy persons.

A wide variety of diseases were associated with a positive Dimer test assay in hospitalized patients many of these diseases have been reported to be associated with an increase in fibrinolytic activity.

Increased levels of D-dimer (cross link fibrin fragment) have been found in patients with deep vein thrombosis, acute myocardial infarction, acute pulmonary embolism,
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unstable angina, and disseminated intravascular coagulation (12,13,14).

Plasma from nearly 40% of pregnant women with pre-eclampsia was reported positive for D-dimer. Patients with D-dimer had more severe disease (15). While the sensitivity of plasma D-dimer measured by ELISA in the diagnosis of DVT is high, the utility of ELISA methods is limited in a clinical setting (16). VIDAS D-dimer is an automated ELISA D-dimer test offering high analytical performance. The single-dose and ready-to-use test format allows VIDAS D-dimer to run individual tests without additional cost. More importantly results can be provided to clinicians in a very short time.

A lower usefulness of D-dimer in elderly patients with suspected venous thromboembolism due mainly to a lower specificity of this test in this subset of patients has been reported (17).

Venous Duplex Imaging often allows direct visualization of the thrombus. Thrombus may be difficult to be visualized in its acute form. The addition of color flow imaging facilitates the identification of non-occluding clots. Thrombus echogenicity increase with age of the clot (18). The presence of an echogenic band within the lumen of the vein has considered as representing of thrombus, however, this phenomenon can frequently be mimicked by turbulent flow condition. When the vein is compressed this artifact is eliminated if there is thrombus (19).

Patients and methods:

A total of 50 patients with clinically suspected DVT admitted to vascular department in Surgical Specialties Hospital/Medical City in Baghdad were included in this study during a period of 12 months (October 2012- October 2013). A fully detailed history and medical examination were performed. Patients with history of old DVT within the last year, patients on anticoagulant therapy, and patients with severe infectious or inflammatory conditions were excluded from the study.

Duplex Sonography: All patients underwent duplex scanning of the symptomatic limb or limbs by a vascular radiologist who was blinded to the results of D-dimer test. The pelvic and inguinal veins, as well as both the deep and superficial femoral veins, were scanned with patient in supine position. The popliteal segment was scanned from a posterior position, with the patient lying on the abdomen. The distal venous segment, including the posterior tibial veins, the peroneal veins, the gastrocnemius veins, and the soleus veins, were scanned with the patient in a sitting position. Duplex sonography was used as the gold standard in this study for diagnosis of DVT.

Sample Collection: 1.8 ml of venous blood was collected by a clean venipuncture from each patient in a collecting tube containing 0.2 ml of 3.2% sodium citrate and centrifuged at 4000 rpm for 15 min within 4 hours after collection. Platelet poor plasma was collected and then frozen at −20°C for a maximum of one month for analysis.

D-dimer Assay: All samples were thawed and recentrifuged prior to analysis. Plasma D-dimer for all samples was assayed by a rapid method VIDAS D-dimer. Tow controls negative and positive have been run with each test.
Results:
The results presented in this chapter were based on the analysis of 50 patients with suspected DVT, in 37 (74\%) of them DVT was confirmed (group 1) and in 13 (26\%) of them DVT was excluded (group 2) by venous duplex sonography (figure 1).

Figure 1:
DVT and non-DVT groups according to the result of venous sonography.
The age of patients with DVT ranged between 18-85 year with a mean of 43 ± 4 years, and standard deviation of 16.64 and standard error 2.35, the majority of patients are between 25-50 years of age (figure 2).

Figure (2): The age distribution of the patients with DVT.
Table (1): The base line characteristics of DVT and non-DVT groups.

<table>
<thead>
<tr>
<th></th>
<th>DVT group</th>
<th>Non-DVT group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean</td>
<td>43.4 years</td>
<td>45.2 years</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 33, Female 5</td>
<td>Male 10, Female 2</td>
</tr>
<tr>
<td>Surgery or Trauma</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Malignancy</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Varicosity</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>No risk factors</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
</table>

The above table shows associated risk factors of DVT: Male sex is significantly associated with DVT (P=0.0005). Surgery and trauma were significantly associated with DVT (P=0.0003). Malignancy risk group includes 5 patients with gastrointestinal tract, 2 patients with pulmonary carcinoma, and one patient with non-Hodgkin lymphoma, malignancy is significantly associated with DVT (P=0.0006). Four pregnant women of different stages of pregnancy, two of them are grand multigravida. Pregnancy is significantly associated with DVT (P=0.0005).only 14 patients of the total 50 patients do not have comorbid condition.

Table (2): D-dimer means, standard deviation, and range in DVT and non-DVT groups

<table>
<thead>
<tr>
<th></th>
<th>D-dimer mean ±SD in mg/dl</th>
<th>MIN in ng/dl</th>
<th>MAX in ng/dl</th>
<th>Range of D-dimer in ng/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVT</td>
<td>5498.021 ± 3266.133</td>
<td>2571.39</td>
<td>11000.350</td>
<td>8428.960</td>
</tr>
<tr>
<td>Non DVT</td>
<td>1906.384 ± 1533.898</td>
<td>375.0</td>
<td>2251.7</td>
<td>1976.7</td>
</tr>
</tbody>
</table>
The use of D-dimer in exclusion of diagnosis

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Figure (3) : The mean level of D –dimer

The table 2 and figure 3 show that the mean level was higher in DVT group (5498.02 ng/dl) compared to non DVT group (1906.38ng/dl). The observed differences in mean D-dimer levels between the two groups was statistically significant (p=0.0003).
Figure (4) : The sensitivity and specificity of D-dimer test
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Figure (5): Receiver Operator Characteristic (ROC) curve plotting sensitivity and the false positive rate across continuum of D-dimer levels.

Figure (5) shows a ROC curve, plotting sensitivity and the false positive rate (1-specificity). Area under the curve is 0.81 with standard error of 0.06 indicate a relationship of D-dimer to the presence of DVT much greater than chance because D-dimer cut off values curve lying above the chance line.
Table (3): Sensitivity, specificity, positive, and negative predictive values at different cutoff points of VIDAS D-dimer.

<table>
<thead>
<tr>
<th>Cut off Of D-Dimer</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>100%</td>
<td>33%</td>
<td>82%</td>
<td>100%</td>
</tr>
<tr>
<td>900</td>
<td>100%</td>
<td>33%</td>
<td>82%</td>
<td>100%</td>
</tr>
<tr>
<td>3000</td>
<td>71%</td>
<td>75%</td>
<td>90%</td>
<td>47%</td>
</tr>
</tbody>
</table>

Using sensitivity, specificity, negative and positive predictive values for specific D-dimer levels as cutoff points sensitivity and negative predictive values were maintained 100% up to the level of 900ng/dl ,while the specificity and positive predictive value were (33% ,82% ) respectively .With increasing D-dimer levels the specificity and positive predictive value will increase while the sensitivity and negative predictive value decrease. At a cutoff point of 3000ng/dl the sensitivity and negative predictive value decrease to (71%, 47%) while the specificity and positive predictive value increase to (75% , 90%) respectively as shown in table( 4)

Discussion:
Deep vein thrombosis has an annual incidence of 1/1000. An estimate of case fatality rate range from 1% - 5% , however , the incidence and the case fatality are very age dependent (20) .Early diagnosis of DVT and the prevention of its complication ,pulmonary embolism ,is highly desirable .While clinical examination cannot relied upon in isolation to make a diagnosis of DVT, its combination with appropriate history taking can provide useful information (21).Duplex scanning ,the present gold standard for the diagnosis of DVT , is relatively time consuming and expensive .A rapid test with high sensitivity and high negative predictive value ,allowing preselection of patients requiring further sonography investigation ,could decrease the number of sonography performed and results in significant cost reduction .
This study use a rapid and quantitative method for individual sample assay which is automated VIDAS D-dimer test to rule out the diagnosis of DVT. It reveals that excellent sensitivity (100%) and negative predictive values (100%) were maintained up to a cut-off level of 900ng/ml a level below which DVT could be safely excluded from a patient. A relatively good specificity of 75% at a level of 3000 ng /dl was found, indicating that the majority of patients with DVT had a level above this value. But patients with levels between 900ng/ml and 3000ng/ml could not be safely excluded from having a DVT.

This study agrees with most of the published literature on D-dimer \(^\text{(12,13,22,23)}\). Four out of fifty patients included in the present study who had D-dimer level below 900ng/dl were found by venous duplex to have no DVT that is mean approximately one tenth (n=4 ) of patients could have avoided a venous imaging study if a level of 900 ng /dl or less had been used to exclude DVT , this would have translated into a significant cost saving ,another potential benefit is the rapid time of the assay ,results being available within 1 hour .

VIDAS D-dimer assay showed a significant difference between the mean levels of D-dimer of patients with and without DVT (P=0.0003) which agree with most studies \(^\text{(24,25)}\). However, in DVT group of patients the finding of high level of D-dimer (>10,000ng/dl) in patients with associated conditions like cancer and recent surgery is interesting but expected since these conditions can independently elevate D-dimer results in absence of obvious thrombosis which makes the test non-specific . Exclusion of patients with risk factors from this study was difficult as it further reduces the sample size and limits the value of the study since only 12 patients (10% )do not have comorbid conditions that would potentially elevate D-dimer .

The mean age of the patients was 43 years and two patients were more than 70 years with D-dimer levels >500 ng/dl .In both the venous duplex results were negative for DVT and they do not have other condition which elevate D-dimer level this finding agrees with the study of (Carlos et al) \(^\text{(26)}\) which suggests a higher cut-off value for elderly patients for exclusion of DVT since the baseline of D-dimer increases with age \(^\text{(17)}\).
Conclusion:

1. VIDAS D-dimer method is a sensitive method that can be used in the initial management of deep vein thrombosis if a level of 900ng/dl is used as a cut-off point for exclusion of deep vein thrombosis.

2. VIDAS D-dimer method is not a specific test so it cannot be used for the diagnosis of deep vein thrombosis.

Recommendation:

It is recommended to use VIDAS D-dimer method in emergency unit as an initial management of DVT by rolling out the diagnosis of DVT in patients with negative result (<900ng/dl).

References:


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The use of D-dimer in exclusion of diagnosis

Lamyaa J. H., Hussein H.A., Rasha T.J.


The use of D-dimer in exclusion of diagnosis

Lamyaa J. H., Hussein H.A., Rasha T.J.


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Distribution of Blood Groups and Rhesus factor among selected sample of Iraqi Students

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²Pathologist, BSc, MSc, PhD/ Medical School/ Malaysia

ABSTRACT

Background: There exists ignorance of blood groups among many people and surprisingly even among the literates. Despite the importance of this health parameter in blood transfusion, it is also one of the requirement of obtaining driving license and national identity card.

Objectives: The objectives of this study were to determine the frequency of the blood grouping (ABO) and Rhesus (Rh) factor of the blood groups and to determine the awareness on the importance of blood grouping among the study’s population.

Subjects and methods: The total number of sample size of this study was 278 students were selected randomly. The study was carried out among two cohort of student's population. First cohort was 168 medical students from Faculty of Medicine, while second cohort included 110 non-medical students from Baquba Technical Institute' students. The study samples have their blood groups determined according to that documented before. While those who don’t know their ABO & Rh blood grouping marked as DK.

Results: The result of this study shows that the rate of blood grouping were 25.5%, 22.3%, 32.0%, 6.1%, and 16.9%, for blood group A, B, O, AB, and DK respectively; while for Rhesus factor blood grouping the results revealed that the rates were 77.0%, 6.1% and 16.9%, for positive, negative and DK respectively.

Conclusion: The blood group O with Rh positive was the most common prevalent among the selected groups. Knowledge of blood group distribution is important for clinical studies, for reliable geographical information and for forensic studies in the population.

Keywords: ABO, blood groups, Rhesus (Rh) factor, students.

Introduction

The ABO & Rhesus (Rh), blood grouping is among the oldest and most important health parameter, most especially in relation to blood transfusion. It is also important in genetics and other heredity determination (¹). Currently motor vehicle driving license and National passport are issued on the basis of one’s blood group determination. It is also one of the needs for the national identification program. Despite of all these, many people are ignorant of this important health parameter (²,³). People have different blood types, known as blood groups. Antigens are hereditary determined and plays a vital role in transfusion safety (⁴). The discovery of the ABO blood groups by Karl Landsteiner was an important achievement.

Original article
Distribution of Blood Groups and Rhesus factor

Salwa Sh.A, Karim Al- Jashamy

Iraqi J. Hematology, November 2015, vol.4, Issue 2

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in the history of blood transfusion followed by 1 discovery of Rh antigen\(^{(4)}\). Since 1901, more than 20 distinct blood group systems have been characterized but the ABO and Rhesus (Rh) blood groups remain the most clinically important\(^{(5)}\). Both these systems are useful in blood transfusion and organ transplantation. The distribution of blood groups has been studied in various populations all over the world during the last half-century\(^{(6)}\). The frequencies of ABO and Rhesus-D showed great variation from one population to another in different geographic locations, reflecting the underlying genetic, ethnicity diversity of human populations\(^{(7)}\). Association of blood groups and different disease states have also been investigated for example people with blood group (O), have high risk of peptic ulcer women with blood group (A) have been reported to have endometrial and ovarian cancers more frequently than women with non-A blood groups. Also people with group A have a substantially increased risk for coronary heart disease\(^{(8)}\). The distribution of ABO blood groups have been shown to work as a strong predictor of national suicide and homicide rates and a genetic marker for obesity\(^{(7,8)}\). The objective of this study was to determine the frequency of different blood groups and determination of the predominant of ABO, Rh blood groups among the study population, and creating awareness on the importance of blood grouping.

Materials and methods

This cross sectional study was carried out among two cohorts of students population. The First one was Diyala Faculty of Medicine' students constituted from (168) medical students selected randomly from the six grades of the Faculty. While the second cohort included (110) students selected also randomly from first and second grades of Baquba Technical Institute. The age of the study samples ranging from 17-25 year. The study samples have their blood groups determined according to that documented before. While those who don’t know their ABO & Rh blood grouping, they marked as DK.

Data collected from the study groups by a special questionnaire designed by the researches. This questionnaire includes information about gender, age, ABO, Rh-factor, blood grouping, and race. Descriptive statistic was used for analysis of the data included numbers, percentages, and tables.

Results :

Table 1: Percentage distribution between races and gender of Iraqi students who are included in the study

<table>
<thead>
<tr>
<th>Race</th>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabic</td>
<td></td>
<td>82</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Kurdish</td>
<td></td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>Turkmen</td>
<td></td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>88</td>
<td>190</td>
</tr>
</tbody>
</table>

Descriptive statistic was used for analysis of the data included numbers, percentages, and tables.
Table 2: Percentage distribution between Rh factor and races among Iraqi students included in the study.

<table>
<thead>
<tr>
<th>Rh factor</th>
<th>Arabic</th>
<th></th>
<th>Kurdish</th>
<th></th>
<th>Turkmen</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Do not know</td>
<td>47</td>
<td>19.5</td>
<td>7</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>Positive</td>
<td>179</td>
<td>74.3</td>
<td>16</td>
<td>64</td>
<td>6</td>
<td>100</td>
<td>214</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>6.2</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>241</td>
<td>100%</td>
<td>25</td>
<td>100%</td>
<td>6</td>
<td>100%</td>
<td>278</td>
</tr>
</tbody>
</table>

Table 3: Percentage distribution between genders and Rh factor among Iraqi students included in the study.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Rh Factor</th>
<th>DO not Know</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>23.4</td>
<td>74</td>
<td>34.7</td>
</tr>
<tr>
<td>Female</td>
<td>36</td>
<td>76.6</td>
<td>139</td>
<td>65.3</td>
</tr>
<tr>
<td>Total</td>
<td>47*</td>
<td>100.0</td>
<td>213**</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*47/278 (17%), **213/278 (76.6%) ***18/278 (6.4%)

Table 4: Percentage distribution of gender among Iraqi study samples

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MALE</th>
<th>%</th>
<th>FEMALE</th>
<th>%</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Medical</td>
<td>48</td>
<td>57.1</td>
<td>120</td>
<td>63.2</td>
<td>168</td>
<td>60.4</td>
</tr>
<tr>
<td>Non-Medical</td>
<td>40</td>
<td>42.9</td>
<td>70</td>
<td>36.8</td>
<td>110</td>
<td>39.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>84</td>
<td>100.0</td>
<td>190</td>
<td>100.0</td>
<td>278</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 5: Percentage distribution of ABO blood group with gender among Iraqi students included in the study.

<table>
<thead>
<tr>
<th>BLOOD GROUP</th>
<th>Medical</th>
<th></th>
<th>Non-Medical</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MALE</td>
<td>FEMALE</td>
<td>MALE</td>
<td>FEMALE</td>
<td>No.</td>
</tr>
<tr>
<td>A</td>
<td>13</td>
<td>33.3</td>
<td>19</td>
<td>21.1</td>
<td>71</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>38.4</td>
<td>23</td>
<td>25.5</td>
<td>62</td>
</tr>
<tr>
<td>O</td>
<td>6</td>
<td>15.4</td>
<td>42</td>
<td>46.6</td>
<td>89</td>
</tr>
<tr>
<td>AB</td>
<td>5</td>
<td>12.8</td>
<td>6</td>
<td>6.6</td>
<td>17</td>
</tr>
<tr>
<td>TOTAL</td>
<td>39</td>
<td>100.0</td>
<td>90</td>
<td>100.0</td>
<td>239</td>
</tr>
</tbody>
</table>

DISCUSSION

ABO and Rh blood groups are most important blood groups in human beings (5). The frequency of four main blood group systems varies in population throughout the world and even in different parts of country (6,7). One of the objectives of current study was to increase awareness about the ignorance of blood groups among people. Surprisingly even among the literates that ignorance is present (7). It
is this ignorance that motivates the researchers for this study. Despite the importance of this health parameter in blood transfusion, it is also one of the requirement of obtaining driving license and national identity card, with the aim of creating awareness on importance of blood grouping and determination of the predominant blood group in the ABO system in the population of the study area (7,8).

The percentage of ABO blood groups were found as were 25.5%, 22.3%, 32.0%, 6.1%, and 16.9%, for blood group A, B, O, AB, and DK respectively. While for Rhesus blood grouping the results revealed that the percentages were 76.6%, 6.4% and 17.0%, for positive, negative and DK respectively. The result revealed that highest percentage was for blood group (O), the least percentage (6.1%) for AB blood group. This is in agreement with Jay Prakash et.al study, where his results revealed that the average percentage of ABO groups were found as O (34.8%), A (34.3%), B (27.0%) and AB (3.9%). The Rh positive and negative distribution in the studied population was 98.6% and 1.4% respectively (1,6). Overall for medical student's population, blood Group (B) was most prevalent among males. While blood group (O), was most prevalent among females. On the other hand blood group (A) was most prevalent among males, while for the female the results were same as for female medical students. Regarding Rh factor distribution also varies among the study population, among over all it was 6.4%, percentage of DK was higher among female student and this can be explained by more ignorance among females than males, also males needs more official documents like driving license, military card, sport, swimming card for sport clubs in addition to the care for males more than females in regard to this issue.

The ABO and RhD pattern in both the male and female population studied correlates with previous studies carried out in other part of Nigeria population: like Ogbomosho, Oyo State; Benin (7).

For second cohort the highest percentage was for blood group (O), followed by A. Blood group AB was least prevalent with 10.9%. Out of total 110 students, 7 (6.4%) students didn’t know their blood grouping. O - Positive blood group is significantly high in our population. It is well established that ABO and rhesus (Rh) genes and phenotypes vary widely between ethnic groups and both within and between geographical areas. Regarding the distribution of ABO according to race, although the students 'number from races other than Arab, very small, constituted 6.8%, 4.3% of the study sample for Kurds and Turkmen respectively. So our percentages may be unreliable. The small percentages of those races due to displacement of most Kurds, and Turkmen to Kurdistan and Kirkuk due to insecurity and hard situation in Diyala, that is why most Diyala population escape from terrorists and military processes and displaced to Northern governorate for security, with particular displacement to Kurds and Turkmen. Mohamad Jaff in his study for ABO blood groups in Kurd stated that the most prevalent blood group was O (37.16%), followed by blood groups A (32.47%) and B (23.84%), whereas the least prevalent blood group was AB (6.53%). The majority 91.73% were Rh positive, and 8.27% were Rh negative. Data showed that among the Rh-positive individuals, 34.03% were O, 29.99% were A, 21.69% were B, and 6.02% were AB. Breakup of the Rh negatives showed that 3.13% were group O, 2.48% were A, 2.15% were B, and 0.51% were AB. Every transfusion center should have a record of frequency of blood group system in their population. It helps in inventory management (8).

In conclusion; Knowledge of blood group distribution is important for clinical studies, for reliable geographical information and for forensic studies in the population.
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Hematological profile of patients with Acromegaly in Iraq

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²National center for diabetes / Almustansiriya University

ABSTRACT

Background: Acromegaly is a disease characterized by growth hormone and insulin like growth factor hypersecretion due mostly to pituitary somatotropic adenoma. The diagnosis of Acromegaly is usually delayed for years exposing patients to slowly evolving chronic complications.

Objectives: To explore the value of performing peripheral blood examination as routine work up in monitoring Iraqi patients with Acromegaly.

Patients and Methods: This study was conducted on 38 patients with Acromegaly attending the national center for diabetes research and management. Peripheral blood indices were done by hematological analyzer and blood film stained by Gemisa stain for proper cells morphology done at hematological unit in Iraqi center for cancer and genetics research.

Results: The patients examined showed higher values compared with control group in platelets indices (MPV and PDW) that were statistically non-significant. The monocyte count was significantly lower in patients compared by control group (p <0.05). Two patients were found to suffer from thrombocytopenia. One male with mild thrombocytopenia, the second is female with moderate thrombocytopenia. One female with moderate iron deficiency anemia.

Conclusion: Peripheral blood exam in patients with Acromegaly is highly indicated, low cost and valuable in follow up patients.

Keywords: acromegaly, hematological profile

Introduction

Acromegaly is a disease characterized by growth hormone (GH) and insulin like growth factor-1 (IGF-1) hyper secretion due to in most cases to a pituitary somatotropic adenoma (¹). The diagnosis of acromegaly is usually delayed four years, exposing patients to slowly evolving chronic complication (²). Diabetes mellitus (DM) and
Peripheral blood leukocytes are composed of granulocytes, monocytes, as well as lymphocytes. Leucocyte can be activated by advanced glycation and products, oxidative stress and cytokines in the state of hyperglycemia. Leucocyte may be activated by tumor necrosis factor α (TNF-α), transforming growth factor -β1 (TGF-β1) to participate in the pathogenesis of diabetic microvascular and macrovascular complications. Elevated differential cell count neutrophils, monocytes and eosinophils also predict the future incident of CAD. Anemia is an independent risk factor for the development of cardiac morbidity and mortality. Decreased hemoglobin levels are known to be associated with an increased risk of coronary atherosclerosis due to increase in blood flow and share stress resulting in endothelial damage and vessel wall thickness.

The aim of our study is to evaluate the peripheral blood finding in Iraqi acromegaly patients on octreotide.

**Patients and Methods:**

Patients with acromegaly who entered a disease management program at center for diabetic from the period of October 2013 to October 2014. We prospectively analyzed (38) patients Male (22) and Female (16) with acromegaly receiving octreotide LAR (Novartis) and control group (20) person matched with age and gender of the patients group. The present study was approved by the human research ethics committee of our center and informed consent was obtained from each patient included in our data. Each patient participated in a detailed history. All of the patients underwent complete physical and medical examination (height, weight, blood pressure, ECG, chest x-rays).

For the study (2 ml) of venous blood was collected in EDTA tubes, the full blood cell indigos (Hb, PCV, RBC counts, MCV, MCH, MCHC, RDW-CV, RDW-SD, WBC count, Neutrophils, Lymphocytes and Monocytes count, platelets count, MPV, PDW and PCT) were performed on ( mindary 3000) hematology analyzer working on principle of light scattering. The counter was maintained according to the manual instructions of manufacturer. Blood smear were performed using Giemsa stain for proper differential WBC.
Hematological profile of patients with Acromegaly count and cell morphology. The test was performed within one hour from blood collection. The test was done at hematological unit at Iraqi center for cancer and medical genetic research.

**Statistical Analysis:**

All Values were expressed as mean ± SD. Comparison between control and patients when performed using two tailed students t-test and were corridors significant if the obtained P value was lower than 0.05.

**Results:**

A total 38 patients Male (22) and Female (16) with age range (25-70) years were included in the present study. Table (1) clearly demonstrated that the lymphocytes count of the acromegaly patients mean (2.4 ± 0.6) were non-significant (P> 0.05) as compared to the control group mean (2.6 ± 0.5). While the monocyte count of the patients group mean( 0.3 ± 0.1) was significantly lower than the control group mean (0.5 ± 0.1) with P value of (< 0.05).

Regarding red cell indices the Hemoglobin of patients mean (13.7 ± 1.9) was higher than control group mean (13.4 ± 1.4) However, non-significant different was noticed. Considering the platelet indices in table (1) revealed that MPV of patients is higher than control group. The PDW of patients (14.7± 0.2) were higher than the control (14.1± 0.6). However non – significant difference were found.

Our data revealed that 16 patients out 38 patients included in our study suffering from diabetes mellitus representing (42%) of all acromegaly patients examined.

Table (2) showed comparison between diabetics group (16 acromegaly patients) and non-diabetic group (22 acromegaly patients). In spite of non-significant difference in the platelets indices (MPV and PDW) however the diabetic group that mean Value of MPV (9.9 ± 0.7) higher than that of non-diabetic group (9.6 ± 0.6) similar by the mean value of PDW in diabetic group (14.8 ± 0.2) higher than that of non-diabetic group (14.7 ± 0.2).

Two patients were found to have thrombocytopenia, the 40 years Male with hypertension, while the second is 57 years Female had co morbidity of hypertension, thyrotoxicosis, and diabetes.

Only one female with 44 years old complains of moderate iron deficiency anemia Hb (8.1 g/dl), iron status were performed iron (85 mg/dl) total iron binding capacity (688 mg/dl) transferrin saturation (12.35 %) moreover the ferritin (10 ng/ml).
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acromegaly (n=38)</th>
<th>Control (n=20)</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>7.4 ± 1.7</td>
<td>7.7 ± 1.1</td>
<td>N.S</td>
</tr>
<tr>
<td>Neutrophil (N)</td>
<td>4.5 ± 1.3</td>
<td>4.5 ± 0.8</td>
<td>N.S</td>
</tr>
<tr>
<td>Lymphocytes (L)</td>
<td>2.4 ± 0.6</td>
<td>2.6 ± 0.5</td>
<td>N.S</td>
</tr>
<tr>
<td>Monocytes (M)</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Hb</td>
<td>13.7 ± 1.9</td>
<td>13.4 ± 1.4</td>
<td>N.S</td>
</tr>
<tr>
<td>PCV</td>
<td>41.4 ± 5.1</td>
<td>40.8 ± 3.1</td>
<td>N.S</td>
</tr>
<tr>
<td>Red blood cells (RBC)</td>
<td>5.2 ± 1.5</td>
<td>4.8 ± 0.2</td>
<td>N.S</td>
</tr>
<tr>
<td>MCV</td>
<td>83.2 ± 6.3</td>
<td>81.7 ± 4.7</td>
<td>N.S</td>
</tr>
<tr>
<td>MCH</td>
<td>28.3 ± 2.7</td>
<td>28.8 ± 3.2</td>
<td>N.S</td>
</tr>
<tr>
<td>MCHC</td>
<td>329.7 ± 52.2</td>
<td>322.3 ± 26.8</td>
<td>N.S</td>
</tr>
<tr>
<td>RDW(CV)</td>
<td>13.3 ± 1.1</td>
<td>13.9 ± 1.8</td>
<td>N.S</td>
</tr>
<tr>
<td>RDW(SD)</td>
<td>37.2 ± 4.9</td>
<td>36.8 ± 1.9</td>
<td>N.S</td>
</tr>
<tr>
<td>Platelets (PLT)</td>
<td>236.8 ± 62.1</td>
<td>236.2 ± 62.4</td>
<td>N.S</td>
</tr>
<tr>
<td>MPV</td>
<td>9.7 ± 0.6</td>
<td>9.3 ± 0.6</td>
<td>N.S</td>
</tr>
<tr>
<td>PDW</td>
<td>14.7 ± 0.2</td>
<td>14.1 ± 0.6</td>
<td>N.S</td>
</tr>
<tr>
<td>PCT</td>
<td>0.2 ± 0.05</td>
<td>0.3 ± 0.05</td>
<td>N.S</td>
</tr>
</tbody>
</table>

N.S: Non-significant.
Table 2: Means of hematological parameters in Diabetic acromegaly patients and Non diabetic acromegaly

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic Acromegaly (n=16)</th>
<th>Non Diabetic Acromegaly (n=22)</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>8.0 ± 2.0</td>
<td>7.0 ± 1.5</td>
<td>N.S</td>
</tr>
<tr>
<td>Neutrophil (N)</td>
<td>10.9 ± 16.3</td>
<td>9.6 ± 17.0</td>
<td>N.S</td>
</tr>
<tr>
<td>Lymphocytes (L)</td>
<td>7.4 ± 13.1</td>
<td>5.1 ± 8.9</td>
<td>N.S</td>
</tr>
<tr>
<td>Monocytes (M)</td>
<td>0.9 ± 1.6</td>
<td>0.6 ± 1.1</td>
<td>N.S</td>
</tr>
<tr>
<td>Hb</td>
<td>13.9 ± 1.4</td>
<td>13.5 ± 2.2</td>
<td>N.S</td>
</tr>
<tr>
<td>PCV</td>
<td>42.1 ± 4.1</td>
<td>40.9 ± 5.7</td>
<td>N.S</td>
</tr>
<tr>
<td>Red blood cells (RBC)</td>
<td>5.6 ± 2.3</td>
<td>5.0 ± 0.4</td>
<td>N.S</td>
</tr>
<tr>
<td>MCV</td>
<td>84.6 ± 4.2</td>
<td>82.3 ± 7.5</td>
<td>N.S</td>
</tr>
<tr>
<td>MCH</td>
<td>28.9 ± 1.6</td>
<td>27.8 ± 3.2</td>
<td>N.S</td>
</tr>
<tr>
<td>MCHC</td>
<td>320.1 ± 78.5</td>
<td>336.7 ± 16.7</td>
<td>N.S</td>
</tr>
<tr>
<td>RDW(CV)</td>
<td>19.8 ± 27.2</td>
<td>13.5 ± 1.4</td>
<td>N.S</td>
</tr>
<tr>
<td>RDW(SD)</td>
<td>36.2 ± 1.9</td>
<td>38.0 ± 6.2</td>
<td>N.S</td>
</tr>
<tr>
<td>Platelets (PLT)</td>
<td>223.7 ± 64.8</td>
<td>246.4 ± 59.8</td>
<td>N.S</td>
</tr>
<tr>
<td>MPV</td>
<td>9.9 ± 0.7</td>
<td>9.6 ± 0.5</td>
<td>N.S</td>
</tr>
<tr>
<td>PDW</td>
<td>14.8 ± 0.2</td>
<td>14.7 ± 0.2</td>
<td>N.S</td>
</tr>
<tr>
<td>PCT</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.05</td>
<td>N.S</td>
</tr>
</tbody>
</table>
Discussion:

Our data revealed that all acromegaly patients that (MCV) values within normal limit, moreover, non-significant differences in MCV values in both the patients and control groups were noticed. It is well known that octreotide LAR has an effect on vitamin B₁₂ metabolism (manual instruction of the drug). Arising in MCV value precedes the anemia by months and non-macrocytic anemia never results from vitamin B₁₂ deficiency unless coexistence of iron deficiency or thalassemia traits (¹³,¹⁴,¹⁵).

In megaloblastic anemia because of the progressive nature of gradual replacement of normocytic cells with macrocytic progeny of megaloblastic bone marrow, the earliest change in red cell indices is an increase in (RDW) that reflects an increase in anisocytosis of red cells (¹⁶) however, an increase in RDW is also found in iron deficiency anemia and thalassemia traits which are prevalent in our population (¹⁷,¹⁸) regarding the WBC count, the present study showed that the value of WBC in acromegaly group is non-significantly difference with control.

The monocytes count is significantly lower in the patients group compared with the control group (P<0.05).

The WBC count is regarded as inflammatory marker; these findings indicate that the inflammatory system is not chronically active in the acromegaly patients.

Also a study conducted by potter et al (2008) when they found that both inflammatory markers, (C-reactive protein) (CRP) level and leucocytes count were similar in patients and controls recently groups, another study performed by Verheist et al (2012) showed that high sensitive c-reactive protein is significantly lower in acromegaly patients compared with the control group (¹⁹,²⁰).

In fact, growth hormone and insulin like growth factor excess induces a specific cardiomyopathy. Rhythm disturbances and valve dysfunction are also frequent in acromegaly but the coronary artery disease is less than expected which had associated with inflammatory process (²¹,²²). The present study identify that the platelets indices the MPV and PDW showed that both are higher in patients groups compared with the control groups. An increase in MPV value is regarded as an independent risk factor for thromboembolism, stroke and myocardial infarction (²³).

Recent study in India showed that both MPV and PDW are significantly higher
Hematological profile of patients with Acromegaly in diabetic patients compared with the control group (24).

The present study showed that (42%) of acromegaly patients had diabetes mellitus, however, when we compare the both platelets indices (MPV and PDW) between the diabetic acromegaly patients and the non-diabetic acromegaly patients a non-significant difference were identified. In spite of higher values in both platelets indices in diabetic group than mean value (9.9) non diabetic groups mean value (9.3) this could be explain that the sample size of the patient is small to have a realistic result.

This study showed two patients suffering from thrombocytopenia, the cause of thrombocytopenia could not be identify and further investigation are needed to clarify the causative agent, only one acromegaly female found to have moderate iron deficiency anemia because of menorrhagia which could be due to hormonal disturbances.

Further studies are recommended to include plasma FVIII, fibrinogen level and high sensitive C - reactive protein with WBC count and differential count as inflammatory markers in acromegaly patients in conclusion peripheral blood examination acromegaly patients is highly indicated, low cost test and valuable in follow up acromegaly patients.

References:

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Hematological profile of patients with Acromegaly


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e-mail: khleed59@yahoo.com
Immunomodulation of Polysaccharides Extracted From Wild *Lycium barbarum* Iraqi plant

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**ABSTRACT**

This study demonstrates the favorable effects of Iraqi wild type *Lycium barbarum* active component as an immunomodulation agent. The fruit of Iraqi *Lycium barbarum* is rich with Polysaccharides, which were investigated qualitatively and quantitatively in the present study. The extracted polysaccharides total content calculated, as glucose was 3.4mg/g dried fruits.

The Immunomodulation effect for the extracted polysaccharides on normal human peripheral blood lymphocytes showed an increasing in lymphocytes proliferation significantly when it tested by MTT assay. The immune stimulating effect of the polysaccharides extract caused alteration in both IL-10 and TNF-α levels. After 2 hours of exposure to the extracted polysaccharides at concentrations (250 and 500 µg/ml), the normal human blood lymphocytes showed an elevation in IL-10 level against TNF-α level while the apposite results developed after 4 hours of exposure and both estimations were done by ELISA technique.

**Key words**: *Lycium barbarum*, polysaccharides, Immunomodulation.

**Introduction**

The discovery and identification of a new drugs, which can potentiate the immune function has become an important goal of researches in immune-pharmacology. The flora of Iraq, the ancient Mesopotamian land of civilization is interesting; about 1500 medicinal plat species, which have been recorded in Iraq, and large number of these plants, are used for medicinal purpose (¹). Studies are in progress to understand how these compounds may or may not provide protection against toxic, mutagenic and carcinogenic activities of chemical compounds. *Lycium barbarum*, a well-known Chinese traditional medicine and foodstuff, contained different active components which have many proposed pharmacological and biological effects; including anti-aging activity (²), immune modulation and
anti-cancer activity\(^3\). The polysaccharides is an important bioactive compound in *L. barbarum* fruits, which has been found to have anti-cancer properties. The α-D (1→4) polygalacturonan from *L. barbarum* polysaccharide (LBP3a) is able to induce T lymphocyte proliferation and to promote an increase in interleukin-2 (IL-2) receptors expressed on isolated human peripheral lymphocytes \(^4\).

All studies and researches on *Lycium barbarum* biological active components were done on the Chinese grown plant, while there are little (if not) researches about the Iraqi wild type plant. Therefore, the study of the effects of polysaccharides on the immune cells is of great significance and the present work was aimed to:

1-Identify the polysaccharides component from the fruits of the Iraqi wild *L. barbarum* plant, qualitatively and quantitatively.

2-Investigate the effect of the extracted polysaccharides towards normal human blood lymphocytes culture (by MTT assay).

3- Determine the cytokines level (IL-10 & TNF-α) in lymphocytes cultured cells by ELISA technique.

**Material and Method**

**i-Plant Collection:**

Ripe orange small fruits from *Lycium barbarum* trees grown as a wild plant were collected from Al-Jadriya district at University of Baghdad, and classified by the herbarium of the Biology Department, collage of Science at University of Baghdad.

**ii-Extraction of Polysaccharides from the Fruits:** \(^5\)

About 25 g of powdered *Lycium barbarum* fruits were mixed with 300 ml distilled water, then boiled for one hour, cooled, and filtered with piece of guise finally centrifuged for 30 minutes at 1500rpm. The filtrate was collected and cold solution of 95% ethanol was added and allow to stand for 24 hours. The precipitated polysaccharide was collected and washed with cold absolute ethanol then acetone and weighted after drying and kept in refrigerator at 4°C.

**iii- Determination of Total Polysaccharides Content in the Fruit** \(^6\)

For total polysaccharide determination, different glucose standard solutions (0.3, 0.25, 0.20, 0.15 and 0.1) mg/ml were prepared from glucose stock solution of 1mg/ml. About 250 mg from the extracted polysaccharides (the precipitate) was dissolved in 50 ml hot water to get solution of (5mg/ml) concentration. The following methods were done to determine polysaccharides content in the fruit.

**A. Qualitative Determination**

A general Benedict's test was done as primary qualitative determination for polysaccharides\(^7\). The reagent contained...
blue copper(II) ions(Cu+2) which were reduced to copper(I)ions(Cu+1) which precipitated as insoluble red copper(I) oxide in the presence of reducing sugar and heating.

**B. Quantitative Determination**

For quantitative determination, a phenol-sulfuric method by Dubois et al (8) was applied as follows:

About 0.4 ml from each standard solutions and 0.4 ml from the extracted polysaccharide were transferred each to separated glass tubes, then 0.4 ml of 5% phenol solution was added to all tubes, mixed with 2ml concentrated sulfuric acid. The mixture was shaken for 30 minutes and finally the absorbance was measured at 490 nm. A standard curve was plotted between concentrations verses absorption then from straight line equation the total polysaccharides concentration was calculated as glucose.

**iv-Immunomodulation Determination** *(in vitro)*

To determine *in vitro* immune effects for *Lycium barbarum* extracts; lymphocytes culturing and viable counting was employed in each step; lymphocytes proliferation, and cytokines level(IL-10 &TNF-α) in the supernatant of lymphocyte culture treated cells were involved.

**A-Lymphocytes Culturing and Viable Counting** *(9,10)*

-About forty milliliters of venous blood were taken from healthy volunteers, their ages in the range of (25-35) year’s old, never taken medication at least 10 days ago.

-Each ten milliliters was transferred into vacuumed tubes containing 0.2% EDTA as anticoagulant with continuous gentle shaking.

-The human peripheral blood was diluted PBS (pH=7.2) in 1:1 ratio.

-About 5 milliliters of the diluted cell suspension was layered onto three milliliter of Ficoll-isopaque separation fluid (lymph prep; specific gravity=1.077g/l, placed into vacuumed tubes(10 ml capacity).

-The tubes were centrifuged at 2000 rpm for 30 minutes.

-The mononuclear cells were collected with sterile pasture pipette, transferred into 10ml vacuumed tubes, suspended with 5ml RPMI 1640, and centrifuged for 10minutes at 2000 rpm. The step was repeated twice.

-The isolated lymphocyte cells were collected again and suspended in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100μg/ml streptomycin, then transferred into appropriate tissue culture plate and incubated for 18 hours at 370C in 5% CO2 incubator.

**B-Determination of cell viable counting for the isolated lymphocytes**

The cell count and viability were determined according to Freshney procedure *(11)*. Trypan blue 1% solution freshly prepared in PBS was used. Dead
cells unlike viable cells took up the dye within seconds which could be easily distinguished under light microscope.

-About 10μl from both Trypan blue stain and lymphocyte cell suspension were mixed for 30 seconds, then 10 μl from the mixture was applied gently into both grooves edge at the two sides of a haemocytometer chamber, underneath the cover slip.

-Under light microscope 40X objective lens all cells were counted in 1mm², then a separate counting of viable(transparence) and non-viable(blue) cells was done.

-Cell concentration(cell/ml), total cell count and %viable cell count were calculated:

% viable cell= number of living cells/ total number of cells.

-The viable counting with Trypan blue result should be more than 90% viable cells count.

C-Measurement the Proliferative Cultured Lymphocytes by MTT Assay (11)

-About 100μl of the suspended cells was seeded in each of the 96 well microtiter plate, about(106cell/well).The plate was incubated at least for 2 hours in a CO2 incubator.

-Serial concentrations from extracted polysaccharide was prepared from stock solution (1000µg/ml) to get (500, 250, 125, 62.5, 31.25, 15.625, and 7.8125) µg/ml, then sterilized with 0.22 μm Millipore filter.

-About 100 μl from each concentration of the extract was added in triplicate to the lymphocytes seeding plate. Control positive was employed as 10 μl of 0.1% PHA solution (phyto-hemagglutinin), while negative control represented by untreated lymphocytes suspended in medium.

-The plate was incubated in a CO2 incubator for 24 hours at 37⁰c.

-About 50 μl of MTT dye(2mg/ml) was added to all wells, then incubate for further 4 hours.

-The MTT-formazan crystals which formed only by live cells were dissolved with100μl DMSO added to all wells.

-Absorbance at 620 nm was recorded immediately by ELISA reader.

-Viable cell Lymphocytes as a percentage was calculated as follow:

[Absorbance of the test /Absorbance of negative control]X 100.

-A comparison between the results of the extracted polysaccharide at different concentrations was statically calculated to choose the most effective dosages of each extract that may cause lymphocytes proliferation that to be used in further experiment as immunostimulants.

D-Determination Cytokine Concentration by ELISA Kit (12)

The work was done following the instruction of US Biological TNF-α and IL10 kit protocol/Biochemical & Biological Reagents, United State Biological. Catalog No(T9160-01). The
supernatants of treated lymphocytes at different concentrations of extracted carotene (previous steps) were applied in this test. At the end of experiment a standard curve for each cytokine different concentrations was plotted with their absorbance at 450nm, then all test reading were calculated according to straight line equation obtained from the standard curve and both TNF-α and IL-10 level of the treated lymphocytes in the supernatant were obtained and evaluated statistically.

Statistical Analysis:
The Statistical Analysis System- SAS (2004) was applied for all results to show effect of difference concentration and other factors in studied parameters. The least significant difference (LSD) test and Duncan test at the comparative between means in this study.

The Results

i-Lycium barbarum Active Components

There is no study about Iraqi wild type Lycium barbarum and its active components had been done before. The polysaccharides are the main constituents of Iraqi Lycium barbarum was estimated qualitatively and quantitatively in the current study.

ii- polysaccharides content in the fruits: The final precipitate yield was one gm from 25g dried powdered fruits. Only 250 mg were dissolved in 50ml hot distilled water for quantitative analysis of total polysaccharide calculated as Glucose.

iii-Total Polysaccharides Content in fruits of Lycium barbarum A. Qualitative Determination: A red precipitate was formed as a result of the Benedict's general test

Figure (1) Benedict's test give red precipitate with the total polysaccharides extracted from Lycium barbarum fruits
B. Quantitative Determination:

Spectrophotometric absorption of the extracted *L. barbarum* were done to determine the total polysaccharides by reading the absorbance of different concentrations of D-glucose standard solution. All readings were plotted for standard curve to estimate the total polysaccharides in the extracted fruits sample, which gave an absorbance of (1.2 nm) Figure(2).

![Figure 2](image.png)

Figure (2) Standard curve for glucose as determined by spectrophotometer, \( R^2 = 0.986 \)

The results showed that the concentration of total polysaccharides in the extracted fruits were 85 mg (3.4 mg/g dried fruit).

Extraction and isolation of polysaccharides even in low concentration is simple, as they are soluble in hot water and the easiest method is first produce a hot water extract of herb using more than one extraction to get most of polysaccharides into solution and then force the polysaccharides out of the solution by adding alcohol in which they are not soluble, then the liquid is separated off and the residue is dried to produce the finished polysaccharides \(^ {13} \). The yield of this procedure is 3.4 mg/g of the dried fruits from the wild Iraqi type, while references showed (5-8) % polysaccharide content in cultivated type as in Chinese desired and more content up
Immunomodulation of Polysaccharides Extracted to 10-15% can be obtained with optimized condition of extraction (14).

**iv-Extracted Components from *Lycium barbarum* as Immunomodulator (In vitro)**

In order to trace immune-modulation and the mechanism for regulation of the immune system; Lymphocyte proliferation (by MTT assay), and cytokines (IL-10 and TNF-α) level were employed. Results of the effect for polysaccharides extracted from *L. barbarum* on these parameters were analyzed statistically.

**Table (1) Effect of purified flavonoid and polysaccharides extracted from *L. barbarum***

<table>
<thead>
<tr>
<th>Conc.(µg/ml)</th>
<th>% viable Lymphocytes treated by the Extracted components</th>
<th>T-test Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Falvonoid</td>
<td>Polysaccharide</td>
</tr>
<tr>
<td>3.91</td>
<td>12.57 ± 0.90 A</td>
<td>115.47 ± 3.63 A</td>
</tr>
<tr>
<td>7.812</td>
<td>13.14 ± 0.34 A</td>
<td>83.01 ± 1.60 A</td>
</tr>
<tr>
<td>15.625</td>
<td>13.50 ± 4.09 A</td>
<td>85.72 ± 5.27 A</td>
</tr>
<tr>
<td>31.25</td>
<td>13.91 ± 0.75 A</td>
<td>99.65 ± 4.23 A</td>
</tr>
<tr>
<td>62.5</td>
<td>11.16 ± 0.27 A</td>
<td>102.49 ± 1.96 Bc</td>
</tr>
<tr>
<td>125</td>
<td>13.19 ± 0.24 A</td>
<td>110.05 ± 3.04 Ab</td>
</tr>
<tr>
<td>250</td>
<td>12.99 ± 0.68 A</td>
<td>112.40 ± 2.97 Ab</td>
</tr>
<tr>
<td>500</td>
<td>15.07 ± 0.76 A</td>
<td>106.79 ± 2.36 Abc</td>
</tr>
<tr>
<td>LSD Value</td>
<td>4.677 ns</td>
<td>10.006 *</td>
</tr>
</tbody>
</table>

PHA (+ve control) = 193.61% ± 12.6 for all readings.
The (-ve control) = 100 % ± 12.6 for all readings.

Mean with different letters at the same column represented significant differences.
Results indicated that a significant differences \((P\leq0.05)\) among the polysaccharides and purified flavonoid as well as within each extract at different concentrations as compared with results of the mitogenic (PHA) as a positive control and the untreated lymphocytes as negative control. As shown in Table(1), the purified flavonoid suppress lymphocyte proliferation with no significances between all concentrations, while the extracted polysaccharides showed stimulating effect for the immune-system by increasing lymphocytes proliferation, specially at high concentrations(500, 250, and 125 \(\mu g/ml\) ) in respect to control result. The proliferative activity by the mitogenic (PHA) appeared to be with a high influence than polysaccharides on normal lymphocytes. The results agreed with studies about the effects of flavonoids on immune cell functions \(^{15,16}\). These studies showed that the aglycon part of well-known flavonoids possessed inhibitory effects on human lymphocytes \(^{17}\) and the derivatives of flavone and flavonol which have 2,3-unsaturated bond and at least 1 hydroxyl group present in the flavonoid structure Figure(1-3) showed the suppressive activity, but when various glycosidic substitutions to A- and/or C-ring of the flavonoid aglycones found , this substitutions will eliminate the suppressive activities of their aglycones, regardless of sugar compositions and positions of substitutions \(^{16}\). The flavonoids possessing 5-hydroxyl,5-methoxyl and 6-methoxyl groups and those with cyclohexyl instead of phenyl substitution (i.e.2-cyclohexyl-benzopyran-4-one), showed the greatest inhibition \(^{15}\) activity to normal lymphocytes. Evidence indicated that selected flavonoids, depending on structure, can affect (usually inhibit) secretary processes, mitogensis and cell-cell interaction including possible effect on adhesion molecule expression and function, moreover certain flavonoids may affect gene expression of cytokines and their effects and cytokine receptors. This due to their capacity to stimulate or inhibit protein phosphorylation that regulate cell function or by counterbalancing the effect of cellular protein tyrosine phosphatases \(^{17}\). As a result of the relatively poor prognosis and response to conventional chemoradiotherapy, there is a great need for new effective agents. Renewed attention in recent years to natural therapies has stimulated a new wave of research interest in traditional practices. Herbs have become a target for the search for new anticancer drugs. About half of the drugs used in clinical practice come from natural products \(^{18}\). Various \textit{in vitro} studies about the mechanism of the plant cytotoxicity were differ from one cell culture to another depend on whether whole plant extract was used or any of the plant component. In fact, many nutritive and nonnutritive phytochemicals with
Immunomodulation of Polysaccharides Extracted

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diversified pharmacological properties have shown promising responses for the prevention and treatment of various cancers, including different types of cancer. Most of the animal studies done on *L. barbarum* explained the anti-cancer effects of the plant were through immune enhancements and prevent the development of complications or even tendency to carcinogenesis by increasing numbers of CD4+ and CD8+ T cells to relieve the immunosuppression and enhance the anti-tumor function of the immune system (19). T lymphocytes play a central role in adaptive immunity (4). At the same time, the percentage of cells in G0/G1 phase was increased, thus because T cells spontaneously arrest in G0 and may remain quiescent for long period of time until exposed to specific antigen or mitogens that initiates a cascade of biochemical events leading the resting T cells to enter the cell cycle then proliferating and differentiating. For this reason the plant active components had been used as immune stimulant or immune adjuvant. (4,20) In China, many types of polysaccharides, among them *lycium* polysaccharides (LBP) and *Astragalus* polysaccharides (APS) are widely used as an immune adjuvant; having been identified as a class of macromolecule that can profoundly affect the immune system, stimulate cell proliferation, induce the expression of surface antigens on lymphocytes, affect the expression of cytokines, and promote the production of antibodies (21). In a study, it was reported that LBP and APS possess effective immunostimulatory effects when used in vaccination programs against Foot and mouth disease virus (FMDV), Newcastle disease virus (NDV) and Infectious bursal disease virus (IBDV) (22). The appropriate concentration and antiviral action of APS on the propagation of H9N2 AIV (Avian influenza subtype H9N2 belongs to the low pathogenic avian influenza virus (AIV) group; considered to be a common cause of disease epidemics) in chick embryo fibroblasts (CEF) was investigated. *Astragalus* polysaccharide (APS) effectively increases the expression of IL-2, IL-4, IL-6, IL-10, LITAF and IL-12, promotes cell growth, and improve humoral immunity, and boosts both T cells and B cells. *L. barbarum* polysaccharides (LBP) can stimulate moderate immune responses therefore could potentially be used as a substitute for oil adjuvant in veterinary vaccines. Ling and his colleagues results showed that the isolated polysaccharides, combined with a DNA vaccine encoding the major outer membrane protein (MOMP) of *Chlamydia abortus*, induced protection in mice against challenge (23).

**Effect of Extracted Components**

*L. barbarum* on Cytokine Levels (IL-10 and TNF-α)

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In order to trace cytokines (IL-10 and TNF-α) level in the supernatant of the treated lymphocytes with polysaccharides at different concentrations and exposure time (2 and 4 hours) as well as control culture and standard solutions, ELISA was used and standard curve for both interleukins was plotted separately Figure (3).

![Standard curve of IL10 for ELISA result](image)

![Standard curve of TNF-α for ELISA results](image)

Figure(3): Standard curve of IL-10 (A) and TNF-α (B) analyzed by ELISA, $R^2=0.98$ And 0.93 respectively
Table (2) Effect of different concentrations of polysaccharides and exposure time (2, 4 hours) on lymphocytes IL-10 & TNF concentrations

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>IL-10 (pg/ml)</th>
<th>Level</th>
<th>LSD Value</th>
<th>TNF Level (pg/ml)</th>
<th>LSD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hr.</td>
<td>4 hr.</td>
<td></td>
<td>2 hr.</td>
<td>4 hr.</td>
</tr>
<tr>
<td>125</td>
<td>140.00 ±7.54c</td>
<td>152.86 ±7.43b</td>
<td>29.41 NS</td>
<td>786.0±28.71a</td>
<td>338.00 ±8.73c</td>
</tr>
<tr>
<td>250</td>
<td>244.20 ±9.37a</td>
<td>129.20 ±7.60c</td>
<td>33.51 *</td>
<td>532.0±19.62c</td>
<td>359.33 ±8.83c</td>
</tr>
<tr>
<td>500</td>
<td>222.4±8.21ab</td>
<td>74.10 ±7.55d</td>
<td>30.98 *</td>
<td>666.4±24.62b</td>
<td>493.16 ±10.11b</td>
</tr>
<tr>
<td>Control</td>
<td>200.00 b</td>
<td>220.00 a</td>
<td>12.67 *</td>
<td>676.0 b</td>
<td>650.0 ba</td>
</tr>
<tr>
<td>LSD Value</td>
<td>23.75*</td>
<td>21.27*</td>
<td>----</td>
<td>69.48*</td>
<td>26.12*</td>
</tr>
</tbody>
</table>

* (P≤0.05).
The means with different small letters at the same column represented significantly different.

Table (2) showed that whenever IL-10 level increased, a clear decrease in TNF-α level was noticed, for the polysaccharides treated lymphocytes after (2 and 4) hours exposure time. The greatest effect of polysaccharides treated lymphocytes for 2 hours exposure caused increasing in IL-10 level to be (244.2 pg/ml), at polysaccharides concentration 250 µg/ml in comparison to control (200 pg/ml), at the same time a significant decreased in TNF-α level to lowest value (532 pg/ml) was shown at the same concentration. The highest TNF-α level after 2 hour treatment was (786 pg/ml) at 125 µg/ml polysaccharides concentration in relative to control value (676 pg/ml), at the same time this concentration affected lymphocytes IL-10 level to be at lowest its value (140 pg/ml) as shown in Table(2). However, after 4 hours exposure, an increase in TNF-α level was combined with decreased in the IL-10 level. Treatment of normal human lymphocytes with different polysaccharides concentrations for 4 hour exposure, showed difference in their effects on both cytokine levels. As shown in Table(2) a large decrease in IL-10 level was dependent on increasing in polysaccharides concentration while in contrast, TNF-α level increased with the increasing of polysaccharides.
concentrations with or without significances results.

Both cytokines showed different levels as time of polysaccharides exposure increased. There was a direct relation between polysaccharides concentrations and IL-10 level after 2 hours exposure, and a reverse relation between them after 4 hour exposure, the opposite relation was found for TNF-α level and polysaccharides concentration with the duration of exposure Table(2). Similar results were obtained in a study by Lei et al. on the effect of Aralia chinesis and Tripterygium wilfordii on serum TNF-α, IL-4 and IL-10 level in rats with adjuvant-induced arthritis. Both plants had significantly increased IL-4, IL-10, levels and markedly reduced TNF-α level compared with control group. The induction of cytokine is a key event in the initiation and regulation of an immune response. Many compounds are now used routinely to modulate cytokine production, and therefore the immune response, in a wide range of diseases, such as cancer.

Interleukin-10 and tumor necrosis factor-α are two important cytokines in antitumor immunity. In a study by Lei et al. showed that the effects of L. barbarum polysaccharides protein complex (LBP3p) on the expression of interleukin-2 and tumor necrosis factor-α in human peripheral blood mononuclear cells were investigated by reverse transcription polymerase chain reaction (RT-PCR). The LBP3p significantly enhanced interleukin-2 mRNA expression at 8 hours, peaking at 12 hours and returning to baseline levels at 15 hours. At 24 h, a marked decrease was observed. With respect to tumor necrosis factor mRNA expression, human peripheral blood mononuclear cells exposed to LBP3p showed a significant increase as early as 2 hours after treatment. The greatest increase was observed at 4 h after treatment, returning to baseline levels at 8 hours, being undetectable at 24 hours. Interleukin-10 (IL-10) is a pleiotrophic cytokine that has an important role in regulating the immune response. This cytokine potently inactivates macrophages, inhibiting the expression of proinflammatory cytokines [e.g., tumor necrosis factor α (TNF-α) and IL-6] and disabling antigen presentation/T cell activation, by inhibiting expression of major histocompatibility complex class II, B7-1, and B7-2.

The anti-inflammatory activity of IL-10 is augmented by enhancing the release of soluble(s) TNF receptors (R) and IL-1R antagonist. In contrast to its activities on macrophages, IL-10 induces the proliferation of mast cells, B and T cells, and enhances T cell responses to IL-2. A major focus of IL-10 research has been to identify the mechanism by which IL-10 mediates suppression of cytokine synthesis. This remains a controversial field; specifically, the ability of IL-10 to
Immunomodulation of Polysaccharides Extracted

Zainab Y. Mohammed, Ahmad R. Abdullah

inhibit lipopolysaccharide (LPS)-induced gene expression has been shown to be transcriptionally mediated via the inhibition of the nuclear factor-κB pathway. However, further evidence also suggests that IL-10 can act through a post-transcriptional mechanism via destabilizing mRNA, in the case of TNF-α and the chemokine KC. This effect requires the AU-rich elements in the 3′ untranslated region. Furthermore, these reports suggest that the effects of IL-10 are indirect and that IL-10 is inducing a gene whose product is responsible for mediating the destabilization of mRNA.

Interleukin-10 and tumor necrosis factor-α are two important cytokines in antitumor immunity. In a study by Lu et al. showed that the effects of L. barbarum polysaccharides protein complex (LBP3p) on the expression of interleukin-2 and tumor necrosis factor-α in human peripheral blood mononuclear cells were investigated by reverse transcription polymerase chain reaction (RT-PCR) (25). The LBP3p significantly enhanced interleukin-2 mRNA expression at 8 hours, peaking at 12 hours and returning to baseline levels at 15 hours. At 24 h, a marked decrease was observed. With respect to tumor necrosis factor mRNA expression, human peripheral blood mononuclear cells exposed to LBP3p showed a significant increase as early as 2 hours after treatment. The greatest increase was observed at 4 h after treatment, returning to baseline levels at 8 hours, being undetectable at 24 hours (25).

In one study used microarray analysis to identify IL-10-inducible genes in the presence and absence of the powerful pro-inflammatory stimulus LPS. These studies have identified 19 inducible genes for IL-10. Three of these genes, IL-1ra, SOCS3, and CD163, have previously been shown as being regulated by IL-10; however, the other 16 represent novel IL-10-inducible genes first identified in a study by Kaur et al. (30). The result of the present study indicated that Lycium polysaccharides acted to reduce tissue inflammation – in part by inhibition of TNF-α gene expression and promote IL-10 production and expression. Although in vitro studies did not necessarily predict results in vivo out comes, such studies have provided insights into molecular targets, and the relative contributions of these activities as a potent immune-modulator agent which need in vivo elucidation and more investigation for their action and mechanism must be subjected to a further studies.

**Conclusion:**

The Immunomodulation effect for the Lycium polysaccharides may play a role through reducing tissue inflammation – in part by inhibition of TNF-α gene expression and promote IL-10 production and expression.
**Reference**


Case report

Case report:

A Ten Year Old Boy with Unexplained Hemolytic Anemia

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ABSTRACT

Babesiosis is a tick-borne zoonotic disease transmitted by the intracellular protozoan from genus Babesia. Infection with is uncommon and symptomatic disease is mostly confined to splenectomized patients. It may be transmitted by blood transfusion.

In splenectomized patients, the disease has an acute onset and is often fatal. Unsplenectomized patients may experience a milder self-limiting disease, although intravascular hemolysis does occur. The diagnosis is made from the peripheral blood film, where the parasites, looking very similar to malaria, are seen inside the red cells.

In this case report, we describe a child with unexplained hemolytic anemia with presumptive diagnosis of autoimmune hemolytic anemia treated with Steroids and immunosuppressive treatment with no response. After review of his investigation, surprisingly, his peripheral blood film revealed Babesiosis.

CASE PRESENTATION

A Ten years old boy presented to the general pediatrician at October 2013 with 2-day history of pallor, jaundice, abdominal distension and dark-colored urine. He received 1 unit of packed red cells once. His condition remained stable for 1 month and deteriorated again with
pallor and change in urine color and later on was admitted to a hospital for investigations and follow up for 1 week, and discharged home without any complications. He kept on follow up only for 2 months. Since 24th of January 2014, treatment started with small dose prednisolone and increase gradually. A consultation to Child Welfare Teaching Hospital Hemato-oncology outpatient clinic was done on 24th March 2014 in which full investigation done and revealed a picture of autoimmune hemolytic anemia. For that reason prednisolone started at a dose of 2mg/kg/day for 2 weeks at the beginning then dose was adjusted according to the clinical criteria and the result of investigations. After 6 months of follow up, immunosuppressive treatment with mycophenolate mofetil (Celcept)® started with gradual tapering of steroids. The anemia worsened with declining in hemoglobin level, increasing in reticulocyte count with deepening of jaundice; so an online consultation to Swinfen Charitable Trust website http://www.humanitariantelemed.org was established to Dr. Peter Wood, who say: "it certainly sounds like a hemolytic anemia although hemoglobin nadir is not too bad (92g/L). I note the initial positive Coomb's test, but then several negative tests subsequently. If the initial positive was only weak, it may not be significant. Certainly the predominantly negative Coomb's suggest it is NOT an immune hemolysis and hence unlikely to respond to immunosuppression. Non-immune cause particularly hereditary spherocytosis should be considered (there may be a family history; other causes would be G6PD or pyruvate kinase deficiency. If you are convinced, the hemolysis is immune then Rituximab is a reasonable option, as is cyclosporine A. The response to Rituximab usually only lasts for a couple of years if it responds".

Our interpretation, we have more than one patient who has negative coomb’s test turned to be positive when checked abroad so we didn’t rely on the lab. In determining the accuracy of Coomb's test. It wasn’t a case of hereditary spherocytosis so we continue treatment as autoimmune hemolytic anemia ignoring the comment of the expert which was a valid one.

On August 2014, courses of Rituximab (375 mg/m² weekly for 4
doses) had been started with steroid and Celcept®; the latter was discontinued with gradual taper. The patient was kept on Prednisolone only after finishing the forth course of Rituximab. Deterioration of the clinical condition with progressive anemia, worsening of jaundice and change in the urine color happened after starting the slow tapering of steroid (5mg per month). In April 2015, Cyclosporine A (Sandimmune)® 5 mg/Kg/day in 2 divided doses had been started with small dose of steroid but the condition remained stationary with element of mild hemolysis (high reticulocyte count and hemoglobin around 9.0 g/dl with jaundice).

At 1st of October 2015, a suggestion was contemplated to transfer the patient outside Iraq for further investigation unavailable in our country to diagnose the type of hemolytic anemia to prescribe the correct treatment according to his investigation.

Consultation to Dr. Azadeh Kiumarsi in Iran had been done and her notes:

"Surprisingly, in his PBS we saw some ringed shaped organisms within the RBCs which we sent it to a parasitologist and he approved that there was evidence for "Babesiosis". We recommend that you treat the organism; it may help the hemolytic anemia to stop!!! but if this surprising diagnosis and its treatment would not stop the lysis we recommend splenectomy as the next step".

At 12.10.2015 Swinfen review for Dr. Peter Wood had been done, who answered:" Babesiosis is a red cell parasite like malaria and can certainly cause intravascular hemolysis as in your case It would have a negative coomb’s test and would not respond to steroid therapy .I have looked at the images and there do appear to be intracellular inclusions that look like Babesia .The treatment of choice is quinine 25 mg/kg/day and clindamycin 20-40 mg/kg/day both for 7 days , you would expect to see a reduction in hemolysis with 1-2 weeks (falling bilirubin, LDH, and RTC )but the Hb. May take a little longer to recover .Splenectomy is a reasonable option and if effective will be simpler in the long term "

On Examination: He had cushingoid face, mild pallor, and jaundice. There is neither lymphadenopathy nor abnormal
pigmentation but there is palpable splenomegaly.

*Results of laboratory investigations and Treatments are shown in tables 1 and 2.*

*Other Investigations*

GUE= normal.

Autoimmune study = Negative

Blood Urea= 21 mg/dl, S. creatinine = 0.8 mg/dl

TSB= 2.8 mg/dl, Direct= 0.8 mg/dl, Indirect= 2 mg/dl, SGPT= 12 U/L, SGOT= 13 U/L

Serum Haptoglobin =377 mg/dl (N=32-205)

Serum LDH= 808 IU/ L (N=207-450)

*Treatment*

Briefly , the patient had been managed by the following treatment for presumed autoimmune hemolytic anemia during the course of his disease : Steroids in the form of prednisolone courses , Mycophenolate Mofetil (Celcept)®, Rituximab( 4 courses) , Cyclosporine A (Sandimmune) ® as well as one unit of packed red blood cell transfusion .

After the diagnosis of Babesiosis has been established, a decision made to start antiparasitic treatment of Babesiosis based on the suggestion of Dr. Azadeh Kiumarsi with quinine 20-40 mg/kg /d & clindamycin 25mg/kg/d for 7-10 days.

**SUMMARY AND DISCUSSION**

* Babesiosis is a worldwide tick borne hemolytic disease that is caused by intra erythrocytic protozoan parasites of the genus *Babesia*. Human infection is accidental; Babesiosis may also be acquired by blood transfusion, particularly in areas endemic for *B. microti* and *B. duncani*. It is a disease with a world-wide distribution affecting many species of mammals with a major impact on cattle and man .

**History:**

Babesiosis was first reported in 1888 by Viktor Babes in Romania who detected the presence of round, intraerythrocytic bodies in the blood of infected cattle.  

**Epidemiology**

Babesiosis has rarely been reported outside the United States. Sporadic cases have been reported from a number of countries including France, the former Yugoslavia, United Kingdom, Ireland, the former Soviet Union and Mexico. In the United
States, infections have been reported from many states but the most endemic areas are the islands off the coast of Massachusetts and New York and in Connecticut (5,6). Transfusion-associated Babesiosis has also been described (7). Patients receiving erythrocyte transfusions are at highest risk, while infection after transfusion of plasma has not been reported (8).

Overall, the risk of acquiring Babesiosis from a blood transfusion is very low. In Connecticut, the risk of acquiring babesiosis from a transfused unit of packed red blood cells was estimated at about 0.17 percent and was even lower from a transfused unit of platelets (9). Transplacental/perinatal transmission has been reported (5,6).

Clinical Manifestations

Mild Illness (10,11,12,13):

- Nonspecific symptoms (Fatigue, malaise, weakness) (common)
- Fever >38°C, Chills & sweats (common)
- Headache (less common)
- Myalgia (less common)
- Arthralgia (less common)

- neck stiffness, sore throat, dry cough, weight loss, vomiting, diarrhea & dark urine (less common)
- Mild splenomegaly and hepatomegaly
- Lymphadenopathy is absent.
- Jaundice (rare)
- Slight pharyngeal erythema (rare)
- Retinopathy with splinter hemorrhage and retinal infarct (rare)
- Parasitemia less than 4-5%
- Hemolytic anemia (low hematocrit, Elevated LDH, low Hemoglobin, elevated total bilirubin, low Haptoglobin &/or reticulocytosis)
- Thrombocytopenia (common)
- White blood cell counts are normal, increased or mildly decreased
- Liver enzyme are elevated

Severe Illness

Severe Babesiosis was defined as a hospitalization ending in death, lasting longer than two weeks, or requiring a stay in the intensive care unit (ICU) of two days or longer (13).

- Parasitemia >4 percent
- alkaline phosphatase >125 U/L
- and white blood cell counts >5 x 10⁹/L
- Malaise (12)
- Arthralgia or myalgia (12)
• Shortness of breath (12)
• Thrombocytopenia (12)
• Elevated liver enzymes are also common (12)

**Differential Diagnosis of Babesiosis** (14)

The diagnosis of Babesiosis should be considered in patients with flu-like symptoms in the setting of appropriate exposure (e.g., residents of endemic areas, or travelers returning from endemic area). These include malaria, meningitis, pneumonia, infective endocarditis, viral hepatitis, and noninfectious causes of hemolytic anemia.

Coinfection with other tick-borne illnesses should also be considered. Acute Anemia, Colorado Tick Fever, Ehrlichiosis, insect Bites, Lyme disease, malaria, Q Fever, relapsing fever in emergency medicine, Rocky Mountain Spotted Fever, tick-borne diseases, typhoid fever.

**Diagnosis of Babesiosis:**

**Microscopy** (10,11,15):

• Definitive diagnosis of babesiosis should be made by microscopic examination of thin blood smears (Wright or Giemsa staining under oil immersion).

• *B. microti* appear round, oval, or pear-shaped. The most common form is the ring, with a pale blue cytoplasm and one or two red chromatic dots. Multiple infections per cell may be observed. Ring forms may be mistaken for *Plasmodium falciparum* trophozoites.

**Distinguishing features of Babesia** include:

• Occasional merozoites arranged in tetrads, referred to as "Maltese Cross"
• Occasional exoerythrocytic parasites (when parasitemia is high)
• Absence of brownish pigment deposits (hemozoin) in ring forms
• Absence of schizonts and gametocytes

**Polymerase Chain Reaction (PCR)**

PCR-based amplification of the babesial gene is more sensitive than blood smear examination and results can be available within 24 hours. (16,17)

PCR is especially useful in the setting of low level parasitemia, (eg, at the **onset of symptoms** and **during convalescence**) (11)

PCR can be used to detect persistent Babesial DNA in the blood even when parasites are no longer visible on blood
A Ten Year Old Boy with Unexplained Hemolytic smear. In one asymptomatic case, Babesial DNA persisted for as long as 17 months \(^{(16)}\)

Serologic diagnosis with indirect immunofluorescent antibody testing (IFAT)

- Is most useful when parasites are not visualized by microscopy and DNA is not detected by PCR. \(^{(18,19)}\)
- In cases of acute infection, serology is best used to confirm the diagnosis made by blood smear or PCR. In patients treated with rituximab (anti-CD20 antibody), B cells are depleted and serology is of no value. \(^{(15,20)}\)

Treatment \(^{(21)}\)

Asymptomatic individuals need not be treated, unless a Babesia species is detected on blood smear or by PCR assay for more than 3 months. Suspected Babesiosis should not be treated if reliable blood smear and PCR reaction results are negative. When Babesia is detected, symptomatic patients should be treated.

Severe Babesia microti Infection

First-Line Therapy

Based on the greatest cumulative clinical experience, it is recommended that patients with severe illness caused by \(B. microti\) be treated with intravenous clindamycin plus oral quinine for 7 to 10 days.

Persistent Relapsing Infection

A single course of standard antimicrobial therapy may fail to cure patients who are immunocompromised by one or several of the following conditions: splenectomy, HIV infection/AIDS, malignancy, and immunosuppressive therapy (in particular rituximab therapy). In immunocompromised patients, antimicrobial therapy should be administered for a minimum of 6 weeks, including 2 weeks during which the parasite is no longer seen on blood smear. Drug regimens other than clindamycin (7-10 mg/kg q6-8h IV or 7-10 mg/kg q6-8h PO (maximum 600 mg/dose)) plus quinine (8 mg/kg q8h PO (maximum 650 mg/dose)) have been used, but no particular regimen is superior to another. In addition to the
standard regimen, atovaquone (20 mg/kg q12h PO (maximum 750 mg/dose) plus azithromycin 10 mg/kg PO on day 1 (maximum 500 mg/dose) and 5 mg/kg/day PO from day 2 on (maximum 250 mg/dose)

*Exchange Transfusion*

Partial or complete RBC exchange transfusion should be considered for patients with high-grade parasitemia (≥10%) RBC exchange transfusion removes *Babesia*-infected RBCs, toxic by-products of RBC lysis (e.g., hemoglobin), and circulating inflammatory mediators. Exchange transfusion also corrects the anemia caused by hemolysis of infected red blood cells as well as clearance of infected and uninfected red blood cells. Prompt use of RBC exchange transfusion is associated with favorable outcome. RBC exchange transfusion is also recommended for patients with severe anemia (hemoglobin ≤10 g/dL) or renal or hepatic compromise.

*Mild Babesia microti Infection*

Patients with non–life-threatening *B. microti* infection should be treated with atovaquone plus azithromycin for 7 to 10 days. In a randomized clinical trial, this regimen was found to be as effective as clindamycin plus quinine in resolving symptoms and clearing parasitemia. In patients with mild Babesiosis, symptoms typically improve within the first 48 hours of therapy and should resolve within 3 months.
### Table 1 Laboratory Investigations and treatment/2014

<table>
<thead>
<tr>
<th>Date</th>
<th>Hb.(g/dl)</th>
<th>WBC /cmm</th>
<th>PLT/cmm</th>
<th>RTC %</th>
<th>Coomb's test</th>
<th>TSB mg/dl</th>
<th>Direct</th>
<th>Indirect</th>
<th>SGPT U/L</th>
<th>SGOT U/L</th>
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Hb.=Hemoglobin, WBC=White blood cell, PLT=platelet, RTC=Reticulocyte count, TSB= Total serum bilirubin, SGPT= Serum glutamic pyruvic transaminase, SGOT=Serum glutamic oxaloacetic transaminase. PRD= prednisolone
Table 2 Laboratory Investigations and treatment/2015

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<th>TSB mg/dl</th>
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<th>SGOT U/L</th>
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Hb.=Hemoglobin, WBC=White blood cell, PLT=platelet, RTC=Reticulocyte count, TSB=Total serum bilirubin, SGPT= Serum glutamic pyruvic transaminase, SGOT=Serum glutamic oxaloacetic transaminase. PRD=prednisolone
**Table 3 Differential Diagnosis of Babiosis**

- Acute Anemia
- Colorado Tick Fever
- Ehrlichiosis
- Insect Bites
- Lyme Disease
- Malaria
- Q Fever
- Relapsing Fever in Emergency Medicine
- Rocky Mountain Spotted Fever
- Tick-Borne Diseases
- Typhoid Fever
- Meningitis
- Pneumonia
- Infective endocarditis
- Viral hepatitis
Table 4: Risk factors for developing severe illness due to Babesiosis

<table>
<thead>
<tr>
<th>Risk Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;50 years</td>
</tr>
<tr>
<td>Splenectomy</td>
</tr>
<tr>
<td>Coinfection with HIV or Borrelia burgdorferi</td>
</tr>
<tr>
<td>Immunosuppression caused by cancer chemotherapy or transplantation</td>
</tr>
<tr>
<td>Blockade of tumor necrosis factor alpha (TNF-alpha) activity (etanercept, infliximab)</td>
</tr>
</tbody>
</table>

(1,2,3-5)
Figure 1 – Peripheral blood film obtained at October 1, 2015 showed: (A) ring forms red cell inclusions of Babesiosis with tetrad (Maltese cross) seen in one red cell (low power, 4x), (B) multiple ring forms red cell inclusions of Babesiosis are seen with Maltese cross seen in one red cell, (moderate power, 8x), (C) Maltese cross seen in one red cell (high power, 100x)
Figure 2 – The life cycle of Babiosis

1. Infected mosquito bites human; sporozoites migrate through bloodstream to liver of human
2. Sporozoites undergo schizogony in liver cell; merozoites are produced
3. Merozoites released into bloodstream from liver may infect new red blood cells
4. Merozoite develops into ring stage in red blood cell
5. Ring stage grows and divides, producing merozoites
6. Merozoites are released when red blood cell ruptures; some merozoites infect new red blood cells, and some develop into male and female gametocytes
7. Another mosquito bites infected human and ingests gametocytes
8. In mosquito’s digestive tract, gametocytes unite to form zygote
9. Resulting sporozoites migrate to salivary glands of mosquito

Sexual reproduction

Asexual reproduction

Intermediate host

Definitive host

Sporozoites in salivary gland

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References


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تقرير الحالة النادرة

صبي ذو عشر اعوام مع فقر الدم الانحلالي غير المبرر

أ. م. د. مازن فصل الجادي: استشاري أمراض الدم، مستشفى حماية الأطفال، كلية بغداد الطب، مدينة الطب، بغداد / العراق.

د. أسيل عبد راشد: اختصاص أمراض الدم والأورام، FIBMS, CABP, مدينة مرجان الطبية بابل / العراق.

البابيزيا هو مرض طفيلي ينتج عن الأصابة الأصابة بالطفيلي من عائلة البابيزيا وهو أحد الأمراض المنتقلة من الحيوان للإنسان، وينتقل للحيوان عن طريق القراد ويمكن أيضا انتقاله عن طريق نقل الدم. وبعد البابيزيا من الأمراض النادرة الحدوث وتكون أعراضه واضحة غالبا لدى المرضى المرفوعي الطحال حيث يكون المرض لديهم فتاكة أو مهددا للحياة بينما يكون المرض قابل للشفاء التام دون علاج لدى المرضى غير المرفوعي الطحال أو قد يصابون بفقر دم انحلالي. يتم تشخيص المرض عن طريق صورة الدم حيث يظهر الطفيلي داخل الكرية الحمراء بصورة شبيهة بالإصابة بالضمي بالمالاريا.

في تقرير الحالة السريرية هذا نصف طفلا مصابا بفقر دم انحلالي ذاتي وعولج بالستيرويدات القشرية والأدوية المثبطة للمناعة دون حدوث استجابة تذكر. بعد أعادة تقييم المرض كانت المفاجأة، حيث تم تشخيص البابيزيا لديه في صورة الدم.
تاثير السكريات المستخلصة من النبات البري ليسيوم بارباروم العراقي على مناعة جسم الإنسان

زينب ياسين محمد ، مركز بحوث التقنيات الإحياءية , جامعة النهرين، بغداد، العراق

أ.م.د.أحمد رشدي عبدالله , كلية الطب الجامعة العراقية، بغداد، العراق

الخلاصة:

توضيح هذه الدراسة الآثار الإيجابية لثمرة بارباروم ليسيوم العراقي على مناعة الجسم. ان نبات بارباروم ليسيوم العراقي غني بالسكريات، تمت التجارب على النبتة نوعيا وكميا في هذه الدراسة. تم حساب محتوى السكريات المستخرجة الكلية حيث كان مستوى السكر في 3.4 مليم/غم من الفواكه المجففة.

أظهر تأثير المناعة للسكريات المستخرجة على الخلايا الليمفاوية العادية الموجودة في دم الإنسان وزيادة في انتشار الخلايا الليمفاوية بشكل ملحوظ عندما تم اختبارها من قبل فحص MTT. تأثير تحفيز جهاز المناعة بالسكريات تم عن طريق تسبب تغيير في مستويات كل من IL-10 و TNF-α. بعد 2 ساعة من التعرض للسكريات المستخرجة بتركيزات (250 و 500 ميكروغرام / مل)، اظهرت الخلايا الليمفاوية في الدم الإنسان ارتفاع في مستوى 10 IL-10 بالضد من مستوى TNF-α في حين أن نتائج مغايرة ظهرت بعدها 4 ساعات من التعرض للسكريات و تم قياس التغييرات والتقديرات بواسطة تقنية ELISA.

الكلمات الرئيسية: بارباروم ليسيوم العراقي, المناعة.
التوصيف الدموي للاشخاص المصابين بداء العملاقة

الخلفية:
داء العملاقة مرض يتصف بأرتفاع نسبة إفراز هورمون النمو وهرمون الانسولين المشابهة لهورمون النمو من الغدة النخامية نتيجة ورم حميد. إن تشخيص داء العملاقة عادة ما يكون متأخر بعد تعرض المرضى لمضاعفات بطيئة وشِيْك مزمن.

الأهداف:
إن الهدف من البحث هو تقييم فحص الدم المحيطي كفحص روتيني لمراقبة حالة المرضى المصابين بداء العملاقة العراقيين.

الطرق والاساليب:
وتضمنت هذه الدراسة (83) مريض مصاب بداء العملاقة الذين يراجعون المركز الوطني لبحث وعلاج السكري، حيث تم فحص الدم المحيطي للمرضى في وحدة أمراض الدم في المركز العراقي لبحث السرطان والوراثة الطبية.

النتائج:
اظهرت النتائج وجود ارتفاع في نسبة الصفحات الدموية (الحجم والشكل) بالرغم من عدم وجود فارق معنوي. ووجد أن اثنين من المرضى يعانون نقص في عدد الصفحات الدموية احدهم ذكر والاخلا اثئي. وانتى أيضا تعاني من نقص الحديد متوسط الشدة.

الاستنتاج:
يُقترح من هذه الدراسة أن فحص الدم المحيطي في المرضى المصابين بداء العملاقة هو ضروري وذو قيمة في متابعة حالة المرضى وتكلفة مادية قليلة.

الكلمات المفتاحية: داء العملاقة، فحص الدم
توزيع مجموعات الدم وعامل Rh لدى عينة مختارة من الطلبة العراقيين

الخلفية:
يوجد هناك جهل في معرفة فصائل الدم بين كثير من الناس والغريب حتى بين المتعلمين. وعلى الرغم من أهمية هذه المعلومات الصحية في نقل الدم، كما أنها واحدة من متطلبات الحصول على رخصة القيادة وبطاقة الهوية الوطنية إلا أن هناك تقليل من أهمية هذا الموضوع.

الIGNORE
الأهداف:
كان الهدف من هذه الدراسة لتحديد وتيرة ونسب مجموعات الدم المختلفة وعامل Rh وتحديد الوعي حول أهميتها بين سكان الدراسة.

الطرق والأساليب:
كان حجم العينة لهذه الدراسة 278 طالبا حيث تم اختيارهم عشوائيا. وقد أجريت الدراسة على مجموعتين من الطلاب. وشملت الدفعة الأولى 168 طالب من كلية الطب، في حين شملت الدفعة الثانية 110 طالباً من طلاب المعهد التقني. في验ين أن DK أولئك الذين لا يعرفون نوع فصيلة الدم أو Rh تم تعريفهم بـ Rh.

النتائج:
إن نتائج هذه الدراسة تظهر أن معدل نوع فصيلة الدم كان 27.5٪، 22.3٪، 0.32٪، 6.1٪، و16.9٪.

الخلاصة:
إن فصيلة الدم O مع Rh إيجابي كان الأكثر شيوعاً بانتشاره بين المجموعات المحددة، عن طريق توزيع مجموعة الدم مهم للممارسات السريرية، للحصول على المعلومات للموافقة. كلمات البحث:
فصائل الدم، عامل Rh، الطلاب.
استخدام D-Dimer في استبعاد تشخيص الاشتباه بخثر الأوردة العميقة

لمياء جعفر حمودي القيسي (1)، حسين حسن عبد (2)، رشا طارق جواد (3)

1. اختصاص أمراض الدم المختبري. مدير قسم المختبرات التعليمية في مركز ابن البیطار التخصصي لجراحة القلب
2. اختصاص جراحة الصدر والأوعية الدموية. مدير قسم النضح في مركز ابن البیطار التخصصي لجراحة القلب
3. اختصاص أمراض الدم المختبري. مدير قسم المختبرات التعليمية في مستشفى الطفل المركزي

الخلاصة

الخلفية: تخثر الأوردة العميقة هو اضطراب شائع مرتبطة بفاعلية كبيرة، ومع كثرة وريدي مزمن وكذلك انسداد رئوي مميت. تصوير الأوردة هو استبانحرية تعتبر الطرق الذهبية في التشخيص، ولكن تم استبدالها في معظم المناطق بواسطة الموجات فوق الصوتية المزدوجة، وهي طريقة جيدة جداً. الأعراض الالتهابية لداء تهاب الأوردة الاصطناعية تعكس كمية الفيبرين المنحل، ومستويات D-Dimer، ميزة خاصة في التشخيص السريع. D-Dimer يمكن قياسه كمياً بواسطة ELISA إما أو نوعياً من خلال تراص اللاتكس.

الأهداف: كان الهدف من هذه الدراسة هو تقييم استخدام D-Dimer في استبعاد تشخيص تخثر الأوردة العميقة.

الطرق والأساليب: تم دراسة مجموع من 50 مريضاً قدموا إلى قسم العيادات الخارجية للأوردة الدموية، المرضى الذين يعانون تخثر الأوردة العميقة، والمرضى الذين يتلقون علاج متعدد التخصصات. جمعت قسم أمراض الدم المختبري بيانات الخصوبة المتعددة الفحوصات. أجريت جميع المريضين على نظام VIDAS لتحديد D-Dimer، وتم تحديد القمة النسبية لل尼亚جرا في كل مريض. انخفاض مستوى D-Dimer يمكن قياسه كمياً بواسطة ELISA. النتائج: كان متوسط أعمارهم مجموعاً تخثر الأوردة العميقة هو 43 سنة. وجدت مجموعات تخثر الأوردة العميقة من 37 مريضاً (74%) كاذبة، واستهدفت في 13 مريضاً (26%) من خلال فحوصات الصيدلة المتعددة لل尼亚جرا. ومن مستوى في المجموعات تخثر D-Dimer الميوثري في الأوردة العميقة كان 5490.021 نانومغ / ديسيلتر بينما في غير مجموعة تخثر D-Dimer للأوردة العميقة كان 1906.384 نانومغ / ديسيلتر. هذه الفروق كانت ذات دلالة إحصائية (P = 0.0003).

الاتصالات: طريقة قياس النياجرا D-Dimer في نظام VIDAS مثيرة للاهتمام، والنتيجة من خلال النياجرا D-Dimer في نظام VIDAS تعمل بناء على التكلفة لـ (500 و 900) نانومغ / ديسيلتر (71% - 90% على التوالي). النتائج: في نظام VIDAS الطريقة لا تعتبر فحص خاص ومحدد لتشخيص تخثر الأوردة العميقة. 

الكلمات المفتاحية: D-Dimer، التشخيص، تخثر الأوردة العميقة

الاستنتاجات: طريقة قياس النياجرا D-Dimer في نظام VIDAS مثيرة للاهتمام، والنتيجة من خلال النياجرا D-Dimer في نظام VIDAS تعمل بناء على التكلفة لـ (500 و 900) نانومغ / ديسيلتر (71% - 90% على التوالي). النتائج: في نظام VIDAS الطريقة لا تعتبر فحص خاص ومحدد لتشخيص تخثر الأوردة العميقة.

الخلاصة: استخدام D-Dimer في تشخيص تخثر الأوردة العميقة. رعاة الرجاء الامتحان.
The albuminuria (Microalbuminuria) in patients with nephropathy who are taking Diferasirox

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Summary:

Background: Treatment of iron-related drugs to prevent or remove excess iron in the body may affect kidney function in patients with nephropathy. The early sign of renal dysfunction and kidney failure in patients with anemia and iron deficiency is microalbuminuria.

Objectives: The aim of this study was to find the recurrence frequency of microalbuminuria in patients with iron deficiency and anemia who are taking Diferasirox and also to find any relationship between the microalbuminuria level and other factors such as age, gender, and type of nephropathy and creatinine and ferritin levels in the blood.

Methods and Approaches: This is a school-based study on patients with nephropathy (401 patients) who are taking Diferasirox in the Haemorrhoid Blood Hospital from April 2012 to February 2014. Information was collected from the patients through a questionnaire and urine samples for microalbuminuria measurement using the immunological enzyme-linked (ELISA) method and blood samples for biochemical tests including creatinine and ferritin.

Results: 100 patients were included in this study (38 males and 62 females, with an average age of 10.59 ± 25.74 years). 31 patients had a level of albuminuria equal to or higher than the cut-off point of 35 mg/mL. 23 patients were older than 21 years (33.3%, 32 mg/mL). No clear relationship was found between the albuminuria level and creatinine and ferritin levels in the blood. Only a clear relationship was found between the albuminuria level and patient age.

Conclusion: We conclude from this study that the frequency of microalbuminuria in patients with nephropathy who are taking Diferasirox is relative. Age, gender, and type of disease affect the microalbuminuria level.

Keywords: Microalbuminuria, nephropathy.

The albuminuria (Microalbuminuria) in patients with nephropathy who are taking Diferasirox
علاقة فيروس حمى الحلأ البشري السادس مع سرطانات اللمف في المرضى بالعراق

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ا. م. د. داوود سلمان وهبي / كلية التقنيات الصحية والطبية / بغداد

الخلاصة:
الخلفية: فيروس حمى الحلأ البشري النوع السادس مرتبط بمرض الوَرْدِيَّة الطِّفْلِيَّة خلال مرحلة الطفولة تتبعها مرحلة الحفاظة التي تبقى مدى الحياة وتحتوي فترات من استعادة النشاط المتكرر في الأشخاص ذوي المناعة المنخفضة. بالرغم من إن العديد من الدراسات تشير إلى الدور الأمراضي لفيروس حمى الحلأ البشري السادس في سرطانات اللمف إلا أن هذه مسألة مازالت خلافية.

الأهداف: تهدف الدراسة إلى تحري عن فيروس الحلأ البشري السادس وعلاقته بسرطانات اللمف باستخدام tecنخات المصلية والجزيئية المختلفة إضافة إلى حساب مستوى الحمولة الفيروسية في البلازما.

المريض وطريقة العمل: أجريت هذه الدراسة ذات الطابع التحليلي المقطعي المستعرض للحالات والشواهد في مركز أمراض الدم في الجامعة المستنصرية ومستشفى بغداد التعليمي في بغداد للفترة الممتدة بين أيلول 2012 وغداة آذار 2012. شملت مجاميع المرضى 110 مريضًا مصابًا بالسرطان هودجكن الملمفي و39 مريضًا مصابًا بالسرطان اللاهودجكن الملمفي من كلا الجنسين تراوحت أعمارهم بين 15 إلى 80 سنة. تشخيص الاصابة السرطانية اعتمد على نتائج اختبارات الدم والاختبارات التشريحية. تتضمن خضع مرضى人群 من الأشخاص الأصحاء طاحية ادخل ضمن الدراسة كمجموعة سيطرة تم اختيارهم من ضمن المتبرعين بالدم. معدل العمر تراوح بين 18 ـ 55 سنة. تم الكشف عن الأجسام المضادة من نوع (ج و م) الخاصة بفيروس الحلأ البشري النوع السادس عن طريق اختبار مقايسة المناعي المرتبط بالإنزيم كما استخدم اختبار التألق المناعي للكشف عن الأجسام المضادة نوع (ج) وتقنية تفاعل السلسلة المتبلمرة لحساب مستوى الحمولة الفيروسية. جميع النتائج خضعت للتحليل الإحصائي واعتبرت قيمة بت الأقل من 0.05 فرقًا معنويًا.

النتائج: أظهرت النتائج أن مستوى إيجابية المصل للأجسام المضادة نوع ج والتي حصلت باستخدام اختبار المقايسة المناعي أعلى فيمرضي سرطان هودجكن من مجموعة السرطان (81.8% مقابل 61.0% حيث قيمة بت = 0.186 و بمقابل 0.758% حيث قيمة بت = 0.038) وفي مرضى سرطان اللمف اللاهودجكن (64.1% مقابل 61.0% حيث قيمة بت = 0.079 و بمقابل 0.534 و ب= 0.173) على التوالي. كانت نتائج الأجسام المضادة من نوع (م) والتي حصلت بطريقة المقايسة المناعي المرتبط بالإنزيم كانت أعلى معنويًا في مرضى سرطان هودجكن مقاومة لمجموعة السرطان (27.2% مقابل 6.8% حيث ب= 0.038) لكنها لم تكن ذات فرق معنويًا في مجموعة مرضى سرطان اللمف اللاهودجكن (17.9% مقابل 8.6% حيث ب= 0.086) مقارنةً بالحالة المضادة من نوع (ج) و المضادة لفيروس الحلأ البشري السادس. بطرق تفاعل السلسلة المتبلمرة وكانت النسبة من 27.3% في مرضى سرطان هودجكن في حين لم تسجل أي حالة موجبة بين مرضى سرطان اللمف اللاهودجكن ولا في مجموعة السرطان. كما كان معدل الحمولة الفيروسية للحالات الموجبة (نسبة/ملليتر) (0.3 ± 1.4*10³)

الاستنتاج: بالرغم من معدل الأكاسيم المضادة لفيروس حمى الحلأ السادس كان عاليا بين مرضى سرطان اللمف من نوع هودجكن ولاهودجكن إلا إن الدور الأمراضي للفيروس في تطور السرطان مازال من الصعب.

الكلمات المفتاحية: فيروس حمى الحلأ البشري السادات, سرطانات اللمف, سرطان هودجكن الملمفي.
المجلة العراقية لأمراض الدم
مجلة علمية محكمة تصدر مرتين في السنة ع---
المركز الوطني لبحوث وعلاج أمراض الدم - الجامعة المستنصرية - بغداد - العراق
تشرين الثاني 2015
المجلد 4 العدد 2

مدير التحرير
أ. د علي محمد جواد المظفر

رئيس التحرير
أ. د علاء فاضل علوان

سكرتير المجلة
د. نضال كريم الرحال

هيئة التحرير

<table>
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صدر العدد الأول في 2005
أول رئيس تحرير د. نبيل سلمان مراد
أول مدير تحرير د. اديب الشامي