# IRAQI JOURNAL OF MEDICAL SCIENCES

#### **CHAIRMAN OF THE EDITORIAL BOARD**

Professor Faeza Aftan Zghair Msc, FIC. Path

#### **CHIEF EDITOR**

Professor Fakhir S. Al-Ani PhD.

#### **EXECUTIVE EDITORIAL BOARD**

Ghassan A. Al-Shamma PhD	Professor	<b>EDITOR</b>
Alaa G. Hussien FICMS	Professor	<b>EDITOR</b>
Nidhal Abdul-Muhymen PhD	Professor	<b>EDITOR</b>
Samir M. Jasim PhD	Asst. Professor	<b>EDITOR</b>
Mutaz A. Al-Qazzaz FICMS	Asst. Professor	<b>EDITOR</b>
Hussam A. Ahmed FRCS	Asst. Professor	<b>EDITOR</b>
Enas T. Abdul-Karim DCH, PhD	Asst. Professor	<b>EDITOR</b>
Atheer J. Al-Saffar FICMS	Asst. Professor	<b>EDITOR</b>
Hasan A. AL-Hamadani FICMS	Asst. Professor	<b>EDITOR</b>
Hala S. Aref CABP	Asst. Professor	<b>EDITOR</b>
Waseem F. Mohammed FICMS	Lecturer	<b>EDITOR</b>
Ali F. Hadi PhD	Lecturer	<b>EDITOR</b>
Suhad M. Salih FICOG	Lecturer	<b>EDITOR</b>

JOURNAL SECRETARY Esraa' S. NAJI

TECHNICAL EDITOR Aliaa' N. Hatam

### **IRAQI JOURNAL OF MEDICAL SCIENCES**

All articles published represent the opinions of the authors and do not reflect the policy of IRAQI JOURNAL OF MEDICAL SCIENCES. All rights are reserved to IRAQI JOURNAL OF MEDICAL SCIENCES. No part of the journal may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or via any storage or retrieval system, without written permission from the journal.

All correspondence and subscription information requests should be addressed to:

The Editor of IRAQI JOURNAL OF MEDICAL SCIENCES

P. O. Box 14222, Baghdad, Iraq.

College of Medicine

Baghdad, Iraq

Tel and Fax: 964-1-5224368

E-mail: Iraqi\_jms\_alnahrain@yahoo.com

#### **ADVISORY BOARD**

Professor Abdul-Hussien M. AL-Hadi (Al-Nahrain University)
Asst. Professor Adeeb A. AL-Zubaidy (Al-Nahrain University)
Asst. Professor Ali Khiralla (Babil University)

Professor Amjad Dawood Niazi (Iraqi Board for Medical Specialization)
Professor Anam Rasheed AL-Salihi (Irf Institute of Embryo Research & Infertility Treatment / Al-Nahrain

University)

Asst. ProfessorAtta Gitti Allawi(Wassit University)Asst. ProfessorFakhraddin N. Nassir(Kirkuk University)

Asst. Professor Faris Abdul kareem (Alkindi collage\ Baghdad University)

Asst. Professor **Farqad Badir Hamdan** (Al-Nahrain University) Asst. Professor Ferhad Suliffan (Duhok University) Asst. Professor Haider J. Mobarak (Al-Nahrain University) **Professor** Hashim M. AL-kadimy (Al-Nahrain University) Asst. Professor Hassan A. Hassan (Al-Nahrain University) **Professor** Hikmat A.R. Hatam (Al-Nahrain University) **Professor** Hussam H. Ali (Al-Nahrain University)

Asst. Professor

Professor

Jassim M. AL-Mahana

(Al-Anbar University)

(Al-Kufa University)

Lecturer

Khudier K. Ibrahim

Asst. Professor

Lamia A.K. AL-Saady

Professor

Maha M. AL-Bayati

(Al-Anbar University)

(Al-Nahrain University)

Professor Mahmood Hayawi Hamash (Jordan)

Professor Mohammed H. AL-Alwan (Al-Mustansiriya University)

ProfessorMuaid N. Majeed(Thiqar University)Asst. ProfessorMuzahim K.Al-Khyatt(Al-Mosul University)

Professor Nazar El-Hasani (Iraqi Board for Medical Specialization)

Professor Rafi M. Al-Rawi (U.A.E)

Asst. ProfessorRahi K. AL-Yasiri(AL-Qadisiah University)ProfessorSami E. Matlob(Al-Nahrain University)ProfessorSarmad Khunda(Baghdad University)ProfessorSawsan S. Al-Haidari(Al-Nahrain University)ProfessorThamir A. Hamdan(Al-Basra University)

Professor Usama N. Rifat (U.A.E)

ProfessorUsama S. Al-Nasiri(Al-Nahrain University)ProfessorYarub I. Khattab(Al-Nahrain University)Asst. ProfessorZuhair A. Eissa(Karbala University)

### IRAQI JOURNAL OF MEDICAL SCIENCES

### Aims and Scope

IRAQI JOURNAL OF MEDICAL SCIENCES is published by College of Medicine, Al-Nahrain University. It is a quarterly multidisciplinary medical journal. High quality papers written in English, dealing with aspects of clinical, academic or investigative medicine or research will be welcomed. Emphasis is placed on matters relating to medicine in Iraq in particular and the Middle East in general, though articles are welcomed from anywhere in the world.

**IRAQI JOURNAL OF MEDICAL SCIENCES** publishes original articles, case reports, and letters to the editor, editorials, investigative medicine, and review articles. They include forensic medicine, history of medicine, medical ethics, and religious aspects of medicine, and other selected topics.

## IRAQI JMS FORMAT INSTRUCTION TO AUTHORS

Iraqi Journal of Medical Sciences (Iraqi JMS) is a periodic, peer-reviewed journal published quarterly by College of Medicine, Al-Nahrain University. Iraqi JMS publishes manuscripts in all fields of health and medicine written in English.

**TYPES OF CONTRIBUTIONS:** Original articles, review articles, case studies, editorials, medical education, history of medicine, ethics, practical points, medical quiz, conferences, meetings and letters to the Editor.

#### MANUSCRIPTS:

- Submission of a manuscript implies that is not being considered for publication anywhere.
- The author should provide a document officially state that the current work was carried out at the site which provides this certification. The document should be signed by the highest authorized member at that location.
- Manuscripts submitted to IJMS are subject to editorial evaluation and revision by three referees.
- The format of IJMS complies with the uniform requirements for manuscripts submitted to Biomedical Journals, published by the International Committee of Medical Journals Editors (ICMJE) (Vancouver, British Colombia, 1979) and its last update in October 2001, available on the web site <a href="https://www.icmje.org">www.icmje.org</a>.
- Manuscript should be typewritten double spaced on size A4 (29.5x21 cm) paper with wide margins. Page should be numbered consecutively. One original and three photocopies including figures, tables, and photographs should be submitted. Begin each of following sections on separate page in the following sequence: Title page, abstract and keywords, text, acknowledgments, references, tables, and legends for illustration.
- Manuscript and figures will not be returned to the authors whether the editorial decision is to accept, revise or reject.
- Manuscripts must be accompanied by a covering paper signed by all authors that the paper has not been published in and will not be submitted to any other journal if accepted in IJMS.
- The page should contain (a) title of the manuscript, (b) names of each author (first name, middle initial and family name) including highest academic degree, (c) official academic and/or clinical title and affiliation (d) name and address of the institution where the work was done (e) name and address (E-mail if available) of the author to whom correspondence should be sent.
- ABSTRACT: manuscript should include an abstract of not more than 150 words. Structured abstract typed on a separate sheet and consist of background, objective, method, results, and conclusion. Translation in Arabic to be included:

  (خلفیة الدراسة، طریقة العمل، النتائج و الاستنتاج).
- **KEYWORDS:** three to ten keywords should be provided on the same page as the abstract in Arabic and English. As far as possible, be selected from the National Library of Medicine Medical Subject Headings.
- The Arabic abstract should follow the United Medical Dictionary (Council of Arab Ministers of Health/WHO/ Arab Medical Union/ALESCO, 3<sup>rd</sup> edition.
- Manuscript format: It should be divided into the following parts: introduction, materials and methods, results and discussion.

• **REFERENCES:** All references should be listed in consecutive numerical order by English numerical, in the order of citation in the text. Once a reference is cited all subsequent citations should be to the original number.

#### **EXAMPLES**

- 1. Standard Journal Article: use et al when the number of authors exceeds 6. Halliwell B, Gutteridge JMC. Oxygen toxicity, Oxygen radicals, transition metals and disease. Biochem J. 1984; 219: 1-14.
- 2. Books: Mann JI, Pyorala K, and Teuscher A. Diabetes in epidemiological perspective. London: Churchill Livingstone. 1983.
- 3. Chapter in book: Phillips SJ, and Whisnant JP. Hypertension and strock. In: Laragh JH, and Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2<sup>nd</sup> ed. NewYork: Raven Press; 1995. p. 465-78.
- TABLES: Each table should be typed on a separate page double-spaced, including all headings, number all tables with English numerals and include a short title. Vertical lines between columns are to be avoided.
- FIGURES: All figures must be suitable for reproduction without being retouched or redrawn. Figure number, name of senior author, and title of the work should be written lightly on the back with red pencil. Photographs must be supplied as glossy black and white prints. The top of the figures should be indicated clearly.
- **LEGENDS:** Captions for figures must be typed; double spaced, and must not appear on the figure.

Proof Reading will be done by the secretarial office of the journal. The principal author will receive a copy of the journal. The authors are responsible for accuracy of all statements, data, and references included in the manuscript.

- After the manuscript has been accepted for publication, authors are required to supply the final version of the manuscript on CD in MS word version 6 or later.
- All corresponding to be addressed to the Chief Editor on the address below:

Chief Editor: Iraqi Journal of Medical Sciences College of Medicine, Al-Nahrain University, P.O. Box 14222, Tel. 5231521, Al-Kadhiymia, Baghdad, IRAQ.

#### **Editorial:**

#### **ACCREDITATION**

Fakhir S. Al-Ani MBChB; MSc; PhD.

Accreditation of medical education has become a necessity for all countries of the region. It will enable medical school graduates in the region to meet the requirements of global standards for medical education and practice. Accreditation also provides support for continuous quality improvement in medical education and safeguards the medical profession, to comply with accepted regional or national standards.

#### What is Accreditation:-

Accreditation for medical education programs is *a voluntary peer-review process* designed to attest the educational quality of new and established medical educational programs.

## Why there is a need for Accreditation:-

The remarkable increase of the number of medical schools around the world over the last decades, and many of which have been established under questionable conditions, as well as the goal of safeguarding the quality of healthcare systems in a world have increased the awareness of accreditation as a quality assurance tool.

#### When: - In

In 2004, WHO and WFME established the international Task Force on Accreditation. In October of the same year 26 members of the task force from 23 countries covering all six WHO – WFME regions assembled

Dept. Physiology, College of Medicine, Al-Nahrain University for three days at a seminar in Copenhagen, Denmark, to discuss how WHO and WFME could contribute to establishing sustainable accreditation systems. The need for guidelines was stressed at the seminar and the present document is based on the discussions at the seminar.

Based on the results of this task force the strategic partnership has formulated a set of guidelines for accreditation of basic medical education institutions and programs. Then the activity for accreditation is globalized to be a world- wide activity (In all the countries). The guidelines for accreditation of basic medical education where established by the WHO/WFME in there meeting in Geneva/Copenhagen in May 2005.

The WHO-WFME guidelines are global, but flexible. WHO and WFME acknowledge the differences between countries and regions regarding governance of medical education, socioeconomic conditions and delivery resources. health care systems, etc. Consequently, the global guidelines WHO-WFME accreditation are flexible and take into account the context in which they are to be used.

A dead line for accreditation is setup according to the different regional offices of the WHO, to reach the standards which are useful for educational institutions as a basis for internal evaluation towards continuous quality improvement.

Evaluation based on generally accepted standards is the short-term

results of accreditation as described in the regional accreditation guidelines, accreditation is usually granted for periods of up to six years.

When schools are found to have serious deficiencies, this should not be the basis for decisions to close schools or deny recognition to graduates, but are granted conditional they accreditation for limited periods contingent upon certain issues to be addressed and actions to be taken by the school. If a school continues to be unable to deliver courses at the accepted standard, its students must be directed to another school to complete an accredited medical course before they can be recognized.

### ACCREDITATION IN COUNTRIES OF THE EASTERN MEDITERRANEAN REGION AND OF IRAO

For most of the 20th century, the *General Medical Council (GMC)* of the United Kingdom was responsible for evaluating and maintaining a register of medical schools

Operating in countries of the Region and teaching in English. The 18 medical schools established in the Region before 1950 were founded by academicians from Europe, United Kingdom or United States. foundation is not only is the curriculum in these schools based on the style used by the founding country, but even in the certificates conferred by these schools also follow the same nomenclature used in the founding country. For example, MBChB degree of Scotland is conferred in Iraq.

The GMC has continued to evaluate medical schools overseas; it no longer evaluates schools in some countries of the Region because of proliferation of new schools. The number of medical schools in the Region has grown considerably, from 18 schools in 1950 to around 251 in

2005 of which more than 50 are private.

In the 1980s, licensing bodies in industrialized countries started to use the WHO World directory of medical schools in determining which medical school graduates from overseas would be allowed to sit for medical license examinations. However, this listing of medical schools is confined to schools that are approved by the related national ministries of health.

Iraq, the awareness accreditation is not a new task, a national system of accreditation of medical schools. which included national unified examinations, implemented in 1992 as part of a wider accreditation program for institutions of higher education. However, the program was stopped in 1999 because of technical constraints.

Moreover; the Arab Board of Medical Specialization, a professional

Postgraduate board affiliated with the Council of Arab Ministers of Health, has successfully administered a sub-regional accreditation system for postgraduate medical education since the early 1980s. But the pioneering work of the Arab Board in this respect has mainly focused on accreditation of training hospitals used for postgraduate studies.

Although all countries of the Region have established procedures to be followed before graduates from a new medical school are recognized and accepted to practice, these procedures are laid out by national professional bodies or ministries of health and are not based on a structured and objective accreditation program.

By assessing medical schools for accreditation purposes, professional medical registration bodies can be assured that a medical school's educational program satisfies agreed national guidelines for basic medical education. Moreover, accreditation of a

medical school facilitates registration of its future graduates in other countries. A graduate of a medical course accredited by a regional accreditation body would be eligible to register in any country of the Region.

Lastly we should know that accreditation provides an incentive for schools to introduce reform aimed at improving their educational performance and ensuring that both the schools and their graduates are fit for the purpose of promoting and improving the health of their communities.

## The relationship between umbilical venous blood flow & fetal weight in the last trimester

Maha M. Al-Bayati<sup>1</sup> MBChB; CABOG, Wasan I. Majeed <sup>2</sup>MBChB; FIBMS, Abir T. Makki <sup>3</sup> MBChB

#### **Abstract**

**Background:** Doppler applications in pregnancy are expanding exponentially. Flow velocity waveforms provide important information from 12 weeks to term, from maternal vessels, placental circulation and fetal systemic vessels. An important application is the quantitative calculation of umbilical blood flow volume.

*Objective:* to assess the relation between the umbilical blood flow at one hand & fetal body weight & placenta weight on the other hand in the last trimester in both term & preterm labor groups

Study design: A prospective study

**Setting:** department of Gynecology & Obstetrics & Department of Radiology at Al-Kadhimyia Teaching Hospital

**Patients & methods:** This study included 50 pregnant women at first stage of labor. The patients were classified into two groups; group A: Infants born at < 37 weeks of gestation and group B: those infants born at  $\geq 37$  weeks of gestation .The diameter of the umbilical vein was determined by ultrasound & spectral Doppler was used to assess velocity of blood in the umbilical vein & the umbilical blood flow

(UBF) per unit fetal body weight & placental weight estimated in both groups. Results: A statistically significant difference was found in the diameter of the umbilical vein (7.84 mm vs. 8.62 mm, p=0.0001), the volume of umbilical blood flow (410.22 mL/min vs. 523.20 mL/min, p=0.0001), UBF/Fetal weight (230.68 mL/min/kg vs. 166.79 mL/min/kg . p=0.0001) & UBF/Placental weight(102.65 mL/min/100gm vs. 87.23 mL/min/100gm , p=0.01) of group A & group B respectively while the mean velocity of blood flow in the umbilical vein showed no statistically significant difference in both groups (14.11 cm/s vs. 14.93 cm/s, P=0.8).

**Conclusion:** The increase in umbilical blood flow is exceeded by the fetal growth and to a lesser degree by placenta growth. A significant reduction in the umbilical blood flow per unit fetal weight & placental weight take place with increasing gestational age.

*Keywords:* umbilical venous blood flow, fetal body weight, placental weight

IRAOI J MED SCI, 2009; VOL.7 (4): 4-10

#### **Introduction**

Doppler applications in pregnancy are expanding exponentially. Flow velocity waveforms provide important information from 12 weeks to term, from maternal vessels, placental circulation and fetal systemic vessels, with implications for both mother and fetus. Reports of blood flow measurement in pregnancy mostly refer to physiological reduction of placental

<sup>1</sup>Dept. Gynecology & Obstetrics, College of Medicine, Al-Nahrain University, <sup>2</sup>Dept. Surgery ,Radiology, College of Medicine, Al-Nahrain University, <sup>3</sup>Gynecology & Obstetrics ,Al-Kadhimyia Teaching Hospital.

Address Correspondence to: Dr. Maha M. Al-Bayati

E-mail: <u>col\_med\_alnahrain@yahoo.com</u>
Received: 6<sup>th</sup> May 2009, Accepted: 4<sup>th</sup>
November 2009.

vascular resistance (1) or to the redistribution of the cardiac output under hypoxic distress (2). Another important application is the quantitative calculation of umbilical blood flow (3-5). During the course of pregnancy, there is a dramatic increase in uterine blood flow from <100 ml / min at 10<sup>th</sup> week of gestation to 700 - 800 ml / min at term <sup>(6)</sup>. Umbilical blood flow increases as well from 100 ml / min at 22<sup>nd</sup> week of gestation to 300 ml / min a the 38<sup>th</sup> week of pregnancy<sup>(7)</sup>. Relative to the fetal weight, a constant umbilical blood flow of 120 ml / min per unit fetal weight has been reported up to 35<sup>th</sup> week of gestation. After that time, it is assumed to decrease slightly (3-5). However other studies have indicated that the increment in fetal body weight exceeds the increment in the umbilical blood flow in the last trimester (8).Normal placental and fetal growth across pregnancy is characterized by cellular hyperplasia, sequential hyperplasia and hypertrophy, finally, hypertrophy alone <sup>(9)</sup>.Placental growth follows a sigmoid curve, with plateau occurring earlier in gestation than fetal curve. Between 16<sup>th</sup> week and term, human fetal weight increase 20 fold (10). Fetal growth is dependent on adequate transfer of nutrients and oxygen across placenta. Thus it is dependent on appropriate maternal nutrition and placental perfusion. We aimed at this study to assess the relation between the umbilical blood flow at one hand & fetal body weight & placental weight on the other hand in the last trimester in both term & preterm labor groups.

#### Patients and methods

This was a prospective study that included 50 pregnant women at the Department of Obstetrics and Gynecology in cooperation with Ultrasound (U/S) unit at Al-kadhimyia Teaching Hospital from July 2007 through August 2008. Informed consent was obtained from all participants. All the participants were at first stage of labor .The patients were classified into 2 study groups based on gestational age at delivery; group A: Infants born at < 37 weeks of gestation were classified as preterm (n = 15), and group B: those infants born at  $\geq$  37 weeks of gestation (n = 35). The inclusion criteria were singleton pregnancy, term or preterm pregnancy, first stage of labor (infrequent uterine contraction) & intact membrane. Those pregnant women with pre-eclampsia, diabetes mellitus, multiple pregnancy ultrasound evidence of congenital abnormalities, polyhydramnios or oligohydramnios or those infants with features of intrauterine growth restriction or

macrosomia (according to standard growth charts ) were excluded .The demographic criteria for each patient was obtained ( age, weight, parity & gestational age determined by reliable last menstrual period or early U/S and confirmed by neonatal examination ). The blood flow in the umbilical vein was determined by pulsed doppler sonography using Siemens sonoline versa pro machine with 3.5 – 5 MHz transducer. The Doppler convex ultrasound was performed in the first stage of the labor for all patients. Umbilical venous frequency shift was recorded at the placental origin of the umbilical vein, with insonation angle of 30-60°. The diameter of umbilical vein (U.V.) determined & the volume of umbilical blood flow (UBF) was estimated as  $Q = V \times d2 \times \Pi \times 0.15$ where (O) is the volume of umbilical blood flow (ml / min), (V) is the mean velocity (cm / s), and (d) is the diameter of the umbilical vein (mm). To avoid inter-observer variation measurements of mean velocity of blood flow and diameter of umbilical vein were performed by the same radiologist. To reduce error due to a solitary measurement, each Doppler shift signal of the umbilical vein and each vessel diameter were measured twice. Thus, both mean flow velocity and the umbilical diameter for the calculation of the volume of umbilical venous blood flow were determined from the means of two consecutive measurements.

After birth, assessment of the neonatal birth weight, placental weight and ABGAR score were performed by the obstetrician and the neonatologist.

#### **Statistical analysis**

Data were collected and described by using number, percentage, mean  $\pm$  SD & correlation .The association was considered to be statistically significant when P value is < 0.05.

#### Results

Figure 1 represents the distribution of the study groups according to the gestational age

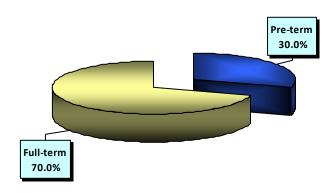


Figure 1: Pie chart represents the distribution of the study groups according to the gestational age

Table 1 shows the demographic characteristics of group A and B are, the mean gestational age at group A

was  $33.47\pm1.88$  while in group B was  $38.86\pm1.33$ 

Table 1: the demographic characteristics of the study groups.

Tubic 1. the demographic characteristics of the study groups.								
Variables	Pre-term (n=15)	Term (n=35)						
	Range (Mean ±SD)	Range ( Mean ±SD)						
Age in years	17-38 (27.13±4.07)	22-42(28.31±3.52)						
Gestational age (weeks)	29-36(33.47±1.88)	37-42(38.86±1.33)						
Parity	1-4(0.80±0.77)	1-5(1.71±1.23)						

Table 2 shows the different Doppler parameters that are assessed in both groups & their relation with newborn body weight & placenta weight.

A statistically significant difference was found in the diameter of the umbilical vein , the volume of

umbilical blood flow, UBF/Fetal weight & UBF/Placental weight of both groups while the mean velocity of blood flow in the umbilical vein showed no statistically significant difference in both groups (P=0.8).

The diameter of the umbilical vein was greater in term than in pre term

infants (P = 0.0001) as a result there was a significant increase in the

umbilical blood flow in term group than in pre term group (P = 0.0001).

Table 2: The relation of different measurements of umbilical vein parameters with gestational age.

	Pre-term (n=15	Full-term(n=35)	P value
Measurement	16	14	
	Mean±SD	Mean±SD	
Mean velocity of blood flow (cm/s)	14.11±1.22	14.93±0.57	0.8NS
(0.11.0)	1	11.50 0.07	0.01 (5
Umbilical vein diameter (mm)	7.84±0.21	8.62±0.23	0.0001*
Volume of umbilical blood flow (mL/min)	410.22±53.94	523.20±31.84	0.0001*
UBF/Fetal weight (mL/min/Kg)	230.68±38.32	166.79±17.04	0.0001*
UBF/Placental weight (mL/min/100 gram)	102.65±8.42	87.23±6.56	0.01*

Figure 2 shows that a positive correlation (r=0.8) exists between the umbilical blood flow & fetal weight with a significant reduction in umbilical

blood flow per unit of fetal weight noted with increasing gestational age (P value = 0.0001).

NS: not significant, \*Significant

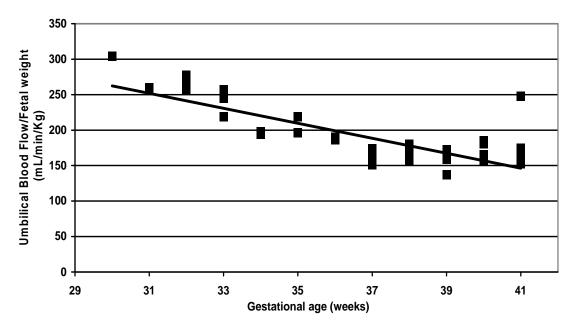


Figure 2: The correlation between gestational age (weeks) and umbilical blood flow per unit fetal weight (ml/min/kg). r= -0.8

Figure (3) shows that a slight but statistically significant reduction in umbilical blood flow per unit placental weight was found in group B in comparison with group A (r=0.5, P=0.01).

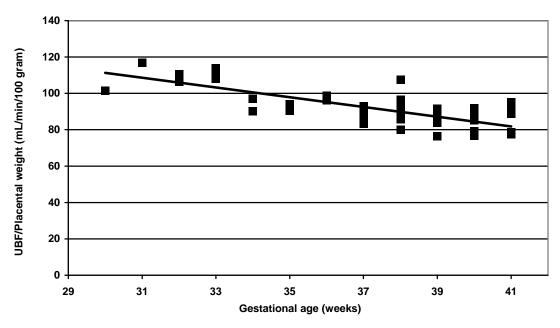


Figure 3: The correlation between gestational age (weeks) and UBF/Placental weight (ml/min/100gr). r=-0.5

#### **Discussion**

For the fetus, placental blood volume flow rate is as important as cardiac output and lung perfusion in adults. The measurement of fetal blood flow velocity using Doppler U /S was introduced into routine obstetric practice many years ago, both to detect fetal circulation in the context of any gestational disease and to assess fetal well-being. (11)

Volume flow rate determinations based on the Doppler principle is being one of the rapidly growing application during pregnancy (12-14). In this study we the relation between investigated umbilical venous blood flow & fetal weight in last trimester. The range of velocity of umbilical venous blood flow in this study was between (11.5 -15.8)cm/s and the mean velocity was similar in group A and B 14.1, 14.9 cm/s, respectively which is consistent with result of Link et al (6) who found that the mean flow velocity was 13.1 cm/s in preterm group and (13.6 cm / s) in term group with no significant correlation with gestational age while Barbera et al (12) found that the

umbilical vein blood flow velocity increases throughout pregnancy a conclusion that was inconsistent with our results.

The umbilical vein diameter was greater in group B (8.6 mm) than in group A (7.8 mm). This result was consistent with result of link et al <sup>(6)</sup> who found that the mean umbilical vein diameter was 8.8 mm in full term group and 8.1 mm in preterm group, and was statistically significant difference .Also this result was consistent with the results of Barbera et al <sup>(12)</sup> who found a significant increase in the umbilical vein diameter throughout pregnancy.

The observation of a constant velocity of blood flow and increasing cross section of the umbilical vein is consistent with result of Erskine et al (3) and Kunzel et al (8), both found that the increase of umbilical venous blood flow throughout gestation was due to and depended on the growth of the umbilical vein in the presence of a rather constant flow velocity.

With respect to the blood flow volume, we found that the blood flow umbilical vein increased markedly in group B, the mean UBF was 523.2 ml / min while in group A it was 410.2 ml / min. This result was in agreement with result of Link et al (6) and kunzel et al (8) who found that the blood flow volume significantly increased with gestation from 432 ml/min in pre term to 515 ml/min in full term. Barbera et al (12) found that the absolute umbilical vein increases exponentially from 97.3 ml/min at mid-gestation to 529.1 ml/min at 38<sup>th</sup> week of gestation which was in agreement with our results.

We found a continuous reduction in the umbilical blood flow per unit of fetal weight with increasing gestation (166.7 ml / min / kg in group B and 230.6 ml /min / kg in group A). This result was in agreement with results of Link et al, <sup>(6)</sup> and of Kunzel et al, <sup>(8)</sup> they found that the blood flow per unit of fetal weight reduced in full term (154 ml / min / kg) compared to (221 ml / min / kg) in preterm group which was a significant difference, whereas Barbera et al, (12) found that the umbilical blood flow per kilogram of weight did not change significantly with gestational age, this result disagree with our result.

Regarding the relation between the placental weight & the UBF, we found that the blood flow per unit of placental weight was reduced with increasing gestation, group A showed a mean UBF of 102.6 ml/min per 100 gm and group B 87.2 ml/min per 100 gm of placental weight. This result was in agreement with result of Link et al <sup>(6)</sup> who found that the umbilical blood flow per unit placental weight was slightly but significantly diminished in full term than in preterm group 90 and 100 ml/min/100gm respectively.

It was concluded that A significant reduction in the umbilical blood flow per unit fetal weight takes place with increasing gestational age & the umbilical blood flow per unit placental weight was reduced with increase gestation.

#### References

- **1.** Ferrazi E, Gementi P and Bellotti M et al. Doppler velocimetry: critical analysis of umbilical, cerebral and aortic reference values. Eur J Obstet Gynecol Reprod Biol.1991; 38:189-196.
- **2.** Arbeille P, Leguyader P, Fignon A et al. Doppler ultrasonographic investigation of fetal cerebral circulation. In: Maulik D. Editor, Doppler ultrasound in obstetrics and gynecology (1<sup>st</sup> ed). Springer-Verlag. New York. 1996; 161-180.
- **3.** Erskine RLA and Ritchie JWK. Quantitative measurement of fetal blood flow using Doppler ultrasound.Br J Obstet Gynecol.1985; 92:600-604.
- **4.** Eik-Nes SH, Marsal K, Brubakk A et al. Ultrasonic measurement of human fetal blood flow. J Bio med Eng.1982; 4:28-36.
- **5.** Gill RW, Kossff G, Warren PS and Garrett WJ. Umbilical venous flow in normal and complicated pregnancy. Ultrasound Med Biol.1984; 10:349-363.
- **6.** Link G, Clark KE, Lang U. Umbilical blood flow during pregnancy: evidence for decreasing placental perfusion. Am J Obstet Gynecol. 2007; 196:489-495.
- **7.** Gill RW, Trudinger BJ, Garrett WJ et al. fetal umbilical venous flow measured in utero by pulsed Doppler and B-mode ultrasound. Normal pregnancies. Am J Obstet Gynecol.1981; 139:720-725.
- **8.** Kunzel W, Jovanovic V and Grubner S. Blood flow in the umbilical vein and artery in pregnancy. Geburtshilfe Frauen heilkd.1991; 51:513-522.
- **9.** Baschat A. Fetal growth disorders. In James DK. High risk pregnancy management options.2006; 240-243.
- **10.** Battaglia FC, Rgenault TR. Placental transport and metabolism of amino-acid. Placenta. 2001; 22:145-161.
- **11.** Marsl K, Persson PH, Larsen T et al. Intrauterine growth curves based on ultrasonically estimated fetal weights. Acta Paediatr. 1996; 85:843-848.
- **12.** Barbera A, Galan HL, Ferrazzi E et al. Relation of umbilical vein blood flow to growth parameters in human fetus. Am J Obstet Gynecol. 1999; 181: 1:174-179.
- **13.** Challis DE, Warren PS, Gill RW. The significance of high umbilical Venous blood

flow measurements in a high-risk population. J Ultrasound Med 1995; 14: 907-912

14. Kohl T, Silverman NH. Evaluation of umbilical venous blood flow by Doppler color flow mapping and conventional ultrasonographic methods. J Ultrasound Med 1996; 15: 465-73)

## Detection of Respiratory syncytial virus infection in a sample of infants in Iraq

Shony M. Odisho<sup>1</sup>PhD, Anton S. Al-Bana <sup>1</sup>PhD, Nahi Y. Yaassen<sup>2</sup>PhD.

#### Abstract

**Background:** Human Respiratory syncytial virus (HRSV) is one of the major causes of bronchiolitis and pneumonia in infants.

**Objective:** detection of Anti HRSV antibodies in infants and children by using indirect ELISA, and detection of HRSV antigen by using RespiRSV test.

Materials and Methods: Hundred and eighty four serum samples (104 with respiratory tract infection, 54 without Respiratory tract infection, and 26 with cancer) also 100 nasal and throat swabs were collected from infants and children from Central pediatric Hospital in Baghdad in year (2005-2006). Indirect ELISA test and rapid test used for detection Human RSV antibodies and Human RSV antigen respectively

**Results:** Detection of anti HRSV antibodies using ELISA in children without respiratory tract infection was 26% with mean titer 494, but the antibodies was higher in patients with cancer which were 96% with mean titer 580, however anti HRSV was detected in79% in infants with respiratory tract infection (bronchiolitis and

pneumonia), their mean titer (1411) was, higher than that of the previous two groups.

Human RSV viral antigen was detected by RSV-Respi kit in 45% of nasal and throat secretion collected from children with respiratory tract infection, detection of HRSV antigen samples was compatible with the detection of antiHRSV antibodies in 50%.

**Conclusion:** The highest percentage and titers of antiHRSV antibodies were detected in infants with respiratory tract infection than in others suffering from different cancers or without respiratory tract infection.

Human RSV antigens were detected in nasal/throat swabs in infants with bronchiolitis and pneumonia.

*Keywords:* Respiratory syncytial virus, bronchiolitis, Respi-RSV.

IRAQI J MED SCI, 2009; VOL.7 (4):11-19

#### Introduction

Human Respiratory syncytial virus (HRSV) is one of the major causes of viral respiratory tract disease in young children and infant in developed and developing, poor and rich countries <sup>(1)</sup>. Severe HRSV bronchiolitis and pneumonia requiring hospitalization typically occur in infant less than 9 months of age <sup>(2)</sup>. The HRSV is mainly associated with bronchiolitis in children suffering from underlying illnesses such

as congenital heart disease and bronchopulmonary dysphasia which are at increased risk for severe infection due to HRSV.

In our country the pediatricians noticed increasing numbers of children under 2 years suffering from lower respiratory infections and bronchiolitis which occurs mainly in winter and in some years presented as epidemic with high morbidity and mortality especially in patient underlying illnesses<sup>(3)</sup>. This study were decided to detection antibodies for human RSV in serum samples from infants and young children by using specific ELISA kits, and detection of viral antigen in nasal and throat swabs obtained from

Address Correspondence to: Dr. Snony M Odisho

E- mail: shony odesho @hotmail

Received: 6<sup>th</sup> May 2009, Accepted: 18<sup>th</sup> October 2009.

<sup>&</sup>lt;sup>1</sup>Dept. Microbiology, college of veterinary medicine, Baghdad University, <sup>2</sup>Iraqi centers for cancer and medical genetic researches. Address Correspondence to: Dr. Shony M.

respiratory tract specimens by using Rapid test.

#### Materials and methods

Blood samples collected from children (54 samples from children without sings of respiratory illness, 26 with different kinds of cancer, and 104 suffering from bronchiolitis and pneumonia) during months (December-March) in the year (2005-2006), they were collected from Central Pediatric Hospital. Hundred and eighty four blood samples were collected in a disposable plastic tubes container, then the sera separated and stored at -20 C° till use. ELISA kits: Imported from DIALAB Company (Germany) used for the detection human RSV antibodies in serum.

Hundred nasal and throat swabs were collected from infants and young child, in central pediatric hospital, the inpatients with severe respiratory tract infection( bronchiolitis and pneumonia) chosen depend on clinical signs and chest x-rays, diagnosis by pediatricians and collected at time of illness; the sterile swabs were immersed into tube containing 2 ml of cooled transport media used for virus isolation- RSV-Respi-Strip test, kit were used for

detection of human RSV antigen in nasopharyngeal specimens which based on the use of homogenous immunochromatographic system with colloidal gold particales. The Kits were imported from CORIS BioConcep Company, (Germany).

#### Statistical analysis

The data were analyzed by Chisquare test, analyses of variance (ANOVA) and least significant difference (LSD) for differentiation among the means of groups<sup>(4)</sup>, P Value  $\leq$  0.05 was considered as significant result.

#### Results

A total of (54) serum samples were collected from patients with different ages ranging from 1 month Infants to 5 years children without signs of respiratory illness, only 14 serum samples had a positive titer of anti HRSV (26%) ranging from (215-875) and mean titer (494). The highest percentage of anti HRSV was (44.4%) in age 1-3 months and the lowest percentage was in 10-12 months (18%).These results showed statistical significant differences among the age groups (P>0.05) (Table 1).

Table 1: Detection of anti HRSV antibodies in children without respiratory tract infection by indirect ELISA.

Tested	<b>A</b> 000		+ve an	ti HRSV*	-ve antiHR	RSV*
sera	Age	No.of-	⊦ve %	mean titer	No.of-ve	%
9	1-3months	4	44.4%	215.7±83.8	5	55.5%
8	4-6months	3	37%	236.6±118.6	5	62.5%
16	7-9months	3	18.8%	$378.6 \pm 163.3$	13	81%
11	10-12months	2	18%	875.5±512.5	9	81.8%
10	1-5 years	2	20%	$763.5\pm650$	8	80%
54		14	26%	494	40	79%

<sup>\*</sup>Cut off value= 67. Over this number is +ve HRSV antibodies and below is -ve L.S.D. = 813.

p > 0.05

Twenty six (26) patients with different kinds of cancer (3 Leukemia, 2 Wilm's tumor (WT), 13 Acute lymphocytic leukemia (ALL),3 Chronic lymphocytic leukemia (CLL) and 5 Non Hodgkin's lymphoma (NHL)), at central pediatric Hospital who were under chemotherapy. Examination of sera of these patients for anti HRSV antibodies revealed that

18 of them were positive which were equal to 69% with a mean titer of 580. All cancer sera from patients aged 1-6 years were suffering from different malignancies positive for anti HRSV antibodies. Statistical analysis of the data results show highly significant differences (P<0.01) mainly in age 1-6 years group of patient with NHL than other groups (Table 2).

Table 2: Detection of anti HRSV antibodies in cancer patients according to disease type and age.

Tested	Type of	Age	+ve Anti HRSV *			Ant	-ve tiHRSV*
sera	cancer	year	No.	%	titer	No.	%
3	Leukemia	1-2 years	3	100%	284.3±120.4	_	_
2	WL	2-3 years	2	100%	214.5±4.5	_	_
5	NHL	1-6 years	5	100%	1495±1116.5	_	_
3	CLL	6-8 years	_		_	3	100%
13	ALL	3-12 years	8	61.5%	$322.6 \pm 98.7$	5	38.5%
26			18	69%	580	8	31%

<sup>\*</sup> Cut off value=67. Over cut off+ve, blow cut off-ve.

One hundred and four Infants and young children suffering from acute bronchiolitis and pneumonia from one month to one years of age. Examination of serum samples of these patients showed that 82 of them were positive (79%) for anti HRSV by indirect ELISA with mean titer of 1411, ranging (354-2867).

All pneumonia patients' sera were positive for HRSV antibodies in the age

7 months to one year, but highest percentage (76%) of broncholitis cases were in age 4-6 months and 10 month- to one year. These data showed statistical significant differences ( P<0.05) among different age groups which was high in 10 months- to one year and low in 1-3 months of bronchiolitis patients, and in pneumonia patients the highest were in 10moths—to one year and lowest in 7-9 months and 1-3 months (Table 3)

L.S.D. = 890.9

P < 0.01

Table 3: Detection of antiHRSV antibodies in Infants with respiratory tract infection by indirect ELISA.

	CIL 1			+ve	Anti HRSV *	-ve	antiHRSV*
Tested sera	Clinical disease	Age	No.	%	Titer ± S.D.	No.	%
26	Bronchiolitis	1-3month	19	73%	1261.1±470.9	7	27%
25	=	4-6month	19	76%	2184.1±916.3	6	24%
11	=	7-9month	8	73%	$1769.2 \pm 1071.1$	3	27%
14	=	10-12month	11	76%	2867.1±1295.4	3	24%
11	Pneumonia	1-3month	9	82%	489.4±231.3	2	18%
7	=	4-6month	6	86%	$1016.6\pm204.5$	1	14%
2	=	7-9month	2	100%	$354\pm80.8$	0	0
8	=	10-12mon	8	100%	1373.8±352.5	0	0
104			82	79%	1411	22	21%

<sup>\*</sup> Cut off value = 67. Over cut off = +ve, below cut off = -ve.

L.S.D = 1570.3

P<0.05

From a total of (184) individual sera samples examined by indirect ELISA showed a high percentage of sera (79%) were positive for anti HRSV and those belong to Infants with respiratory infections followed by sera from cancer patients (69%). However, sera from patients without

respiratory tract infection were (26%) positive for HRSV. These results showed highly significant statistical differences (P<0.01) among the different groups, which was higher in the group of respiratory tract infection (Table 4)

Table 4: Percentage of anti HRSV antibodies in individuals with or without respiratory tract infections and individuals with cancers.

Subjects	No. of sample	+ ve Anti	HRSV %	- ve A	nti HRSV%
Infants without R.T.I	54	14	26%	40	74 <b>%</b>
Infants with R.T.I	104	82	79%	22	21%
Individuals with cancer	26	18	69%	8	31%
	184	114	62%	70	28%

 $X^2 = 25$ 

P<0.01

Hundred nasal and throat swabs collected from Infants and young children suffering from acute respiratory tract infection were examined by Respi test kit. Forty five (45%) tested swabs were positive for HRSV antigens. Nasal-throat swabs collected from Infant

patients in December 2005 showed the highest positive percentage (70%), and the lowest was in March 2006 (25%). These data showed highly significant differences (P<0.01) among the months of the year (Table 5).

Table 5: Detection of HRSV antigen in nasal/throat swabs by using immunochromatographic assav.

Month/year	No. of patient sample	+ve HRSV antigen	-ve HRSV antigen	%
December 2005	39	27	12	70%
January 2006	26	8	18	44%
February 2006	20	6	14	30%
March 2006	15	4	11	25%
Total	100	45	55	45%

 $X^2=30$  P<0.01

The positive result of RSV-Respi-Kits appears as two dark red lines due to presence of RSV- antigen in test samples,

compared to negative result which shows only one dark red line (Figure 1).

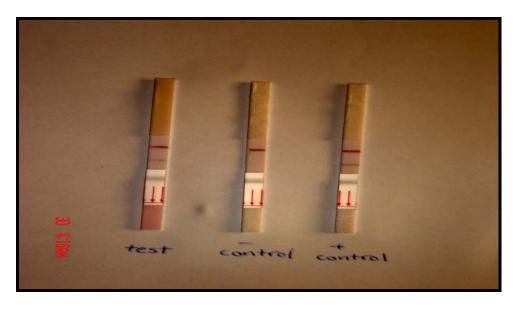


Figure 1: RSV-Respi-test appears as two dark red lines due to presence of RSV-antigen in nasal/ throat swabs, compared to negative result which shows only one dark line.

Forty one Infants who were suffering from acute bronchiolitis and pneumonia were examined for presence of both HRSV antigen and antibodies. Results revealed that (54%) of patients were positive HRSV antigen in the nasopharyngeal swabs by using Respi-test kit but 46% samples were negative for HRSV antigen which was 46%. Thirty four (34) serum samples of the same patients showed positive result for anti

HRSVantibodies which make 83% of the tested sera. Twenty patients were positive for both HRSV antigen and antibodies constituted about (50%) of the tested sera. The result showed statistically high significant difference (P<0.01) among different age groups, which was high in group 10 months to one year than other groups (Table 6).

Table 6: Detection of HRSV antigen and antibodies in Infants with respiratory illness.

No. of sample	Respiratory infection	Age		HRSV itigen		HRSV tibody			antigen tibody
	infection		No.	%	No.	%	No.	%	titer
11	Broncholitis	1-3 months	5	45%	8	73%	4	36%	263.5±125
9	=	4-6 months	5	65%	8	89%	5	56%	527±196
3	=	7-9 months	1	33%	3	100%	1	33%	67
2	=	10-12 months	1	50%	2	100%	1	50%	308
6	Pneumonia	1-3 months	3	50%	4	67%	2	33%	120±21.2
3	=	4-6 months	2	67%	2	67%	2	67%	489±379
2	=	7-9 months	2	100%	2	100%	2	60%	354±180
5	=	10-12 months	3	60%	5	100%	3	60%	1410±829
41			22	54%	34	83%	20	50%	440

L.S.D=766 P<0.01

#### Discussion

Human respiratory syncytial virus has been recognized as most important causative agent of serious respiratory tract infection in infants and young children <sup>(2)</sup>. It account for approximately 50% of all pneumonias <sup>(5)</sup> and about 70% of bronchiolitis, hospitalization are associated with HRSV infection in developed world and in Europe, HRSV accounts for 42 - 45% of hospital admissions for lower respiratory tract infections in children younger than 2 years of age<sup>(6)</sup>.

In Iraq, pediatricians recognized an increasing number of infants with bronchiolitis and pneumonia as reported in hospital admission in winter months <sup>(3)</sup>. Our study is the first in the country to records the presence of RSV in respiratory tract infection in infants by detection of RSV viral antigens and specific viral antibodies in ill Infants.

The antibodies which was found in children with other disease condition, could be due to previous RSV infection or may be a maternal type of antibody as reported in other studies<sup>(7)</sup> who

mentioned that the presence of specific HRSV antibodies in the sera of all full-term newborn due to transplacental transfer of maternal antibodies. The level of these antibodies decline slowly during the first few months of life and it is believed that after 7 month of age the detectable antibodies are usually due to natural HRSV infection<sup>(8)</sup>. Also breastfed Infants have the added benefit of maternal antibodies in form of colostrom and milk <sup>(9)</sup>. However, the presence of antiHRSV in Infants without signs of respiratory tract may be due to asymptomatic infection with HRSV <sup>(10)</sup>.

The detection of antiHRSV in sera of patients with different kind of cancer indicated that these patients were probably infected with HRSV; this result is higher than the result obtained in other study (11), who reported 37% in cancer patients, and agreed with other studies (12), who explained the high infection in immunocompromized patients since they are more susceptible to HRSV infection. Also these patients are able to shed the virus for a longer period of time than the normal Infants (13). In addition, the patients usually immunosupressing drug therapy which suppression of immunity causes accompanied by slow recovery and causes prolonged shedding of the virus from the respiratory tract (14).

The highest percentage of antibodies was detected in infants with pneumonia. Our result agreed with other study <sup>(5)</sup> who indicated that 70% of broncholitis associated with HRSV, but our results were higher than others <sup>(6)</sup> who found 42 - 45% and this may be due to difference in the virulence of the virus and many other stress factors.

Also we noticed differences in titer of anti HRSV antibodies depending on the age of the patients tested which was

low in infants from 1-3 months but the titer increased when infants becomes older, this may be due to developing antibodies which received as low titer and also to low of transfer maternal antibodies from mother to infants through the placenta<sup>(15)</sup>, also the blunted antibody response may be due to the relative antibody response or due to relative secondary immunologic immaturity of infants or due to of suppressive effect maternally transmitted transplacental antibody which cause a non responder for the RSV<sup>(16)</sup>.

The titer of antibodies is significantly higher in infants with bronchiolitis infection than with pneumonia, these may depends on the disease condition as in acute or active phase than in advanced phase (pneumonia), these results agree with other studies<sup>(17)</sup>.

From the accumulative total samples examined (184) serum samples the incidence of HRSV infection was 62% and high percentage was in infants with respiratory tract infection (79%) followed by cancer patients (69%).

The second part of our study was to detect RSV antigen in nasopharyngeal secretion using Respi-test and indirect ELISA. The result of present study is higher than that recorded who detected 31% RSV by using Respi- test and 34% by using viral culture method, but in agreement with other (19), who detected HRSV in 41% of patients samples using light cycler RT-PCR in Australia, and with <sup>(8)</sup> who found 42% to 45% of tested samples by using RT-PCR in European children, but less than that identified<sup>(6)</sup> who recorded 70% bronchiolitis patients hospitalized in U.S.A. by using RT-PCR, studied the infection rate in Huston state showed that 68.8% of infected infants were less than 12 months and 82.6% during 1-2 years of their life<sup>(20)</sup>.

The high detection rate of HRSV was in December which is one of the cold months in Iraq; this agreed with others <sup>(21)</sup> whom found that RSV is a primary infantile RSV infection and typically represented as a winter respiratory tract infection.

Further studies on HRSV in developing countries such as Indonesia (22) and South Africa (23) and Gambia (24) indicated that RSV is a seasonal prevalent agent and has most frequent occurrence at the coldest times of year. These studies are in agreement with our results.

Results show that 50% of Infant patients (20 out of 40) were positive for both HRSV antigens in the nasal/throat secretion and antiHRSV antibodies in their sera. These results indicate that presence of humeral antibodies doesn't appear to play a role in resolution of viral infection and clinical recovery from RSV, with virus shedding continues from the upper respiratory tract. These results agreed with others which proved that humeral antibodies did not stop viral shedding from infected mucosa (25). Similarly studies recorded that viral shedding was blocked in presence of specific secretary immunoglobulin (IgA) at time of clinical recovery (26). Also the frequency of lower respiratory tract infection caused by RSV is significantly among children with RSVneutralizing antibodies and mainly when the titer of neutralizing antibodies lower than  $100^{(27)}$ .

We recommended to isolate the RSV in infant and identified the strain and type present in Iraq which causes illness.

#### **References**

- **1.** Selwyn BJ. The epidemiology of acute respiratory tract infection in young children comparison of findings from several developing countries. Rev. Infect. Dis. 1990; 12:5870-5888.
- **2.** Collins PL, McIntosh K and Chunock RM. Respiratory syncytial virus. Fields virology 3<sup>rd</sup> Ed. Vol. 1. Lippincott-Raven, Philadelphia, pa.1996;p 1313-1351.
- **3.** Abu-Diab A, et al. Comparison between per nasal flocked swabs and nasopharyngeal aspirates for the detection of common respiratory viruses in samples from children. J. Clin. Microbiol. 2008. **46**:2414-2417.
- **4.** Snedecon GW and Cochron WG. Statistical method, Jowa ststo. Uni-Press. 1968.
- **5.** Henrickson KJ, Hoover S, Kehl KS, and Hua W. National disease burden of respiratory viruses' detection in children by polymerase chain reaction. Pediatr. Infect. Dis. 2004; 23:11-18
- **6.** Simoes EA. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory disease. J. Pediatr. 2003; 143:118-126.
- **7.** Parrot RH, Kim HW and Arrobio JO. Epidemiology of respiratory syncytial virus infection in Washington, D.C. Infection and disease with respect to age, immunologic status race and age. Am. J. Epidemiol.1973; 98: 289-300
- **8.** Beem M, Geezer R and Anderson J. Respiratory syncytial virus neutralizing antibodies in persons residing in Chicago, Illinois. Pediatrics.1964; 34:761-770.
- **9.** Watt PJ, Zardis M and Lambden PR. Age related IgG subclass response to respiratory syncytial virus fusion protein in infected infants. Clin. Exp. Immunol.1986; 64:503-509.
- **10.** Hall CB, Long CE, Schanbel KC. Respiratory syncytial virus infection in previously healthy working adults. Clin.Infect.Dis. 2001; 33:292-296.
- **11.** Anaissie EJ, Manfouz TH, Allan T, Pouli A, Desikan R, Fassas, A and Barlogie B. The natural history of respiratory syncytial virus infection in cancer and transplant patients' implication for management. Blood. 2004; 103:1611-1617.
- **12.** Falsey AR, Formica MA, Hennessy PA, Criddle MM, Sullender WM and Walsh EE. Detection of Respiratory syncytial virus in adult with chronic obstructive pulmonary disease. Am. J. Resp. Crit. Med. 2006; 173:639-643.
- **13.** Chadwani S, Borkowsky W, Karasinki K, Lawrence R and Welliver R. Respiratory

- syncytial virus infection in human immunodeficiency virus infected children. Pediater.1990; 117:251-254.
- **14.** Taylor CE, Croft AW and Kernaham J. Local antibody production and respiratory syncytial virus infection in children with leukemia. J. Med. Virol.1990; 30:277-281.
- **15.** Murphy BR, Alling DW and Snyder M. Effect of age and pre-existing antibody on serum antibody response of infants and children to F and G glycoprotein during respiratory syncytial virus infection. J. Clin. Mocrobiol. 1986; 24:894-898
- **16.** Chan K H, Peiris J S, Lim W, Nicholls JM and Chiu SS. Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children. J. Clin. Virol. 2008.**42**65-69.
- **17.** Graham BS, Henderson GS, Tang YW, Lu Y, Neuzil KM. and Colley DJ. Priming immunization determines T helper cytokine mRNA expression patterns in lung of mice challenged with respiratory syncytial virus. J. Immunol. 1993; 151:2032-2040.
- **18.** Van den Wijngoert S, Defoor M, Van Beers D. Comparison of rapid immunochromatographic diagnostic test with viral culture to detect respiratory syncytial virus in nasopharyngeal specimens. Microbiology Laboratory, Hopital universitaire St-pieme; rue haute, Brussels, Belgium.2004; 322-100.
- **19.** Whiley DM, Sgrnies MW, Mackay IM and Stoots T. Detection of human RSV in respiratory samples by light cycler Reverse transcriptase PCR (LC-RT-PCR). J. Clinicl. Micro.2002; 40: 4418-4422.
- **20.** Loens K,\* Van Heirstraeten L , Malhotra-Kumar S , Goossens H, and Ieven M . Optimal Sampling Sites and Methods for Detection of Pathogens Possibly Causing Community-Acquired Lower Respiratory Tract Infections . J Clin Microbiol .2009:47(1):21-31.
- **21.** Openshaw PJ. and Trooping JS. Immune response and disease enhancement during respiratory syncytial virus infection. Clin. Micobiol. Rev.2005; 18:541-555.
- **22.** Djelantik LG, Gessner BD, Soewignjo S, Steinhoff M, Sutanto A, Widjaya A. Incidence and clinical feature of hospitalization because of respiratory syncytial virus lower respiratory illness among children less than two years of age in a rural Asian setting. Pediatr. Infect. Dis. J. 2003; 22:150-157.
- **23.** Madhi SA, Ventre M, Alexandra R, Lewis H, Kara Y, Karshagen WF, Greef M and Lasse N. Respiratory syncytial virus associated in high

- risk children and national characterization of the circulating virus genotype in South Africa. J. Clin. Virol.2003; 27:180-189.
- **24.** Weber MW, Milligan P, Sanneh M, Awemoyi A, Dakour R, Schneider G. An epidemiological study of RSV infection in the Gambia, Bull. W. H. O.2002; 80:562-568.
- **25.** Hall CB, Douglas RG and Geiman JM. Respiratory syncytial virus infection in infants: quantization and duration of shedding. Pediatr. 1976; 89:1-5.
- **26.** Meert KL, Sarnaik AP, Gelmini MJ and Lieh MW. Aerosolized ribavirin in mechanically ventilated children with respiratory syncytial virus lower respiratory tract disease: a prospective double blind, randomized trial, crit. Care Med.1994; 22:566-572.
- **27.** Law B, Wang EL and McDonald N. Dose ribavirin impact on the hospital course of children with respiratory syncytial virus infection. Pediatrics 1997; 99.

## Comparison between Serum Prolactin Levels Determined by VIDAS and RIA Techniques

Rayah S. Baban PhD, Yahya Y.Farid PhD.

#### Abstract

**Background:** Human prolactin can be determined in human serum or plasma quantitatively by many techniques such as ELISA, RIA, and ELFA (miniVIDAS).

**Objectives:** To estimate the strength of association of total serum prolactin and free serum prolactin values measured by two different methods [RIA and VIDAS]. And to predict the prolactin value measured by RIA corresponding to a given value by VIDAS.

Methods: Two technical methods VIDAS and RIA were used in determination of prolactin level in sera of twenty five women with uterine fibroid conducted at two laboratories admitted at Al-Khademyia Teaching Hospital during the period January 2008 to April 2009. Total and free serum prolactin was measured by both VIDAS and RIA using their assay kits. Statistical methods of correlation and regression were used to compare between the two methods.

**Results**: The study revealed a highly significant positive correlations between VIDAS and RIA total and free serum prolactin, r=0.999,

 $R^2=0.998$ , P<0.001, r=0.998,  $R^2=0.997$ , P<0.001 respectively. High linear regression equations were found between VIDAS and RIA total and free serum prolactin, y= 0.358x+ 0.57,  $R^2=0.998$  and y= 0.355x+0.49,  $R^2=0.997$  respectively. The recovery percentages of prolactin (R %) in two methods were approximately equal to each other, VIDAS R%=50.52±0.89% and RIA R%= 50.38±1.57% respectively.

**Conclusion:** A highly significant positive correlation was found between RIA and VIDAS for both total and free serum prolactin. And high linear regression equations were found between the two methods to predict RIA values corresponding to a given VIDAS value.

**Keywords:** VIDAS prolactin, RIA prolactin, PEG precipitation, macroprolactinemia, uterine fibroid.

IRAQI J MED SCI, 2009; VOL.7 (4): 20-26

#### Introduction

Prolactin is synthesized as a prehormone with a molecular weight of 26 kDa: when the pre-prolactin is cleaved, the resulting polypeptide has a molecular weight of 23 kDa. This monomeric form, biologically active, accounts for approximately 85-95% of the total PRL present in normal individuals <sup>(1)</sup>, but other circulating species are identifiable on gel filtration chromatography (GFC): the "big" PRL (50-70 kDa), a dimeric/ trimeric form variably glycosylated, and

<sup>1</sup>Dept. Chemistry and Biochemistry, College of Medicine, Al-Nahrain University. Address Correspondence to: Dr. Rayah S.

E-mail: r baban@hotmail.com

Received: 16<sup>th</sup> August 2009, Accepted: 18<sup>th</sup> October 2009.

the "big big" PRL or "macroprolactin" (150- 170 kDa), mainly a complex between monomeric PRL and an anti-PRL immunoglobulin G (IgG) (2-4), in some cases a covalently or noncovalently bound aggregate monomeric PRL molecules with increased glycosylation (5,6). In normal population the big PRL accounts for less than 10% of circulating PRL, whereas the macroprolactin represents a small (less than 1%) amount of total PRL<sup>(7)</sup>.

In particular cases (mainly in sera from hyperprolactinemic subjects) the relative proportions of circulating forms can be quite different: macroprolactin can represent even the 90% of circulating PRL <sup>(8)</sup>. Macroprolactin is cleared more slowly than monomeric

PRL and hence accumulates in the serum. It is generally believed that macroprolactin has a low or absent biological activity in vivo, because it cannot cross the endothelium and reach the cell surface receptors (9-11)

The reference method for demonstration and quantification of high molecular mass forms of PRL is the GFC, but it is time-consuming and expensive for routine use. An alternative technique, more usually employed, is the polyethylene glycol (PEG) precipitation.

The PEG is able to precipitate large molecular mass proteins; in this way, after the precipitation of macroprolactin by PEG, the supernatant should contain prevalently the monomeric PRL form.

Afterwards, the PRL levels are measured, in serum and in supernatant, with the immunometric assay used in the laboratory. The results of PEG test, interpreted on the basis of the percentage of PRL recovery (R %), allow to detect the macroprolactin interference and, furthermore, to obtain an estimate of monomeric, biologically active, PRL (12).

Prolactin can be determined in human serum or plasma quantitatively by many techniques such as ELISA, RIA, and ELFA (miniVIDAS). The assay principle of VIDAS prolactin is an automated quantitative test for use on the VIDAS instruments, for the enzyme immunoassay using ELFA technique (Enzyme linked fluorescent Assay) [VIDAS Prolactin Kit, REF 30 410] while the assay principle of RIA prolactin is based on the competition between unlabelled hPRL and fixed quantity of [125 I] labeled hPRL for a limited number of binding sites on a specific antibody [125] RIA KIT (REF: RK-553).

#### Subjects and Methods

The study was conducted during the period from January 2008 to April 2009 on twenty five women diagnosed with uterine fibroids in Obstetrics and Gynecology department at Al-Khademyia Teaching Hospital. They had different clinical presentations like amenorrhea, menstrual disturbance, and galactorrhea. All of them were sent for MRI to exclude the presence of pituitary adenoma and abdominal ultrasound to confirm the diagnosis of uterine fibroid.

Ten milliliters of blood were aspirated from each patient in order to estimate the level of prolactin in their sera prior to the operation [myomectomy or hysterectomy]. Blood sample was left to clot at room temperature and then separated by centrifuging at 800 xg for 10 minutes. Each patient serum was divided into two parts and sent to two different laboratories to evaluate the total serum prolactin and free prolactin after precipitating with Polyethylene glycol (PEG 8000) by miniVIDAS and RIA techniques. The VIDAS Prolactin was determined by using ELFA technique (Enzyme Linked Fluorescent Assay) (VIDAS Prolactin Kit, REF 30 410), while the RIA Prolactin was determined using [125 I] RIA KIT) (REF: RK-553).

The PEG 8000 precipitation test was performed according to the method proposed by Fahie-Wilson and Soule <sup>(13)</sup>. Two hundred micro liters of a 25% PEG 8000 solution was added, at room temperature, to equal volume of serum and was centrifuged (after thorough vortex mixing) at 1800 x g for 30 min at 20 °C. Prolactin was measured, without delay; in the supernatant obtained after PEG precipitation using the miniVIDAS and RIA, after correction for dilution, were compared with those obtained from untreated serum.

The results of PEG test were expressed as PRL recovery. agreement with literature data (7, 11, 13, 14). we assumed an R% value <40% as indicative of presence of substantial amounts of macroprolactin in serum (macroprolactinemic subjects); on the contrary, a R% value >60% was considered indicative of substantial absence of macroprolactin hyperprolactinemic subjects).

#### Results

Table 1 shows the mean±SD for total serum prolactin and free serum prolactin measured by two methods. The mean±SD of total serum prolactin measured by VIDAS technique was found to be higher than that of the same sample measured by RIA technique (389.14±160.85 ng/ml, 138.86±58.76 ng/ml respectively). And also after precipitating with PEG 8000, VIDAS free serum prolactin mean±SD was higher in same sample measured by RIA technique (192.55±80.04 ng/ml, 68.34±28.80 ng/ml) respectively.

A highly significant positive linear correlation was found between VIDAS

and RIA total serum prolactin (r=0.999,  $R^2 = 0.998$ , P<0.001). And after treating samples with PEG 8000, the VIDAS and RIA free serum prolactin measured also revealed a highly significant positive linear correlation (r=0.998,  $R^2 = 0.997$ , P<0.001) (Table 2).

In order to confirm these correlations, a highly significant linear correlations was found between VIDAS and RIA total serum prolactin with linear regression equations (y= 0.358x+0.57,  $R^2 = 0.998$ ) (Figure 1) as well as between VIDAS and RIA free serum prolactin when measured by the same two techniques (y= 0.355x+0.49,  $R^2 = 0.997$ ) as shown in (Figure 2).

The mean values of recovery prolactin percentage (R %) were approximately the same in both VIDAS and RIA techniques for all patients (50.52±0.89, 50.38±1.57) respectively (Table 3). It means that all samples do not contain macroprolactin and both techniques give the same results approximately.

Table 1: A descriptive analysis for total serum prolactin(Total S.PRL) and free serum prolactin (Free S.PRL) measured by VIDAS and RIA techniques. (n=25)

Serui	m prolactin	mean±SD (ng/ml)	Range(ng/ml)
	Total S.PRL	389.14±160.85	101.34-589.43
VIDAs	Free S.PRL	192.55±80.04	51.76-295.99
	Total S.PRL	138.86±58.76	33.26-213.83
RIA	Free S.PRL	68.34±28.80	17.52-107.1

Note: mean $\pm$ SD is mean  $\pm$  standard deviation.

Table 2: Correlations for both total serum prolactin and free serum prolactin measured by VIDAS and RIA techniques.

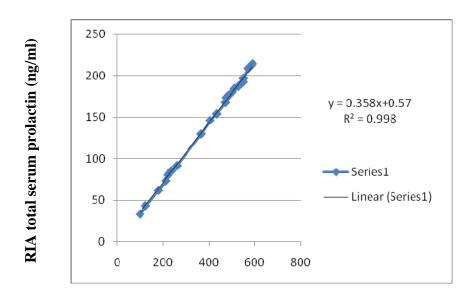
111	casurcu D	y vidas	and KiA	icciniiques.		
serum prolactin	r	P	t	95% Confidence Interval		$R^2$
				Lower	Upper	
Total S.PRL(ng/ml)*	0.999	< 0.001	119.218	0.359	0.372	0.998
Free S.PRL(ng/ml) >	0.999	<0.001	88.639	0.351	0.368	0.997

<sup>❖</sup> Correlation between total serum prolactin measured by VIDAS and RIA technique.

Table 3: Recovery prolactin percentages measured by VIDAS and RIA techniques (n=25).

Recovery prolactin percentages	mean±SD	Range	P value
VIDAS (R%) prolactin	50.52±0.89	48.92-52.62	Not
RIA (R%) prolactin	50.38±1.57	46.61-53.05	Not

Note: R%= Recovery prolactin percentage



VIDAS total serum prolactin (ng/ml)

Figure 1: Correlation with linear regression line between VIDAS and RIA total serum prolactin levels.

<sup>&</sup>gt; Correlation between free serum prolactin measured by VIDAS and RIA technique.

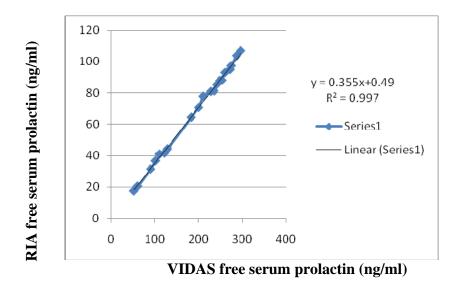


Figure 2: Correlation with linear regression line between VIDAS and RIA free serum prolactin levels.

#### Discussion

Hyperprolactinemia is a common problem encountered in reproductive disorders <sup>(15)</sup>. The understanding that prolactin hypersecretion not only causes galactorrhea and amenorrhea but also gonadal dysfunction and infertility led to the wider use of prolactin estimations <sup>(16)</sup>

All methods for measuring prolactin cross-reactivity show some with macroprolactin, and the extent to which it contributes to the measured prolactin concentration depends on the choice of reagent antibody. Because the presence of macroprolactin does not appear to contribute to the hyperprolactinemic syndrome in the majority of patients, it is important to raise awareness of the problem in clinical chemistry laboratories and with physicians (17).

Confirmation of the presence of macroprolactin can be made by gel filtration chromatography, but this is time-consuming, costly, and beyond the scope of most clinical laboratories.

Laboratories in the United Kingdom have been using mainly PEG

precipitation techniques to identify macroprolactinemia<sup>(17)</sup>. As highlighted in these twenty-five clinical cases, macroprolactin needs to be identified early in a patient's work-up to avoid unnecessary, costly, and invasive procedures.

All patients in this study were with elevated prolactin level in their sera (table-1) although they were all non pituitary adenoma patients and they were all with uterine fibroid (ectopic or extra pituitary secretion). Nowak et al. 1993 have investigated the actions of several hormones known to stimulate PRL secretion by the pituitary gland or decidua on **PRL** secretion leiomyoma-derived smooth muscle cells (SMC) in monolayer culture. They conclude that leiomyomas express PRL mRNA in vivo and that leiomyomaderived SMC in culture continue to express the PRL mRNA and secrete PRL in the absence of ovarian steroids. PRL secretion by SMC in culture appears to be modulated primarily by changes in cell density (18). Szécsi *et al* 2006 investigated via **HPLC-RIA** determinations of intratissular concentrations of eleven main steroid hormones. The data verify that the determination of intratissular steroid concentrations by HPLC-RIA methods may identify even the most peculiar hormone sources and the hormone profiles facilitate studying pathophysiology of ectopic endocrine tumors (19).

PEG precipitating method was used to determine the prolactin R% and to identify the prolactin profile present in their serum. PEG pretreatment yielded results that correlated best and are recommended as the first-choice alternative to GFC (gel filtration chromatography) (20).

Two techniques in this study were used to predict which one is faster in clinical diagnosis. VIDAS prolactin assay needed only 40 minutes to generate results while RIA prolactin assay needs 120 minutes and 2 days to generate the results. So time consuming is less in VIDAS than RIA technique. Although both VIDAS and **RIA** techniques gives almost the same linear regression equations (figure-1 and 2) and significant positive correlations (table-2) when samples underwent assay before and after PEG 8000 treatment.

As mentioned in VIDAS prolactin kit leaflet, when VIDAS compared with other test methods, a correlation was established between VIDAS PRL kit and enzyme immunoassay (x), r=0.984, and y VIDAS PRL= 1.10 x + 5.81 (Biomérieux, VIDAS PRL, 07325J-GB-2004/09).

Batra *et al* 1989 made a comparison between HPLC and RIA methods in measuring the AZT (Zidovudine) level after oral administration. The results of the two methods did not correlate statistically (correlation coefficient = 0.79). The zero, one and two hours post administration of Zidovudine serum compared. levels were also correlation coefficients were 0.20, 0.75, and 0.68 respectively. They concluded that results obtained by the RIA method did not correlate well with the HPLC method. The variation was consistent. The RIA method values were consistently higher for the one hour and two hours post ingestion levels (21).

Barlow *et al* 1986 used an enzymelinked immunosorbent assay (ELISA) to evaluate serum alpha-fetoprotein in the antenatal screening for fetal open neural tube defects. They concluded that The ELISA method was simple, required about one quarter less operator time than the RIA and enabled results to be generated in one day rather than the two days required by RIA. The ELISA method is a suitable alternative to RIA for routine use in screening for fetal neural tube defects (22).

#### References

- **1.** Sapin R, Gasser F, Grucker D. Free prolactin determinations in hyperprolactinemic men with suspicion of macroprolactinemia. Clin Chim Acta 2002; 316(1-2):33-41.
- **2.** Cavaco B, Leite V, Santos MA, *et al.* Some forms of big big prolactin behave as a complex of monomeric prolactin with an immunoglobulin G in patients with macroprolactinemia or prolactinoma. J Clin Endocrinol Metab 1995; 80(8): 2342-6.
- **3.** De Schepper J, Schiettecatte J, Velkeniers B, *et al.* Clinical and biological characterization of macroprolactinemia with and without prolactin-IgG complexes. Eur J Endocrinol 2003; 149(3):201-7.
- **4.** Hattori N, Ikekubo K, Nakaya Y, *et al.* Immunoglobulin G Subclasses and Prolactin (PRL) Isoforms in Macroprolactinemia Due to Anti-PRL Autoantibodies. J Clin Endocrinol Metab 2005; 90(5): 3036-44.
- **5.** Fahie-Wilson MN, Soule SG. Macroprolactinaemia: contribution to hyperprolactinaemia in a district general hospital and evaluation of a screening test based on

- precipitation with polyethylene glycol. Ann Clin Biochem 1997; 34:252-8.
- **6.** Fahie-Wilson MN, Ahlquist JA. Hyperprolactinaemia due to macroprolactins: some progress but still a problem. Clin Endocrinol 2003; 58(6):683-5.
- **7.** Olukoga AO, Kane JW. Macroprolactinaemia: validation and application of the polyethylene glycol precipitation test and clinical characterization of the condition. Clin Endocrinol 1999; 51(1):119-26.
- **8.** Smith TP, Suliman AM, Fahie-Wilson MN, *et al.* Gross variability in the detection of prolactin in sera containing big big prolactin (macroprolactin) by commercial immunoassays. J Clin Endocrinol Metab, 2002; 87(12):5410-5.
- **9.** Bonhoff A, Vuille JC, Gomez F, *et al.* Identification of macroprolactin in a patient with asymptomatic hyperprolactinemia as a stable PRL-IgG complex. Exp Clin Endocrinol Diabetes 1995; 103(4): 252-5.
- **10.** Leite V, Cosby H, Sobrinho LG, *et al.* Characterization of big, big prolactin in patients with hyperprolactinaemia. Clin Endocrinol 1992; 37(4):365-72.
- **11.** Schlechte JA. The macroprolactin problem. J Clin Endocrinol Metab 2002; 87(12):5408-9.
- **12.** Germano L, Mormile A, Filtri L, *et al* .Evaluation of polyethylene glycol precipitation as screening test for acroprolactinemia using Architect immunoanalyser . LigandAssay 2006; 11 (2).
- **13.** Fahie-Wilson MN, Soule SG. Macroprolactinaemia: contribution to hyperprolactinaemia in a district general hospital and evaluation of a screening test based on precipitation with polyethylene glycol. Ann Clin Biochem 1997; 34:252-8.
- **14.** Leslie H, Courtney CH, Bell PM, *et al.* Laboratory and clinical experience in 55 patients with macroprolactinemia identified by a simple polyethylene glycol precipitation method. J Clin Endocrinol Metab 2001; 86(6):2743-6.
- **15.** Choudhary SD, Goswami A. Hyperprolactinemia and reproductive disorders a profile from north east. J Assoc Physicians India 1995; 43:617-8.
- **16.** Avasthi K, Kaur J, Gupta S, *et al.* J Obstet Gynecol India Vol. 56, No. January/February 2006 Pg 68-71.
- **17.** Rhys J, Ian F.W. McDowell, Maurice Fet al. Macroprolactin Reactivities in Prolactin Assays: An Issue for Clinical Laboratories and Equipment Manufacturers Clinical Chemistry . 2000; 46: 884-885.

- **18.** Nowak RA, Rein MS, Heffner LJ, *et al.* Production of prolactin by smooth muscle cells cultured from human uterine fibroid tumors.J Clin Endocrinol Metab. 1993 May; 76(5):1308-13
- **19.** Batra K, DeSouza M, Stephy E, *et al.* Comparison of radioimmunoassay (RIA) and high performance liquid chromatography (HPLC) methods for serum zidovudine (AZT) level measurements. Int Conf AIDS. 1989 Jun 4-9; 5: 279.
- **20.** Lucille K. Joseph McKenna, Michael N, *et a l*. Specificity and Clinical Utility of Methods for the Detection of Macroprolactin. Clinical Chemistry 52: 1366-1372, 2006.
- **21.** M. Szécsi, I. Tóth, J. Gardi, *et al.* HPLC-RIA analysis of the ectopic cortisol production in a cancerous pancreas tumor. Journal of Biochemical and Biophysical Methods. 2006; November, Volume 69, 1-2, 30, Pages 51-55.
- **22.** Barlow RD, Thompson SG, Cuckle HS, *et al.* Comparison of an ELISA with a RIA method for serum alpha-fetoprotein determination in screening for fetal neural tube defects. Ann Clin Biochem. 1986 May; 23 (Pt 3):334-9

### Genotyping of HLA-class-II by PCR-SSP of Iraqi Breast Cancer Patients

Ahmed A. Al-Hassan<sup>1</sup> MSc; PhD, Nidhal Abdul Muhymen<sup>1</sup> MSc; PhD, Ala'a Ghany Hussien<sup>2</sup> FICMS, Ameera J. Al-Nnema<sup>3</sup> MBChB; MSc, Khalifa Mehdi <sup>3</sup> BSc.

#### Abstract

**Background:** Breast cancer incidence differs among women of different racial/ethnic groups. Several HLA alleles are associated with susceptibility or protection in Breast cancer, the particular allele varies depending on the racial groups.

*Objectives:* This study was established to shed light on the possible association of HLA class II alleles with BC in Iraqi female patients.

Subjects and Methods: The study included 60 subjects: 30 breast cancer patients, 12 patients with benign breast lesions as first control and 18 apparently healthy subjects as second control. Polymerase chain reaction-specific sequence primers (PCR- SSP) assay was conducted to assess HLA-typing.

**Results**: A survey of the distribution of HLA-DR and HLA-DQ alleles frequencies yielded

no evident of positive association between class II alleles and BC as compared with both control groups, but there was appreciable significant decrease in the frequency of DR\*010101,0102,0201-0204,04-13 and DQB1\*0401,02 alleles in BC patients as compared with healthy control (P=0.031).

Conclusions: These findings demonstrated that HLA- DR\*010101, 0102, 0201-0204, 04-13 and DQB1\*0401, 02 alleles may confer protective effects against BC.

Keywords: Breast cancer, HLA allele, PCR.

IRAQI J MED SCI, 2009; VOL.7 (4):27-32

#### Introduction

Human based studies have suggested that the host genetics predisposition is important in disease pathogenesis and protection, considering the importance of immune surveillance during tumorigenesis, some individuals who inherit specific alleles or haplotypes of the highly polymorphic human leukocyte antigen (HLA) system may be exposed or may resist to specific types of cancers (1-5).

Breast cancer incidence differs among women of different racial and/or ethnic groups, and accordingly, several HLA associations have linked HLA system with susceptibility or protection in the disease. However, the

Address Correspondence to: Dr. Nidhal AbdulMohymen.

E-mail: <u>dr.nidhalmohammed@yahoo.com</u> Received: 1<sup>st</sup> June 2008, Accepted: 8<sup>th</sup> June 2009. studies have been consistent with respect to the influence of these alleles in immune clearance of tumor cells in could affect BCway that development. Therefore, HLA genotypes have been suggested to be a biologically based risk factor for BC (6, the present study, polymorphism of HLA-DR and -DQ alleles was analyzed in a sample of Iraqi females with BC using the PCR – SSP method.

#### <u>Subjects and Methods</u> Subjects:

Thirty breast cancer female patients (invasive ductal carcinoma, invasive lobular carcinoma and in situ ductal carcinoma), with an age range of 28 - 73 years, were eligible for this study. The patients were admitted for surgery at Al-Kadhimiya Teaching Hospital and Nursing Home Hospital (Medical City) in Baghdad, for the period March 2006 - March 2007. Pathological data (histologic tumor type grade, tumor stage and lymph

<sup>&</sup>lt;sup>1</sup>Dept. Medical Microbiology, College of Medicine, Al-Nahrain University, <sup>2</sup> Dept. Pathology, College of Medicine, Al-Nahrain University, <sup>3</sup> Medico – Legal Institute.

node status) were obtained from the medical records of patients and validated by an experienced histopathologist.

Two control groups were included: 12females with benign breast lesions (BBL) (6 cases with fibrocystic disease and 6 with fibroadenoma) and 18 apparently healthy females. The latter subjects had no history or clinical evidence of any breast lesions and matched by age and ethnic backgrounds to BC patients.

#### Methods:

#### Blood collection:

Two milliliters of venous blood with EDTA as anticoagulant were collected from each subject.

#### DNA extraction:

Extraction of DNA from peripheral blood was done according to the modified method of Miller <sup>(8)</sup>, using the EXTRA-GENE-I kit (BAG-Germany), which is the most suitable method for isolation since pure DNA can be obtained from whole blood in a short time without the use of toxic chemicals or solvents.

#### PCR amplification:

HLA-genotyping was performed by PCR-SSP according to a method presented by Olerup and Zetterquist (9, 10), using low resolution typing kits (HISTO TYPE / DNA-SSP Kits-BAG-Germany). Appropriate amounts of **DNA** Taq polymerase and (Recombinant Taq polymerase from QIAGEN-company) were added to pre-aliquoted primers and **PCR** conditions were set according to the manufacturer instructions.

#### Detection of PCR products:

PCR products were loaded in 2 % agarose gel containing 0.5 mg/ml ethidium bromide, electrophoresed for 25 min at 12 V/cm, and examined under ultraviolet light. The individual

alleles were assigned for the specific pattern of appropriately sized bands.

#### Statistical analysis

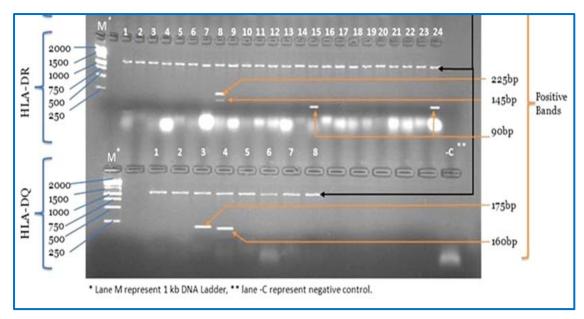
The results were presented in terms of percentage frequencies, and alleles showing variations between patients and controls were further presented in terms of odds ratio (OR), etiological fraction (EF) and preventive fraction (PF). The significance of these differences were assessed by fisher's exact probability (P) (11,12).

#### Results

In this study the mean age of BC patients was 48.1 years with a range of 28 - 73 years, while the mean age of BBL was 35.33 years with a range of 21 - 50 years.

In the PCR-SSP method, 24 HLA-DR and 8 HLA-DQ specific primer mixes were employed as well as, a negative control and ladder mixes. A successful amplification resulted in the generation of a defined length band as a positive internal control in all lanes except the negative control lane, and when there was no amplification, there was no band. In addition, a positive specific amplification resulted in the generation of a specific band in addition to an internal control band (Figure 1).

The observed percentage frequencies of HLA-DR and DQ alleles in the investigated groups are given in tables 1 and 2 respectively, while allele showing a significant variation is presented in table 3. A survey of the distribution of HLA-DR and HLA-DQ alleles frequency yielded no evident of positive association between class II alleles and BC, but there was significant decrease in the frequency of DR\*010101, 0102, 0201-0204, 04-13 and DOB1\*0401, 02 alleles in BC as compared with healthy control (P=0.031) table 3.



**Figure 1:** Electrophoresis of HLA-DR and DQ alleles' amplification by PCR-SSP. The first line represent the HLA-DR genotyping was obtained by using primers that detect the following alleles as they were present in the numbered wells, respectively: 1=DR1,-, DR103; 2=DR15(2),-, DR2, DR16(2),2,-; 3=DR17(3),3,-, DR18(3), DR 17(3); 4=DR4,-, DR4; 5=DR7,-; 6=DR9,-; 7=DR8,-, -, DR8; 8=DR10; 9=DR 11(5),-, DR11(5); 10=DR12(5),-; 11=DR13(6),-; 12=DR13(6); 13=DR,- 14(6); 14=DR14(6),-; 15=DR14(6),-; 16=DR1403,14(6),-, DR13(6); 17=DR 1404,14(6),-; 18=DR14(6),-; 19=DR-6; 20=DR 14(6); 21=DR8; 22=DR6; 23=DR14(6),-; 24=DR14(6),-. The second line represent the HLA-DQ genotyping was obtained by using primers that detect the following alleles as they were present in the numbered wells, respectively: 1=DQB5(1), 10=DR16(1), 10

Table 1: Observed percentage frequencies of HLA-DR alleles in healthy controls, breast cancer patients and benign breast lesion.

Specificities	Healthy controls (n=18)		Cases (bro		Benign breast lesion (n=12)		
HLA-DR-Allele		,,	- '	, ,	N	%	
DR*010101,0102,0201- 0204,04-13	4	22.2	0	0	2	16.7	
DR*030101,0102,04- 07,09,11-16,18-26,28	4	22.2	3	10	3	25	
DR*040101,0102,0301- 11,13,16,17,19-20,23,24,26- 30,32-35,38-53	4	22.2	9	30	3	25	
DR*070101-9,10N	4	22.2	9	30	3	25	
DR*080101-07,10-14,16- 19,22-24,26-30	2	11.1	3	10	1	8.3	

DR*100101,0102	2	11.1	3	10	1	8.3
DR*110201,0202w,1401,140	2	11.1	3	10	1	8.3
2,21						
DR*131402w	1	5.6	3	10	1	8.3
DR*1317,67	0	0	3	10	0	0
DR*1320,24,29,63	1	5.6	3	10	1	8.3
DR*1346	1	5.6	3	10	1	8.3
DR*140301,0302,12,12,27,4	1	5.6	3	10	1	8.3
0/*1318						
DR*150101-12,14-16	5	27.8	9	30	3	25
DRB5*0205	1	5.6	3	10	1	8.3
Blank	4	22.2	3	10	2	16.7

Table 2: Observed percentage frequencies of HLA-DQ alleles in healthy controls, breast cancer patients and benign breast lesion.

Specificities	Healthy controls (n=18)			(breast a) (n=30)	0		
HLA-DQ-Allele	N	%	N	%	N	%	
DQB1*030302,030303,06,12,15	0	0	3	10	0	0	
DQB1*020101-0102,0202-04	4	22.2	3	10	3	25	
DQB1*030101,030102,09,16	2	11.1	9	30	1	8.3	
DQB1*030201,030202,030501-	2	11.1	6	20	1	8.3	
0503,07,08,11							
DQB1*0310	1	5.6	3	10	1	8.3	
DQB1*0401,02	4	22.2	0	0	2	16.7	
DQB1*050101-050302,0504	3	16.7	6	20	2	16.7	
DQB1*060101-0103	1	5.6	3	10	1	8.3	
DQB1*0602-18,20-22,24-26N	3	16.7	6	20	2	16.7	
Blank	16	88.8	21	70	11	91.7	

Table 3: HLA- DR\*010101, 0102, 0201-0204, 04-13 and DQB1\*0401, 02 alleles showing significant variations between breast cancer patients and healthy controls.

HLA-allele	Patier	nts	Healthy controls		Statistical evaluations			
	N	%	N	%	Odds ratio	PF	P	
DR*010101, 0102, 0201-0204, 04-13	0	0	4	22.2	0.1	0.22	0.031	
DQB1*0401, 02	0	0	4	22.2	0.1	0.22	0.031	

PF: Preventive fraction: P: Fisher's exact two-sided probability.

#### **Discussion**

The role of genetic factors in the etiology of BC was documented decades ago. As a result. investigative efforts have focused on the genetic markers of susceptibility to this disease. In particular, HLA system play a pivotal role in cellular immunity and may be an important genetically determined host trait (13, 14). This study is the first attempt on the association between HLA class II alleles and BC in Iraqi patients, in which the alleles were characterized by PCR-SSP.

Although in the current study, there high frequency DR\*040101,0102,0301-11,13,16,17,19-20,23,24,26-30,32-DR\*070101-9.10N 35.38-53 and alleles with patients as compared to control groups, it was statistically not significant. Different results regarding this association was reported, Ghaderi et al., in (2001) revealed statistically significant association between the disease and DRN1\*12 (15). On the other result revealed that hand, present DR\*010101,0102,0201-0204,04-13 and DQB1\*0401,02 alleles showed significant low frequency in BC patients when compared with healthy control, while there was no significant differences as compared with second control group.

Present result was in disagreement with the finding of other studies about the type of protective allele. The HLA DRB1\*1101 and DQB1\*03032 alleles have been suggested to be protective against breast cancer (16). This difference may be partly explained by the fact that the patients studied by Chaudhuri *et al.*, all had early-onset breast cancer; they were all younger than 40 years of age. Furthermore, both groups of patients were belonged to very different racial groups (17).

Also, the discrepancies emerged in this study compared with other studies could be, in part, probably due to the highly modified techniques they used for the same purposes, e.g, PCR, however, if the environmental and genetic factors excluded, which yet to be elucidated, the small number of our study groups may be a considerable contributor to these discrepancies, however, further investigations are certainly required to shed further light on this association.

In conclusion, these data suggested that HLA– DR\*010101, 0102, 0201-0204, 04-13 and DQB1\*0401, 02 alleles may confer protective effects against BC.

#### References

- **1-** Bidwell JL, Soong TW, Raymond PA. HLA genotyping of colorectal carcinoma in the Chinese population. Hum Immunol 1992; 34:19-23.
- **2-** Igney FH, Krammer PH. Death and antideath: tumor resistance to apoptosis. Nat Rev Cancer 2002; 2:277-288.
- **3-** Wu MS, Hsieh RP, Huang SP. Association of HLA-DQB1\*0301 and HLA-DQB1\*0602 with different subtypes of gastric cancer in Taiwan. Jpn J Cancer Res, 2002; 93:404-410.
- **4-** Shankarkumar U, Ghosh K, Badkere S, Mohanty D. Novel HLA Class I Alleles Associated with Indian leprosy Patients. J Biomed Biotechnol 2003; 3:208-211.
- **5-** Harrath AB, Loueslati BY, Troudi W, Hmida S, Sedkaoui S, Dridi A, Jridi A. HLA class II polymorphism: Protective or risk factors to breast cancer in Tunisia Pathology. Oncology Research 2006; 12,No 2, 79-81.
- **6-** Glaser S. Immune –function genes and racedifferences in breast cancer. NorthernCaliforniaCancerCenter;2002.http://www.cbcrporg/research/PageGrantasp?grant\_id=2275
- 7- Glaser S, Join EM, Clarke CA, Erlich HA. Human leukocyte antigen genotype as a contributor to racial/ethnic differences in breast cancer: A population based, molecular epidemiologic study. Northern california cancer center unioncity 2004; Annual rept 2 June 2003-1 June 2004.
- 8- Miller SA, Dykes DD, Polesky IF. Asimple sahing out procedure for extracting DNA from human nucleated cells. Nucl Acid Res 1988; 16:1215.
- 9- Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with PCR-SSP in two hours. Tissue Antigens

- 1992; 39:225-235.
- **10-** Olerup O, Zetterquist H.DR low-resolution PCR-SSP typing-a correction and an update. Tissue Antigens 1993; 41:55-56.
- **11-**Emery AEH. Methodology in medical genetics: An introduction to statistical methods. First edition, UK, Churchill Livingstone 1976; 98-102.
- **12-**Sorlie DE. Medical biostatistics and epidemiology: Examination and Board review First ed, Norwalk, Connecticut, Appleton and Lange 1995; 47-88.
- **13-**Fossum B, Breivik J, Meling GI. A K-ras 13Gly-->Asp mutation is recognized by HLA-DQ7 restricted T cells in a patient with colorectal cancer Modifying effect of DQ7 on established cancers harboring this mutation. Int J Cancer 1994; 58, 506-11.
- **14-**Bateman A, Howell WM. Human leukocyte antigens and cancer: is it in our genes. J Pathol 1999; 188: 231-6.
- **15-**Ghaderi A, Talel A, Gharesi-Fard B, Farjadian S, Amirzargar A, Vasei M. HLA-DRB1 alleles and the susceptibility of Iranian patients with BC 2001; Vol 7, No 1, pp 39-41. **16-**Chaudhuri S, Cariappa A, Tang M, Bell D, Haber D, Isselbacher K, Finkelstein D, Forcione D, Pillai S. Genetic susceptibility to breast cancer: HLA DQB\*03032 and HLA DRB1\*11 may represent protective alleles. Proc Natl Acad Sci 2000; 10; 97(21):11451-
- **17-**Iaffaioli RV, Maio M, Ruggiero G, De Felice M, Ungaro A, De Vecchio L, Rosato GB, Bianco AR, Zappacosta S. HLA and prognostic factors in primary breast cancer. International Journal of Cancer 1985; Vol 35, Issue 5, pp 581-585.

11454.

# Proplast in oral and maxillofacial surgery

#### Ayad A. Hasan FICMS.

#### Abstract

**Background:** Proplast is a material designed for tissue implantation commercially available through the Dow-corning Corporation. It is a gray black laminated felt of vitreous or glassy carbon and Teflon (polytetrafluoroethylene). The vitreous carbon also called hyperpure or elemental carbon or pyrolytic graphite, a pure molecular form of carbon that is pyrolytically derived from hydrocarbon such as rayon. Proplast was 1<sup>st</sup> prepared by Homsy in 1970, invented in 1968 especially for surgical implantation. It's manufactured as a tin felt sheet which is then layered and rolled under high heat and pressure to form the laminated block in common clinical use.

*Objective:* To evaluate the Proplast implant material in restoration of facial bony contour.

*Methods:* There were a total of 18 cases with proplast implant insertion. The mean age of the patients was 27.9 years. The range was 18-35 years. Data was obtained by prospective study and follow-up records of patients with the proplast implant at the department of maxillofacial surgery at Al-kadhymia Teaching Hospital for 6 years follow-up duration from 2000-2006.

Result: Proplast is a useful implant material for the restoration of facial bony contour (success rate was 88.9%). Sixteen implants were judges to be stable (88.8%), and 2 implants (11.2%) were judged to be unstable (removed) due to infection. In the two infected cases the fixation was done by wire fixation instead if suture fixation or spontaneous fixation with an intraoral approach. Of these 16 stable implants 3 were mobile (18.75%) and 13 implants were immobile (81.25%), and this is appeared to depend on the technique of proplast insertion.

Conclusion: Proplast is a useful implant material for the restoration of facial contour, There are some technical difficulties, when it is inserted over areas that are convex such as the malar prominence and orbital margins, in that it is difficult to eliminate the edge effect, but this can be overcome by proper feathering of the edges of the implant with a sharp scalpel.

*Key words:* Proplast, porous, alloplastic, implant.

IRAQI J MED SCI, 2009; VOL.7 (4):33-39

#### **Introduction**

A number of alloplastic materials, which are an inert foreign substances implanted within living tissue, have been used in oral and maxillofacial surgery during the last century, none of which have proved to be entirely satisfactory. Physicians have been implanting non-viable substances into human body since 1565.

Heterogeneous transplants have a history dating from 1668 when Van Meekeran reported the successful transplantation of part of dog's skull to a cranial defect in Russian soldier.

Dept. Maxillofacial surgery, Al-kadhymia Teaching Hospital.

Adress Correspondence to: Dr Ayad A. Hasan.

E-mail: Ayadoo2000@yahoo.com

Received: 5<sup>th</sup> April 2009, Accepted: 4<sup>th</sup> November 2009.

Proplast is microporous implant material, has a porosity of between 70 & 90 volume %, and has high surface energy. The actual surface area is approximately 1200x apparent surface area. The 100-500 µ pore size and the 200-250 dendritic interpore μ connection allow sufficient permeability of tissue for effective metabolic activity; tissue maturation is demonstrable to the point of osteoid or osseous tissue within the implant. The ultra porosity enabling as much as 80% of the implant volume to become tissue (5-12)

Proplast does appear to match up closely with the criteria of scales (1953) laid down for implants, those they:

**1.** Should not be physically modified by tissue fluids.

- **2.** Should not excite an inflammatory or foreign body cell response in the tissues.
- **3.** be chemically inert.
- **4.** be non-carcinogenic.
- **5.** Do not produce a state of allergy or hypersensitivity
- **6.** be capable of being fabricated in the form required with reasonable ease and relatively low cost.
- **7.** be capable of being sterilized <sup>(13-16)</sup>.

Until 1981 there was only one type of proplast. In 1981 proplast II was introduced , it is a PTFE/aluminum oxide (which substitute the vitreous carbon), therefore, the conventional proplast was then called proplast I & the new one is proplast II, which is white, and , therefore, more suitable for superficial implants.

It's particularly indicated where the implant is placed under thin skin, such as the nasal ridge. Proplast II offers a number of advantages over other commonly used silicone & polymethylmethocrylate. It is light, porous, resilient, malleable and easy to shape. It can be readily sterilized after shaping. It has been found to integrate with the surrounding tissues, thereby minimizing the risk of subsequent implant migration and extrusion (17-22).

#### Patients and Methods

Data was obtained by prospective study records of 18 patients with the proplast implant at the department of maxillofacial surgery at Al-kadhymia Teaching Hospital.

The mean age of the patients was 27.9 years. The range was 18-35 years.

The study protocol included the patient name, age, sex, site, cause and duration of the defect. Clinical examination, radiographs, investigations, photographs in anterior and profile view (preoperatively and postoperatively), preparation proplast I and II, placement of the implant either supraperiosteally or subperiosteally. Fixation of the implant either spontaneously, or with absorbable suture (dexon 3:0), or by wire fixation (0.35 gauge) by drilling small holes either side of the implant with straight hand piece and fissure bur with irrigation with normal saline, careful closure in two layers, pressure pack with gauze for 5 days and prescription of antibiotics and follow-up result for 6 years.

Parameters of evaluating the implant postoperatively as good, satisfactory and poor result was done. Good results mean the deformity was completely corrected to the satisfaction of both the surgeon and patient.

Satisfactory mean the defect was corrected but there was dissatisfaction of either the patient or the surgeon. Poor results, when the problems arose necessitating removal of the implant.

The defective area was examined and assessed clinically, and radiographically; these areas include the Orbit, Zygoma, Nose, Chin and mandible.

In case of defective orbital bones. we examined the entire orbital rim, if there is any scar and tethering in the area, the position of the eye lid as well as the presence or absence of the globe and compared to the sound area. We examined if there is associated diplopia and the level of the two orbital sockets. Ocular mobility was examined and facial nerve was assessed by function facial expression.

The Zygoma was examined for the degree of bone loss, any associated tissue loss, scarring and tethering of the tissues. The infraorbital nerve sensation was test by blunt object for any sign of paresthesia or anesthesia. Intraoral examination was done for the state of periodontium and teeth, and if there is any communication with the maxillary sinus. The nose was examined for the defect, if it is

associated with bone loss or with cartilage loss.

The chin area is assessed clinically depending on the true Meridian of the face according to the profile of the patient, and preoperative judgment was done to the size of the implant. Examination of the mental nerve sensation was done by a blunt object. examination done for Intraoral periodontitis, calculus, presence of non-vital teeth especially interiorly, the interdental papillae, as well as the gingival margins, and the presence of partial denture or bridge.

In case of defective orbit, the indications were for cosmetic as well as elimination of diplopia; in others due to blow-out fracture.

For the defective zygoma, nose and chin, the indication was absolutely cosmetic. For mandibular implant, the indication was for augmentation of mandible in conjunction with osteotomy mandibular (intraoral sagittal split) in order to obtain symmetry of the face as a result of unilateral hypoplasia of the mandible in a young female due to fracture sustained during childhood.

In case of orbital defects, the numbers of patients were 4. Two were operated on for the defective roof and 2 were operated on for the defective floor. For the roof a subperiosteal pocket was created to receive the implant by a traditional approach through the eye brow. The remaining 2 patients were operated on for the defective floor of the orbit by an infraorbital approach with the skin crease and insertion of the implant subperiosteally.

For nasal bone defect the numbers of the patients were 4. Two were approached through the skin by supranasal flying bird incision at the nasion, and 2 were approached intranasally by intercartilaginous approach.

zygomatic bone For defects, number of patients were 4, all were approached intraorally be a horizontal incision through the mucosa, a lightly below the depth of the vestibule on the lip side above the canine- premolar teeth. For chin implants, numbers of patients were 5. All approached intraorally by degloving incision, a horizontal incision one inch long through the mucosa midway between the depth of the vestibule and the wet line of the lower lip. Only one patient was operated on for mandibular augmentation. This proplast was used in conjunction with sagittal split of the mandible (intraoral approach, RT. Side), and the augmentation done to LT. side by intraoral approach through the mucosa, midway between the depth of the vestibule and the wet line of the lower lip, slightly to the left side.

#### Result

There were a total of 18 cases with proplast implant insertion. Seventeen were males (94.4%) only one female (5.6%). The mean age of the patients was 27.9 years; the range was 18-35 years. We operated on the orbit (4 cases = 2.2%), nose (4 cases = 22.2%), chin (5 cases = 27.7%), zygoma (4 cases = 22.2%), and mandible (1 case = 5.5%). The higher number of proplast was inserted in chin area, and the least number of proplast was inserted in mandible.

The causes of the defects are war injuries, road traffic accidents, congenital (contour lack), civilian injuries and fracture mandible. The higher cause was due to war injuries (55.5%) = 10 cases.

We used proplast type I (black color) in one case only (mandible) and proplast type II (white color) in 17 cases (94.4%). All proplast II are preformed and did not require any carving, while proplast I required preoperative carving.

Two implants (11.1%) were impregnated preoperatively with blood, one for nose and the other for orbital floor. Two other implants were impregnated with antibiotic solution (penicillin), one for the roof of the orbit and the other for the nose. The 14 remaining implants (77.8%) were unimpregnated with any solution.

Fifteen implants (83.3%) were inserted subperiosteally, and 3 implants (16.7%) were inserted supraperiosteally. The supraperiosteal implants were inserted in cases of zygomatic implant (1 case), and chin implants (2 cases).

The implants were stabilized in their places by three methods; 8 implants (44.4%) stabilized spontaneously, and these include; roof of orbit (2), nose (2), chin(#) and mandible(1), 8 implants were fixed by sutures as follows: floor of orbit (2), both fixed with dexon suture 3:0 with the soft tissues, nose (2), with silk suture 4:0 transcutaneously and in one case; as well as suture fixation, it was fixed with a T-shaped gypsona on the nose for 14 days.

Zygomatic implants (3), with dexon suture 3:0 with the soft tissues, chin (1), with dexon suture 3:0 with soft tissues. Wire fixation (gauge 0.35) was used in 2 cases (11.2%), one for the chin and the other for the zygomatic implant.

Regarding the contour evaluation of the implant postoperatively, a good visible contour was one which was asymmetry in cases where there had been asymmetry or bony defect. We included here the nose and chin for proper contouring. There were 10 implants (55.6%) evaluated as a good contour, these include: orbit (3), 2 for the roof and 1 for the floor, nose (2), zygoma (2) and chin (3 cases). 8 implants evaluated as improved contour (44.4%), these include: orbit (1

case) for the floor, nose (2 cases), zygoma (2), chin (2) and mandible (1).

There was no implant evaluated as worse, or no change occurs.

The colour change of the skin overlying the implant was regarded either satisfactory (no visible colour through the skin), or unsatisfactory (visible colour through the skin). Satisfactory colour was 100% postoperatively stable implants were 16 (88.9%), unstable (removed) were 2 (11.1%), one chin implant and one zygomatic implant, of these 16 stable proplast, 13 (81.25%) were immobile, and 3 (18.75%) were mobile (2 nasal &1 chin implant). Mobile proplast here means not true mobility, but shifting of its position (migrated but still fixed). Of the 18 implants there were 8 (44.5%) palpable margins of the proplast (floor of the orbit 2, nose 2, zygoma 3 and chin 1. Regarding the visibility of the palpable margins, there were 3 visible margins (37.5%) and 5 not visible margins (62.5%). The visible margins were present in nasal implants (2 cases) and in zygomatic implants (1 case).

In evaluating the degree of satisfaction, good results obtained in 7 implants (38.9%), satisfactory results were 50% (9implants) and poor results were 11.1% (2 implants). Good results obtained for the following areas: Mandible (1 case), Orbital floor (2cases), Nose (2 cases) and Chin (2 cases). Satisfactory results obtained for the following areas: Roof of the orbit (2), Nose (2), Zygoma (3) and chin (2).

Poor results occurred with the chin implant (1 case) and zygomatic implant (1 case).

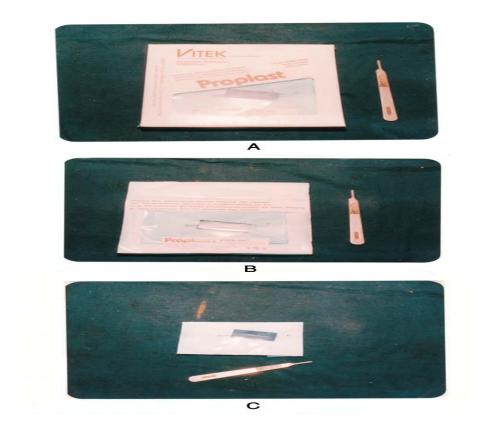


Figure 1: A. Proplast 1. implant in a form of block, as it appears form it 's box . B. The Proplast implant is supplied non-sterite in an autoclavable duble wrap package. This is the 1st wrap package. C. The 2nd warp package.

#### Discussion

Proplast is a useful implant for the restoration of facial contour. There are some technical difficulties when it is inserted over areas that are convex such as the malar prominence and orbital margins, in that it is difficult to eliminate the edge effect; in posttraumatic cases it seemed that slight under building of the contour gave a better appearance than the reverse. When it came to restore concave defects it was excellent. In 17 cases we inserted proplast II (white colour) and in a case we used proplast I (dark colour). The cause of this large difference is due to mandatory use of the proplast II in thin skin areas which include the orbit (4 cases), nose (4 cases) and zygoma (4 cases), and in 5

cases of the chin implants we also used proplast II due to its preformed shape availability in our department. Only in one case we used proplast I (dark colour) for the mandible, this is because here we can use proplast I due to presence of thick skin over the implant, and also we used it because we want a special thickness to get symmetry of the face in the patient we operated on, and this is achieved by careful carving of the implant and compared to the contralateral area until we get the desired shape and thickness. In this study, 2 implants were impregnated preoperatively blood, and this is taken from the same patient by intravenous aspiration of 5cc with a needle and injected it directly to the proplast. In another 2 implants we impregnated them by an antibiotic solution (penicillin) by using vacuum pressure in a 50cc syringe. In the remaining 14 implants we didn't impregnated them in any solution.

Reasons for impregnating only 2 implants with blood is due to the thought of some investigators who suggested that preimpregnating the proplast with blood might promote granulation tissue penetration into the sponge interstices. But this thought is intriguing and there has been no clear experimental evidence to support this concept. In addition, because does not rapidly "soak up" fluid like a sponge, blood must either be injected directly into it or forced into it by vacuum impregnation. The former technique may not give uniform perfusion and it may be laborious; the latter technique is cumbersome in an operating room. If these maneuvers were shown to have no effect on the fibrous ingrowths and fixation, then the additional operating time and risk of contamination would constitute contraindications to such pretreatment of proplast implant. In addition all the investigations did not confirm any advantages pretreatment of the proplast with blood; therefore, we chose only 2 implants for pretreatment with blood, to be compared with the dry implants. Only 2 implants were infused preoperatively with antibiotic (penicillin) for the thought that these measures might decrease the risk of infection, but this is not clear cut, as well as some investigators advocated no pretreatment and confirmed that the dry implants were superior than others which impregnated with antibiotics or blood. The stability of the implant was judged both with regard to mobility on palpation and for any tendency for it to slip completely. Only 2 implants (11.1%) had to be removed (poor stability) because of infection. We

agree with Whitaker, 1987 (11.3% poor stability), and Epstein, 1979 (9.9%). Our results were less than that of Moss, 1979 (19.2% poor stability), the cause was due to different indications. Moss, 1979 used proplast for augmentation of the temporal regions in cases of hypertelorism, also he used the proplast for patients with cleft lip secondary and palate, hypoplastic maxillae, and used once in a post- Le Fort II osteotomy of the anterior maxillae and infranasal area.

#### **References**

- **1-** Georgiade GS, Georgiade NG, Riefkohl R, Barwick WJ. text book of plastic, Max. fac. And Reconstructive surg. 1992; (2), 93-101.
- **2-** Moss KF, Jackson I T, Henderson D, Gibbs P M. The use of proplast in oral and maxillofacial surgery. Br.J. oral surg. 1978-1979; 16,187-197.
- **3-** Connor RJ and Svare GW. Proplast-coated high-strength magnets as potential denture stabilization devices. J. prosthet. Dent. March, 1977; 37 (3), 339-343.
- **4-** Radell BL and Cassingham RJ. A clinical evaluation of proplast as a periodontal implant material. J. periodontal. 1980; 15 (2), 110-114.
- **5-** Kent JN, Homsy CA, Gross BD, and Hinds EC. pilot studies of a porous implant in dentistry and oral surgery. J. oral surg. 1972; 30, 608-615.
- **6-** Arem AJ and Madden JW. soft tissue response to blood-impregnated proplast. Plastic and Reconstructive surgery.1976; 58 (5), 580-586 .
- **7-** Burton DJ, Scheffer RB. Proplast grafting: A new method for stabilization of maxillary advancements. Oral surg- Oral Med- Oral Pathol. 1980; 50 (5), 387-389.
- **8-** Kent JN and Westfall RL. Presurgical infusion of proplast; Primate facial augmentation. J. Oral Surg. 1979; 37 (9), 637-645.
- **9-** Epstein LI. Clinical experiences with proplast as an implant. Plastic and Reconstr. Surg. 1979; 63 (2), 219-223.
- **10-**Dann J J and Epker NB. Proplast genioplasty: A retrospective study with treatment recommendation. Oral Surg.1977; 47 (3), 173-185.
- **11-**Freeman BS. Proplast, A porous implant for contour restoration. British J. plast. Surg. 1976; 29, 158-164.
- **12-**Kent JN, Westfall RL and Carlton DM. Chin and zygomaticomaxillary augmentation

- with proplast: Long-term follow-up. J. Oral Surg. 1981; 39 (11), 912-919.
- **13-**Freeman BS. Proplast [letter]. Plastic-Reconstr. Surg. 1982; 69(5), 902-903.
- **14-**Homsy CA. Porous alloplastic implants. Rev. Laryngol. Oto. Rhinol. Bord. 1981; 102 (1-2), 77-80.
- **15-**Shaber EP. Vertical interpositional augmentation genioplasty with porous polyethylene. Int. J. Oral Max. Fac. Surg. 1987; 16, 678-681.
- **16-**Westfall RL, Homsy CA, Kent JN. A comparison of porous composite PTFE/Graphite and PTFE/Aluminum Oxide facial implants in primates. J. Oral Max. Fac. Surg. 1982; 40 (77), 771-775.
- 17- Shah S, Rhatigan M, Sampath R, Yeoman G, Sunderland S, Brammer R et al. Use of proplast II as a subperiosteal implant for the correction of an ophthalmic enophthalmos. Br. J. Ophthamolol. 1995; 79 (9), 830, 833.
- **18-** Kent J N and Homsy, Temporomandibular joint reconstruction after failed Teflon-proplast implant. Internal journal of oral and maxillofacial surgery. 2008; 16,187,197.
- **19-** Berghaus A and Stelter. Links alloplastic materials in rhinopasty. Curr opin otolaryngology head and neck surgery .2006; (14)4:207-7.
- **20-** Wik R M and Block. Evaluation of the lnion CPS system for the fixation of mandibular fracture in trauma patients. Protoco, 2002; December, CR00002 375.00.
- **21-** Trumpy I G, Roald B and Lybreg T .Morphologic and immunohistochemical observation of explanted proplast-Teflon T M J interpositional implants J. oral Max. Fac. Surgery, 54(1), 63-70.
- **22-** Whear N M,Cousley R R,Liew C, Henderson D. postoperative infection of proplast facial implants. Br. Oral. Max. fac. Surg. 31(5), 292-295.

# Association between TORCH agents and recurrent spontaneous abortion

# Nidhal Abdul Mohymen<sup>1</sup>PhD, Amal Hussien<sup>2</sup>PhD, Farouk K.Hassan<sup>2</sup>PhD.

#### Abstract

**Background:** Toxoplasmosis, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV) (TORCH), that can cause illness in pregnant women and may cause birth defects in their newborns. These entire infectious agents induce a shift of immune response during pregnancy from Th2 to Th1 and apoptosis which can be observed clinically as an abortion process.

*Objective:* To find out the significance of TORCH infection in patients with recurrent spontaneous pregnancy loss.

*Materials and method:* A total of one hundred and nineteen women, ranged from the mean age (23.9 - 28.5)years, were enrolled in the current study and were further classified into three categories: Group A- Recurrent spontaneous abortion (RSA): n= 62 women, with a mean age of (28.5 + 0.68); Group B- non- recurrent spontaneous abortion (non-RSA): n= 34 women, with a mean age of  $(26.4 \pm 0.85)$ and group C- Control (successful pregnancy): n= 23

women, with a mean age of  $(23.9 \pm 0.88)$ . From each patient and control, blood sample was collected. Enzyme linked immune sorbent assay (ELISA),using anti CMV/IgG and IgM, Rubrlla/IgM/IgG ,HSV/IgM and Toxoplasma/IgM/IgG was used.

**Results:** the current study revealed a significant difference in the levels of each of Toxoplasma gondii as well as Cytomegalovirus specific circulating IgM antibodies between group A and group C (p< 0.05) based on their respective enzyme linked immuno sorbent assay (ELISA) Conclusion: In TORCH infections, there was a significant difference between RSA and control in acute infection of T.gondii and in the primary infection of CMV.

**Key words:** Torch, Recurrent Spontanious Abortion, Elisa

IRAQI J MED SCI, 2009; VOL.7 (4):40-46

#### Introduction

Recurrent spontaneous abortion (RSA) is one of the important complications in pregnancy, incidence is 0.5–1%, and the etiology of RSA is varied, and includes maternal or paternal chromosomal aberrations, uterine anatomic abnormalities. endocrine disorders, infections, autoimmune reproductive defects. However, the etiology is undetermined in 40-60% of women with recurrent abortion (1-3). About half of the concepts of RSA have an abnormal karyotype (4), even though the risk for a spontaneous abortion in a subsequent pregnancy is

<sup>1</sup>Dept. Medical Microbiology, College of Medicine, Al-Nahrain University, <sup>2</sup> Dept. Medical Microbiology, College of Medicine, Al-Mustansiryia University.

Address Correspondence to: Dr. Nidhal Abdul Mohymen.

E- mail: <u>dr.nidhalmohammed@yahoo.com</u> Received: 8<sup>th</sup> April 2008, Accepted: 11<sup>st</sup> June 2009. increased when a normal embryonic karyotype is found in abortus material (5). Infection of the uterine lining or slow endometrium with bacteria has also been associated with pregnancy loss in 5-10% of women with RSA. Certain infectious agents have been identified more frequently in cultures from women who have had a spontaneous pregnancy loss; these include Ureaplasma urealyticum, Mycoplasma hominis, and Chlamydia. Other less frequent pathogens include Toxoplasma gondii, Rubella, HSV, Measles, CMV, Coxsackie virus and Listeria monocytogenes, though none have convincingly. At all any severe maternal infection which leads to bacteraemia or viraemia can cause miscarriage. TORCH {toxoplasmosis, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV)}, that can cause illness in pregnant women and may cause birth defects in their newborns. These entire infectious agents induce a shift of immune response during pregnancy from Th2 to Th1 and apoptosis which can be observed clinically as an abortion process <sup>(6)</sup>. Thus, the aim of our study is to find out the significance of TORCH infection in patients with pregnancy loss.

# Materials and methods

A total of One hundred and nineteen women attending the Obstetrics and Gynecology department of Al-Kadhimyia Teaching Hospital in Baghdad between December 2004 and August 2005 were the subject of this study.women, ranged from the mean age (23.9 - 28.5) years, were enrolled in the current study and were further classified into three categories: Group A- Recurrent spontaneous abortion (RSA): n= 62women, with a mean age of (28.5 + 0.68);Group B- nonrecurrent spontaneous abortion (non-RSA): n= 34 women, with a mean age of  $(26.4 \pm 0.85)$ and group C- Control (successful pregnancy): n= 23 women, with a mean age of  $(23.9 \pm 0.88)$ .

From each patient and control blood sample was collected and serum was seperated for the estimation of antibodies against TORCH infection.

Enzyme Linked Immuno Sorbent Assay (ELISA) Was used according to the instruction for the detection of:

CMV/IgG/IgM(BioCheck, Inc. Foster City, CA).

Rubella/IgM/IgG (BioCheck, Inc. Foster City, CA).

HSV/IgM(BioCheck, Inc. Foster City, CA).

Toxoplasma/IgM/IgG (BioCheck, Inc. Foster City, CA).and results were regesrited as mean optical density (OD) readings. The mean gestational age (GA) at the time of abortion for group A and B was (13.94± 0.550) and (15.18±0.937) weeks; respectively, but the mean gestational age at the time of delivery in group C was (38.65±0.135) weeks.

#### **Statistical Analysis**

The ANOVA analysis program, chi-square and the relationship between the indicators was measured qualitatively by using the correlation coefficient.

#### Results

There was a significant difference (p<0.05) in the serum level of *Toxoplasma gondii* specific IgM among the three investigated patients groups (Table 1). The number of positive acute infection of *T.gondii* was 15(24.2%) in group A (RSA) and 5(14.7%) in group B (non-RSA).

Table 1: Prevalence	of TORCH infection	in the three	studied groups.
i abic i. i i cvaichee	or roncin infection		bludica El Jups.

		Groups					
Variable	Results	A (n=62) No (%)			Total (n=119)	Chi-Square P value	
T.gondii	Negative	52 (83.9)	29 (85.3)	21 (91.3)	102		
/IgG	Equivocal	5 (8.1)	3 (8.8)	0	8	0.693	
	Positive	5 (8.1)	2 (5.9)	2(8.7)	9		
T.gondii	Negative	44 (71)	25 (73.5)	23 (100)	92		
/IgM	Equivocal	3 (3.3)	4 (11.8)	0	7	0.023*	
	Positive	15 (24.2)	5 (14.7)	0	20		

D I II	Negative	52 (83.9)	24 (70.6)	19 (82.6)	95	
Rubella	Equivocal	6 (9.7)	3 (8.8)	0	9	0.153
/IgG	Positive	4 (6.5)	7 (20.6)	4 (17.4)	15	
Rubella	Negative	59 (95.2)	31 (91.2)	23 (100)	113	
/IgM	Equivocal	0	2 (5.9)	0	2	0.177
/IgWI	Positive	3 (4.8)	1 (2.9)	0	4	
CMV	Negative	56 (90.3)	33 (97.1)	20 (87)	109	
CMV	Equivocal	3 (4.8)	1 (2.9)	1 (4.3)	5	0.565
/IgG	Positive	3 (4.8)	0	2 (8.7)	5	
CMV	Negative	51 (82.3)	28 (82.4)	23 (100)	102	
/IgM	Equivocal	0	1 (2.9)	0	1	0.128
/IgNI	Positive	11 (17.7)	5 (14.7)	0	16	
HCV	Negative	56 (90.3)	29 (85.3)	23 (100)	108	
HSV	Equivocal	1 (1.6)	1 (2.9)	0	2	0.464
/IgM	Positive	5 (8.1)	4 (17.4)	0	9	

<sup>\*=</sup>significant (p<0.05)

A significant difference was noticed between group A and group C (p<0.001) concerning acute infection with T.gondii and in acute infection with CMV (p<0.05); but no significant

difference (p>0.05) in the mean values of ODs was noticed with the other infections and groups of patients (Table2).

Table 2: Comparison between positive TORCH infections in the studied groups.

Variable	Groups	n=119	MeanOD readingngs ± SE	F test  P value	Sig. betwo	een
				1 value	groups	P value
Tandii	A	62	$0.55 \pm 0.04$		A –B	0.914
T.gondii IgG	В	34	$0.05 \pm 0.54$	>0.05	A –C	0.814
igG	C	23	$0.06 \pm 0.53$		В-С	0.899
Tandii	A	62	$0.05 \pm 0.74$	>0.05	A –B	0.608
T.gondii IgM	В	34	0.06±0.65		A –C	0.000**
1givi	C	23	$0.03 \pm 0.53$		В-С	0.317
D 1 11	A	62	$0.04 \pm 0.59$		A –B	0.151
Rubella	В	34	0.06 ±0.69	>0.05	A –C	0.493
IgG	C	23	$0.08 \pm 0.53$	1	В-С	0.080
Deele elle	A	62	$0.04 \pm 0.45$		A –B	0.837
Rubella	В	34	$0.04 \pm 0.43$	>0.05	A –C	0.315
IgM	C	23	$0.03 \pm 0.51$		В-С	0.284
CNAV	A	62	$0.03 \pm 0.57$		A –B	0.275
CMV	В	34	$0.04 \pm 0.51$	>0.05	A –C	0.619
IgG	C	23	0.06±0.53		В-С	0.678

CMV	<b>A 62</b> $0.05 \pm 0.64$			A <b>–</b> B	0.808	
CMV	В	34	$0.06 \pm 0.58$	>0.05	A –C	0.029*
IgM	C	23	$0.04 \pm 0.47$		<b>B</b> –C	0.388
*****						
HCV	A	62	$0.04 \pm 0.51$		A <b>–</b> B	0.925
HSV IgM	A B	62 34	$0.04 \pm 0.51$ $0.06 \pm 0.48$	>0.05	A –B A –C	0.925 0.999

<sup>\*=</sup> significant different (p<0.05); \*\*= highly significant different (p<0.01); SE= standard error.

incidence The of **TORCH** infection in the first; second trimester abortion and control was compared. There was no significant difference (p>0.05) in the mean values of infection by*T.gondii* (IgG); (IgG); HSV (IgM), between 1st or 2nd trimester abortion and control and between 1st and 2nd trimester abortion, there was no significant difference between infection (p>0.05)trimester of abortion, except in acute infection with CMV and T.gondii when we compared between first trimester abortion and control, as shown in table 3.

In addition, it was found marginally significant difference  $(0.05 \le p \le 0.1)$  in the mean of acute and chronic infection of Rubella between first and second trimester abortion and when compared between first trimester abortion and controls. Furthermore, this study showed a significant difference (p<0.05) in the mean of acute infection of CMV between first trimester abortion  $(0.7\pm0.06)$ and control  $(0.5\pm0.04)$ .

Table 3: Comparison between TORCH infection in first and second trimester abortion and control.

X7	C	110	Mean OD ±	F test	Sig. between	groups
Variable	Group	n=119	SE	p value	groups	P value
T. gondii	1st	53	0.7±0.06		1st -2nd	0.786
(IgM)	2nd	43	0.7±0.05	>0.05	1st -C	0.017*
	C	23	0.5±0.03		2nd – C	$0.079^{a}$
CMV	1st	53	0.7±0.06		1st -2nd	0.138
(IgM)	2nd	43	$0.6\pm0.06$	>0.05	1st -C	0.028*
	C	23	0.5±0.04		2nd – C	0.334
Rubella	1st	53	0.7±0.04		1st -2nd	$0.086^{a}$
(IgG)	2nd	43	0.6±0.05	>0.05	1st -C	0.082a
	C	23	0.5±0.08		2nd – C	0.750
Rubella	1st	53	0.6±0.05		1st -2nd	0.062a
(IgM)	2nd	43	0.5±0.03	>0.05	1st -C	0.073 <sup>a</sup>
	C	23	0.4±0.03		2nd – C	$0.078^{a}$

1st= first trimester abortion; 2nd=second trimester abortion;

<sup>\*=</sup>a significant difference; a= marginally significant difference. For HSV no notable results was found

#### **Discussion**

In the current study there was a significant difference (p < 0.05), in the serum level of Toxoplasma gondii among specific IgM the investigated patients groups. In Iraq, a similar result was obtained by Abbas (2002) (7), showed that 21.5% of women with first abortion have positive only IgM by ELISA test. Al-Fertosi (2006) (8) and Salman (2006) (9) showed that 19.17% of women with single or repeated abortion by using ELISA test. In addition, there is more than one T. strain with gondii difference virulence among isolates in the nature (10). This strains difference could be a potential explanation regarding to the high prevalence of toxoplasmosis.

In the present study, the relatively high frequency of toxoplasmosis in women with abortion could be due to the sample selection. The samples were collected from Al-Kadhimyia Teaching Hospital which is a reference hospital for the surrounding rural areas where they have habits in favor of acquiring toxoplasmosis by eating unwashed raw vegetables or unpadded fruits. addition, in the rural cities there is close contact with cats and consequent exposure to sporulated oocysts by ingestion of these oocvsts that contaminate soil during gardening, or eating undercooked meat contaminated with cysts. Moreover, the low level of education in the women about the risk factors for toxoplasmosis may play an important role in the high rate of infection (11).

Furthermore, in the current study showed a highly significant difference between group A and group C (p<0.001) in acute infection of T.gondii, but no significant different in the mean value between group A and B and between group B and C. It has been proposed that during pregnancy ,systemic maternial immune response is biased in favor of Th2 cytokine

(12,13). Moreover, Th2 cytokines pattern of pregnancy induces the susceptibility to toxoplasmosis infection together with risk of placental infection and congenital transmission (14). Evidence from murine and human pregnancy showed that since Th1 type cytokine mediated pregnancy loss, a shift towards Th1-type immunity during T.gondii infection may help to explain (15,16).Thus, failure pregnancy evidence considerable amount of suggests that Th1 cytokine might well be implicated in adversely affecting pregnancy, directly by interfering with trophoblast survival and function, and indirectly by activating cell-mediated immune effecters (17).

This study, showed a significant difference (p<0.05) in the mean value of acute infection of T.gondii between first trimester abortion and control ,and found marginally significant difference (0.05<p<0.1) in the mean of acute infection of T.gondii between second trimester abortion and control ;because when infection occurs in the first trimester, hormone levels are low and there is little Th2 bias, the chance of transmission to the fetus is low, although the chance of abortion is high (15)

Conversely, infection during the third trimester, when there is a strong Th2 bias, is unlikely to induce abortion more frequently results congenital transmission. There is very likelihood that the Th1 response induced early during T. gondii infection will induce abortion early in pregnancy. In contrast, during the late stages of pregnancy, the strong Th2 bias and the diminished NK cell, macrophage, and CD8+ T-cell function may facilitate parasite survival and increase the likelihood of congenital transmission (15). The significant difference between groups might be associated with placental blood flow, the virulence and amount of T. *gondii* acquired and the immunological ability of the mother to restrict parasitemia.

A significant difference between RSA (group A) and group C (p<0.05) in acute infection of CMV was seen but no significant different in the mean OD values between group A and B or between group B and C was noticed. There are many confounding studies about the association between CMV infection and pregnancy loss; the studies showed that HCMV can result in abortion or stillbirth  $^{(18, 19)}$ .

HCMV act as an immune modulator through elaborating an array of immune evasion strategies to avoid elimination from the host, and its viral proteins an involved in the regulation of cellular gene expression and induction of pro-inflammatory cytokine (20).

In the current study, there was no significant difference (p>0.05), in the serum level of HSV specific IgM among the three investigated groups. Lutwick *et al.*, (2006) (21) reported, that in the world about one million pregnancies occur each year in women who have been infected with HSV-2, but complications occur in only .01% to .04% of all infected pregnant women (22).

#### References

- 1. Stray-Pederson B and Stray-Pederson S. Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. *Am J Obstet Gynecol*. 1984; 148: 140-151.
- **2.** Ogasawara M, Aoki K, Okada S, and Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *Fertil Sterilt*. 2000; 73:300–4.
- **3.** Griebel CP, Halovrsen J, Golemon T B, and Day A A. Management of spontaneous abortion. *AAFP*.2005; 72(7).
- **4.** Stern J J, Cerrillo M, Dorfmann A D, Coulam C B, and Gutierrez-Najar A J. Frequency of abnormal karyotypes among abortuses from women with and without a history of recurrent spontaneous abortion. *Fertil Steril*. 1996; 65:250 –3.
- **5.** Morton N E, Chiu D, Holland C, Jacob P A, and Pettay D. Chromosome anomalies as predictors of recurrent risk for spontaneous

- abortion. Am J Med Genet. 1987; 28:353-60.
- **6.** Campbell S and Lees C. Perinatal infections in obstetrics by ten teachers. 17<sup>th</sup>(ed). Arnold. London. 2000. pp.219-241.
- 7. Abbas M M. Seroepidemiological study on toxoplasmosis among women with history of abortion. M.Sc. thesis. College of Medicine, Al-Nahrain University. 2002.
- **8.** Al-Fertosi R B. Possible cellular expression of IFN-γ and IFN-γ R1 (CD119) in aborted women infected with *Toxoplasma gondii*. M. Sc. Thesis, Coll. Med., Univ. AL-Nahrain. 2006.
- **9.** Salman S L. Correlation between apoptosis and Toxoplasma in abortion induction: relevance of TUNEL assay and caspases. M. Sc. Thesis, Coll. Med., Univ. AL-Nahrain. 2006.
- **10.** Bhopale G M. Review, pathogenesis of toxoplasmosis. *Comp Immunol Microbiol Infect Dis.* 2003; 26: 213-222.
- **11.** Nash J Q, Chissel S, Jones J, Warburton F and Verlander N Q. Risk factors for toxoplasmosis in pregnant women in Kent, United kingdom. *Epidemiol Infect.* 2005; 133: 475-483.
- **12.** Wegmann T G, Lin H, Guilbert L and Mosmann T R. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today*.1993; 14:353–356.
- **13.** Marzi M, Vigano A, Tabattoni D, Villa M L, Salvaggio A, et al. Characterization of type 1 and type 2 cytokine profile in physio-logic and pathologic human pregnancy. *Clin Exp Immunol*.1996; 106: 127-133.
- **14.** Shirahata T, Muroyo N, Ohta C, Goto H and Nakane A. Correlation between increased susceptibility to primary *T.gondii* infection and depressed production of gamma interferon in pregnant mice. *Microbiol Immunol*. 1992; 36: 81-91.
- **15.** Roberts C W, Walker W and Alexander J. Sex-associated horm-ones and immunity to protozoan parasites. *Clin Microbiol Rev.* 2001; 14: 476–488.
- **16.** Denkers E Y and Gazzinelli R T. Regulation and function of T-cell-mediated immunity during T.gondii infection. *Clin Microbiol Rev.* 1998; 11: 569–588.
- **17.** Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M and Farhat B. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spont-aneous abortion. *Hum Reprod.* 2000: 15: 3: 713-718.
- **18.** Fairly J A, Baillie J, Bain M and Sinclair J H. Human cytomegalovirus infection inhibits epidermal growth factor (EGF) signaling by targeting EOF receptors. *J Gen Virol*. 2002; 83:

2803-2810.

- **19.** Fowler K B and Pass R F. Sexually transmitted diseases in mothers of neonates with congenital cytomegalovirus infection. *J Infect Dis.* 1991; 164: 259-264.
- **20.** Mocarski E S. Cytomegaloviruses and their replication. Fields Virology. 3r ed. Fields BN, Knipe DM, Howley PM, Chanock RM. Melnick JL, Monath TP, Roizman B and Straus SE eds. Lippincott-Raven, Philadelphia. 1996; pp. (2447-2492).
- **21.** Chan G, Stinski M F and Guilbert L J. Human cytomegalo-virus induced up-regulation of intercellular cell adhesion molecule- 1 on villous syncytiotrophoblasts. *Biol Reprod.* 2004; 104: 1-10.
- **22.** Lutwick L I, Seenivasan M, Marrie T, Sanders C and Cunha B A. Herpes Simplex. *eMedicine* .2006;29:section 1-10

# Glimpse on Hemostatic Changes Produced By Plasmapheresis

Zainab Mohammad Hasan <sup>1</sup>MBChB, Mayada Saleem Mahmood Al-Niami <sup>2</sup> MBChB; DCP; M.Phil, Haider Hasan Jaleel AL-Shammari <sup>3</sup>MBChB, FICMS.

#### Abstract

Background: The basic idea of aphaeresis is efficient removal of a circulating blood component, either cells (Cytopheresis) or plasma solute (plasmapheresis, plasma exchange). Thus, the treatment goal of aphaeresis is to remove the circulating cell or substance directly responsible for the disease process by automated cell separators in that ensure selectively removal of one or more of blood components from the blood and return remainder to the individual. Plasmapheresis is separation of plasma from blood cells which are returned to the body. It is accompanied by changes in many haemostatic parameters were found when two different replacement fluids were used.

*Objective:* To determine the effect of:

1. Therapeutic Plasma Exchange (TPE) on selected parameters of hemostasis with each

Replacement fluid used and comparing between the effects of the two solutions.

2. Total volume of Plasma Exchanged (PE), spacing between sessions and number of sessions on coagulation screening tests.

**Patients** and Methods: This clinicohaematological study was conducted during a period of six months, from February 2004 at the National Blood to July 2004 Transfusion Center / Baghdad & 50 patients underwent Therapeutic Plasma Exchange for various disorders for 3-12 sessions with two different replacement fluids were used & two types of automated blood cell plus separators(Haemonetics MCS Fresenius AS.TEC 204) were applied. Venous blood samples were collected immediately before & after the first session & after the last

session. Control group of 20 persons were included in this study.

**Results:** The changes in Prothrombin Time, Partial Thromboplastin Time, Thrombin Time, Fibrinogen, Platelets count, Haemoglobin and Packed Cell Volume were significant after TPE. There was no significant difference in changes in crystalloid group from that in Fresh Frozen Plasma (FFP) group. In crystalloid group, significant correlation was observed between Prothrombin Time, Activated Partial Thromboplastin Time, Thrombin Time & volume of PE /session, while spacing between sessions and the number of sessions was significantly correlated with Thrombin Time. Plasma fibrinogen concentration and platelets count were decreased in the patients included in this study.

Conclusion: There is no significant difference in changes in haemostatic system whether crystalloid or diluted FFP was used as replacement fluid. Thus, crystalloid, solution devoid from coagulation material can be used as a replacement fluid in the TPE if the volume of PE is small which will minimize the usage of blood components as a safer replacement fluid substitute.

*Key words:* fresh frozen plasma, therapeutic plasma exchange.

IRAQI J MED SCI, 2009; VOL.7 (4):47-60

#### Introduction

The primary objective of aphaeresis is efficient removal of a

<sup>1</sup>Dept. Hematology, Al- Yarmook Teaching Hospital, <sup>2</sup> Dept. pathology, College of Medicine, Al-Mustansyriah University, <sup>3</sup>Dept. Pathology, College of Medicine, Baghdad University.

Address Correspondence to: Dr. Haider Hasan Jaleel AL-Shammari .

E- mail: <a href="mailto:haideralshammari66@yahoo.com">haideralshammari66@yahoo.com</a>
Received: 9<sup>th</sup> July 2009, Accepted: 8<sup>th</sup> December 2009.

circulating blood component, either cell, (Cytopheresis) or plasma solute (plasmapheresis, plasma exchange). For most disorders, the treatment goal is to remove the circulating cell or substance directly responsible for the disease process (1, 2) (antibodies, immune complexes, abnormal RBCs, malignant WBCs, platelets, protein-bound drugs or toxins). (3) Current

automated aphaeresis instrument use microprocessor technology administer an anticoagulant, collect the treated blood, separate component either by centrifugation or by filtration, isolate the desired component or recombine the remaining component for return to the patient or donor (2). Therapeutic Plasma Exchange (TPE) is generally associated with rapid (and repeated) removal of large quantities of plasma and its associated coagulant (1, 4, 5, 6) When coagulant proteins protein-deficient replacement fluid such as albumin, saline, or colloidal starch are used, an acute fall in clotting factor activity, varying from 40% to 70% of baseline, can be observed immediately after exchange. depletion is usually associated with a prolongation in measured prothrombin time (PT) and activated partial thromboplastin time  $(PTT)^{(1,5,6)}$ . The total amount of PE, spacing in days between sessions and number of sessions in the full course of exchange are the three factors directly related to the procedure of plasmapheresis & are expected to affect the outcome of procedure especially the part related to derangement of coagulation parameters

#### Patients and Methods

From February 2004 to July 2004, a patients underwent total of 50 Therapeutic Plasma Exchange (TPE) for various disorders referred to National Center for Blood Transfusion during this study period were included. The study groups included 22 females and 28 males, with age range from 6 years to 64 years .Those patients include the followings: Myasthenia Gravis; (25) Guillian Barre Syndrome (GBS); (5) Chronic **Inflammatory** Demyelinating Polyneuropathy (CIDP) (1) Pemphigus; (1) Renal failure transplantation (1) Thrombotic Thrombocytopenic Purpura (TTP);

(1) Multiple Sclerosis (MS) & (6) Rhisoimmunized pregnants. Two types of automated blood cell separators (Haemonetics MCS + which represent an intermittent flow centrifugation technique – IFC & Fresenius AS. TEC 204 which represent the continuous flow centrifugation technique - CFC) were used in this study. No instrument specific effects on coagulation screening tests were detected in this study.

A course of Therapeutic Plasma Exchange (TPE) includes 3 to 12 sessions at 1 to 11 days interval. About 1000 ml of plasma was exchanged each session. A 9% Normal saline used as a replacement fluid for 25 patients and FFP was used for remainder (i.e. other 25 patients). Blood samples were collected from the patients before and immediately after 1<sup>st</sup> session and immediately after the last session. PT, PTT, TT, fibrinogen, platelets, Hb and PCV performed immediately.

The control group of consist of 20 age & sex matched individuals, for whom screening tests (Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), Fibrinogen (FNG), Platelets count, Haemoglobin (Hb) and Packed Cell Volume (PCV) had been performed.

#### Sampling:

#### Patient Group:

Blood samples were collected the patients before immediately after the first session and immediately after the last session. A 5 ml of venous blood was collected by clean venepuncture in two collecting tubes, 1.8 ml dispensed in plain plastic tube containing 0.2 ml of 0.11u aqueous trisodium citrate dehydrate for study. The remaining coagulation blood was dispensed in 2.5 ml ethylendiamine tetra-acetic acid (EDTA) containing tube with

concentrate of 1.5 mg EDTA per one ml of blood for determination of platelet count, Hb, PCV and antibody titration. The citrated blood was centrifuged at 2000 g for 15 minutes to get platelet poor plasma (PPP), the later was used for performing time(PT), Activated Prothrombin Partial Thromboplastin time(APTT), Thrombin time(TT) and fibrinogen level(FNG). All the tests performed at Quality Control laboratory in National Blood Transfusion Center - Iraq.

#### Control Group:

This study includes a control group of 20, age and sex matched healthy individuals. Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), Fibrinogen (FNG) had been performed. *Haematological screening tests:* 

All haematological screening tests were done on the study group include

Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), Fibrinogen (FNG), Platelets count, Haemoglobin (Hb) and Packed Cell Volume (PCV) according to the standard technical procedures recommended by the manufacture & consistent with the international references of practical hematology (22). All coagulation studies were performed immediately after blood collection.

#### Statistical analysis

Data were translated into a computerized database structure. Statistical analyses were compute assisted using SPSS ver. 10 (Statistical Package for Social Sciences).

Frequency distribution for selected variables was done first. For variables that were assumed to be normally distributed the statistical significance of mean change observed after plasmapheresis was assessed by Paired t-test, while for a non-normally distributed variable like Antibody titer

Wilcoxon signed rank test was used. The statistical significance difference in mean between the 2 groups was assessed by Independent samples t-test. The statistical significance of association between 2 categorical variables in a small sample size was assessed Fisher's exact test. P value less than the 0.05 level of significance considered was statistically significant.

Multiple regression models was used to study the independent and net effect of the total amount of plasma exchange, number and spacing between plasmapheresis sessions on the response variable, which is the change in coagulation parameters after the last session compared to baseline.

#### Result

According to the type of fluid used to replace the exchanged plasma subtracted by plasmapheresis in this study, our patients were divided into two grouped: First group (Crystalloid group): 25 patients were included & for whom the volume of the plasma subtracted by plasmapheresis was substituted by crystalloid while the second group (FFP group): 25 patients were included & for whom the Fresh Frozen Plasma (FFP) was used to substitute the volume of plasma subtracted by plasmapheresis had been utilized.

The frequency distribution of the study sample by reported reason for plasmapheresis, age, sex & the type of replacement fluid used were tabulated (See table 1 & table 2)

Table 1: Frequency distribution of the study sample with different diagnoses for

plasmapheresis in crystalloid & FFP groups.

Diagnosis (rosson for plasmonhorosis)	Crystall	oids group	FFP group		
Diagnosis (reason for plasmapheresis)	No.	%	No.	%	
Guillian Barre Syndrome	12	48	13	52	
Myasthenia Gravis	3	12	7	28	
Chronic Renal Failure	0	0	1	4	
TTP	0	0	1	4	
Pemphigus	0	0	1	4	
Polyneuropathy	3	12	2	8	
Multiple sclerosis	1	4	0	0	
Isoimmunization	6	24	0	0	
Total	25	100	25	100	

Table 2:Frequency distribution of the study sample for plasmapheresis by age and sex in crystalloid & FFP groups.

A go in years	Crystall	oids group	FFP group		
Age in years	No.	%	No.	%	
Children (<15)	3	12	0	0	
Young Adults (15-49)	17	68	20	80	
Older adults (50+)	5	20	5	20	
Gender					
Female	15	60	7	28	
Male	10	40	18	72	
Total	25	100	25	100	

In this study, patients within the crystalloid group having abnormally prolonged PT, PTT, TT after a course of TPE were as in the followings: 12(60%), 6(25%), 1(4%) respectively,

versus 11(50%) of patients showing abnormally prolonged PT with no change in PTT & TT in FFP group(See table 3)

Table 3: Abnormal prolonged coagulation times with a baseline normal value for PT, PTT & TT parameters after the first and last session of plasmapheresis in crystalloid & FFP groups.

	Crystalloids group					FFP group				
Subjects		Abnorm	ally	ally Abnormally			Abnormally		Abnormally	
with		prolonged	after	prolo	nged after		prolonge	ed after	prolonged after	
normal	Total	the first se	ession	the la	st session	Total	the first session		the last session	
parameter	No.	No.	%	No.	%	No.	No.	%	No.	%
PT	20	11	55	12	60	22	9	40.9	11	50
PTT	24	1	4.2	6	25	25	0	0	0	0
TT	25	2	8	1	4	25	0	0	0	0

On the other hand, patients within the crystalloid group of this study having abnormally low value of fibrinogen, platelets count, Hb and PCV were as in the followings:

2(8%), 2(9.5%), 3(21.4) and

3(15.8) respectively versus 1(4%), 4(17.4%), 4(19%) and 7(31.8%) patients respectively in FFP group as it listed in Table 4.

Table 4: Abnormal low value for Fibrinogen, Platelet count, Hb & PCV selected parameters after the first and last session of plasmapheresis in crystalloid & FFP groups.

	Crystalloids group						FI	FP group		
		Abno	rmally low	Abnorm	ally low		Abnorma	ally low	Abnor	mally
Subjects		afte	er the first	after th	ne last		after the first		low afte	er last
With normal	Total	S	session	sess	ion	Total	sess	ion	session	
parameter	No.	No.	%	No.	%	No.	No.	%	No.	%
Fibrinogen	25	0	0	2	8	25	0	0	1	4
Platelet										
count	21	2	9.5	2	9.5	23	0	0	4	17.4
Hb	14	0	0	3	21.4	21	1	4.8	4	19
PCV	19	1	5.3	3	15.8	22	1	4.5	7	31.8

The changes in the all selective parameter (PT, PTT, TT, fibrinogen, platelets count, Hb and PCV) are significant after TPE in crystalloid group with a P value of  $\leq 0.001$  but they are minimal and many values remain with normal range. There is no significant difference in the changes in crystalloid from that in FFP group.

The underlying reasons for the inability to test all the parameters for all the groups members included in this study was due to many reasons mainly due to insufficient preliminary blood samples, needs for additional blood samples for repetition of tests due to technical errors & others with impossibility to recall the patients within the proper time limits because of poor compliance of the patients &

the real difficulties because of the current situation of the country.

In crystalloid group there is significant correlation between total amount of plasma exchanged in the full course of PE and PT, PTT, TT with a P value 0.004, 0.008 and <0.001 respectively. There is significant correlation between spacing between sessions in days and number of sessions with TT only with a P value 0.04 and 0.01 respectively. In FFP group all the three factors (total amount of PE, spacing between sessions and number of sessions) had no significant correlation with the coagulation screening tests. bleeding or thrombotic sequel reported in this study. (See table 5).

Table 5: The range and mean or median of selected variables by type of replacement fluid used.

Total amount of plasma avahanga (ml)	Crysta	alloids group	FFP group	
Total amount of plasma exchange (ml) in the full course of exchanges	(Rhesus iso- immunization)	(others)Autoimmune disease	Immune diseases	
Range	(3020 - 6050)	(628 - 5128)	(2420 - 13764)	
Mean	4953.3	2746.8	4316.7	
SE	542	293	459	
Mean amount of plasma exchanged per session (ml)				
Range	(500 - 560)	(188 - 1026)	(600 - 1966)	
Mean	512.3	602.6	849.4	
SE	10	57	54	
Duration of the full course of exchanges(days)				
Range	(30 - 112)	(5 - 11)	(4 - 30)	
Median	82	7	7	
Spacing between sessions in days				
Range	(5 - 11)	(1 - 3)	(1 - 3)	
Median	7	2	1	
Number of sessions in the full course of exchange				
Range	(6 - 12)	(3 - 7)	(4 - 12)	
Median	11	5	5	

# Coagulation screening tests

## Control group:

A 20 age and sex matched control group were selected as volunteers. Their PT ranged between 12-14 seconds, PTT ranged between 30-40 second, the TT ranged between (13-21 sec.) while fibrinogen ranged between (1.5-4.0 g/L). These represented a normal values of the control group to which the study groups were compared statistical calculation whether significant or not.

## Study group:

The changes in the PT after plasmapheresis after first and last session are shown in Table 6.

Table 6: The change in PT after plasmapheresis after the first (acute change) and last session (chronic change) in crystalloid & FFP groups.

PT (seconds)	Baseline	After the first	Acute change	After the last session	_	after the last mpared to
		session			First session	(Chronic change) - baseline
Crystalloid group						
Range	(11 to 16)	(12 to 17)	(0  to  4)	(12 to 18)	(-2 to 3)	(-1 to 6)
Mean	13.4	14.9	1.5	15.3	0.4	1.9
SE	0.25	0.27	0.2	0.35	0.3	0.39
*P (Paired t-test)			< 0.001		$0.22^{[NS]}$	< 0.001
FFP group						
Range	(12 to 16)	(13 to 18)	(0  to  3)	(13 to 17)	(-2 to 2)	(0  to  3)
Mean	13.3	14.8	1.4	14.6	-0.1	1.3
SE	0.21	0.28	0.14	0.25	0.2	0.17
*P (Paired t-test)			< 0.001		$0.56^{[NS]}$	< 0.001
**P (Student's t-test)=	$0.72^{[NS]}$		$0.81^{[NS]}$		$0.18^{[NS]}$	$0.2^{[NS]}$

<sup>\*</sup> Paired t-test for the statistical significance of mean change after plasmapheresis.

In both crystalloid group and FFP group the mean of acute change was increased by 1.5s versus 1.4s, while the mean of chronic change was 1.9s versus 1.3s, these changes are statistically significant [P=<0.001] however they are clinically not significant.

The mean of acute & chronic changes in PT in the crystalloid group was not significant from that of FFP group with P value = 0.81, 0.2 respectively

The mean of acute change and chronic change in PTT in the crystalloid group was not significant

from that of FFP group with P value =0.07, 0.09 respectively. In crystalloid group the relative frequency of subject in whom the PTT increased by more than 7s after last session compared to baseline was 5 (20%) (3 patients PTT increased by 8s, 1 patient by 9s and 1 patient by 10s). Patients who received FFP as replacement fluid show no significant increase in the PTT. The change in PTT after plasmapheresis after first (acute change) and last session (chronic change) in both crystalloid group and FFP group are shown in Table 7.

<sup>\*\*</sup>Independent samples t-test for the statistical significance of difference in mean between the 2 groups.

Table 7: Change in PTT after plasmapheresis after the first (acute change) &

last session (chronic change).

PTT (seconds)					Change after the last session compared to	
	Baseline	After the firstsession	Acute change	After the last session	First session	(Chronic change) baseline
Crystalloids group						
Range	(30 to 41)	(33 to 42)	(-1 to 4)	(31 to 45)	(- 4 to 7)	(-4 to 10)
Mean	34.2	36.2	2	37.6	1.4	3.4
SE	0.59	0.5	0.27	0.71	0.61	0.74
*P (Paired t-test)			< 0.001		0.031	< 0.001
FFP group						
Range	(32 to 37)	(33 to 40)	(0  to  3)	(34 to 39)	(-1 to 2)	(0  to  3)
Mean	34.2	35.6	1.4	35.8	0.2	1.6
SE	0.29	0.33	0.21	0.23	0.19	0.15
*P (Paired t-test)			< 0.001		0.31 <sup>{N S}</sup>	< 0.001
**P (Student's t-test)=	$0.95^{[NS]}$		$0.07^{[NS]}$		$0.07^{[NS]}$	$0.09^{\{N S\}}$

<sup>\*</sup> Paired t-test for the statistical significance of mean change after plasmapheresis.

In both crystalloid and FFP group the mean of acute change was increased by 2s versus 1.4s, while the mean of chronic change was 3.4s versus 1.6s, these changes statistically significant with a P value of <0.001 however they are clinically not significant.

The change in TT after plasmapheresis after first (acute change) and last session (chronic change) in both crystalloid group and FFP group are shown in Table 8.

Table 8: The change in TT after TPE after the first (acute change) and last session (chronic change).

		After		After	Change after the lass session compared to	
		the first	Acute	the last	First	baseline
TT (seconds)	Baseline	session	change	session	session	
Crystalloids group						
Range	(11 to 21)	(13 to 22)	(0  to  4)	(12 to 22)	(-3 to 5)	(-3 to 8)
Mean	15	16.4	1.5	17.6	1.1	2.6
SE	0.54	0.53	0.17	0.5	0.49	0.57
*P (Paired t-test)			< 0.001		0.032	< 0.001
FFP group						
Range	(11 to 18)	(12 to 20)	(0  to  3)	(13 to 21)	(-1 to 4)	(0 to 6)
Mean	13.4	14.8	1.4	15.6	0.8	2.2
SE	0.28	0.33	0.15	0.48	0.28	0.33
*P (Paired t-test)			< 0.001		0.008	< 0.001
**P (Student's t-						
test)=	0.012		$0.86^{[NS]}$		$0.57^{[NS]}$	$0.59^{[NS]}$

<sup>\*</sup> Paired t-test for the statistical significance of mean change after plasmapheresis.

<sup>\*\*</sup>Independent samples t-test for the statistical significance of difference in mean between the 2 groups

<sup>\*\*</sup>Independent samples t-test for the statistical significance of difference in mean between the 2 groups.

In both crystalloid and FFP groups the mean of acute change was increased by 1.5s versus 1.4s, while the mean of chronic change was 2.6s 2.2s, these changes versus statistically significant with P value<0.001 however thev are clinically not significant. The mean of acute and chronic changes in TT in the crystalloid group was not significant from that of FFP group with P value =0.86 and 0.59 respectively.

In crystalloid group the relative frequency of subject in whom the TT

increased by more than 5s after last session compared to baseline was 5 (20%) patients (2 patients TT increased by 6s, 2 by 7s and 1 patient by8s), while in FFP group 2 patients increased more than 5s (2 patients increased by 6s). Table 3-12

The change in fibrinogen after plasmapheresis after first (acute change) and last session (chronic change) in both crystalloid group and FFP group are shown in Table 9.

Table 9: Change in fibrinogen concentration after TPE after the first (acute change) and last session (chronic change).

		After		After	Change after the last session compared to	
Fibrinogen conc.		the first	Acute	the last	First	
(gm/L)	Baseline	session	change	session	session	Baseline
Crystalloid group						
Range	(1.9 to 3.7)	(1.8 to 3.3)	(-0.7to -0.1)	(1.4 to 2.8)	(-1.1to -0.1)	(-1.7 to -0.2)
Mean	3	2.6	-0.4	2.1	-0.5	-0.9
SE	0.09	0.08	0.03	0.07	0.05	0.07
*P (Paired t-test)			< 0.001		< 0.001	< 0.001
FFP group						
Range	(1.9 to 3.7)	(1.6 to 3.4)	(-0.6 to 0.7)	(1.4 to 3.1)	(-1.9 to -0.1)	(-2.1 to 0)
Mean	2.9	2.7	-0.2	2.2	-0.5	-0.7
SE	0.11	0.09	0.06	0.08	0.08	0.09
*P (Paired t-test)			< 0.001		< 0.001	< 0.001
**P (Student's t-test)=	$0.82^{[NS]}$		$0.1^{[NS]}$		$0.94^{[NS]}$	$0.36^{[NS]}$

<sup>\*</sup> Paired t-test for the statistical significance of mean change after plasmapheresis.

In both crystalloid and FFP group the mean of acute change was reduced by 0.4gm/l versus 0.2gm/l respectively while the mean of chronic change was reduced by -0.9 versus -0.7, these changes are statistically significant with P value<0.001 however they are clinically not significant. The mean of acute and chronic change in fibrinogen

in the crystalloid group was not significant from that of FFP with P valve of 0.1 and 0.36 respectively.

The change in platelets count after plasmapheresis after first (acute change) and last session (chronic change) are shown in Table 10.

<sup>\*\*</sup>Independent samples t-test for the statistical significance of difference in mean between the 2 groups

Table 10: Change in Platelets count after TPE after the first (acute change) & last

session (chronic change).

					Change after the last session compared to		
Platelets count		After the	Acute	After the	First		
(X1,000,000,000/L)	Baseline	first session	change	last session	session	Baseline	
Crystalloid							
group							
Range	(121 to 477)	(113 to 440)	(-97 to -7)	(103 to 401)	(-134 to -3)	(-159 to -11)	
Mean	295	263.9	-31.1	220.6	-43.3	-74.4	
SE	17.18	15.3	4.34	14.04	5.65	6.67	
*P (Paired t-test)			< 0.001		< 0.001	< 0.001	
FFP group							
Range	(132 to 403)	(120 to 388)	(-87 to -8)	(106 to 310)	(-150 to -13)	(-209 to -26)	
Mean	284.8	252.5	-32.4	202.8	-49.7	-82	
SE	13.76	11.94	3.96	10.07	7.19	9	
*P (Paired t-test)			< 0.001		< 0.001	< 0.001	
**P (Student's t-	$0.65^{[NS]}$		$0.83^{[NS]}$		$0.49^{[NS]}$	$0.5^{[NS]}$	
test)=	$0.65^{11.07}$		$0.83^{11.03}$		$0.49^{110}$	$0.5^{i \cdot i \cdot j}$	

<sup>\*</sup> Paired t-test for the statistical significance of mean change after plasmapheresis.

In both crystalloid and FFP group the mean of acute change was reduction by  $-31.1 \times 10^9 / L$  versus  $-32.4 \times 10^9 / L$ , while the mean of chronic change was a reduction by  $-74.4 \times 10^9$ /L versus - $82\times10^9$ /L, these changes are statistically significant with a P value<0.001 clinically however they are significant. The mean of acute and

chronic change in platelet count in the crystalloids group was not significant from that of FFP with a P value of 0.85 and 0.5 respectively.

The change in Hb concentration after plasmapheresis after first (acute change) and last session (chronic change) are shown in Table 11.

Table 11: Change in blood Hb concentration after TPE after the first (acute change)

& last session (chronic change).

		x lust session (	(0111 01110 011	<del></del>		
						ge after the on compared to
Blood Hb conc. (gm/L)	Baseline	After the first session	Acute change	After the last session	First session	Baseline
Crystalloid group						
Range	(104 to 154)	(103 to 155)	(-3 to 3)	(94 to 151)	(-27 to 1)	(-27 to 1)
Mean	127.1	126.8	-1.8	122.7	-4.1	-4.4
SE	3.38	3.32	0.26	3.68	1.26	1.21

<sup>\*\*</sup>Independent samples t-test for the statistical significance of difference in mean between the 2 groups

*P (Paired t-test)			0.003		0.003	0.001
FFP group						
Range	(71 to 157)	(68 to 157)	(-11 to 4)	(65 to 154)	(-7 to 1)	(-15 to 4)
Mean	133.6	132.2	-1.5	129.7	-2.5	-4
SE	3.76	3.93	0.49	3.99	0.42	0.72
*P (Paired t-test)			0.006		< 0.001	< 0.001
**P (Student's t-	ING		(M.C.)		INCI	ING
test)=	$0.2^{[NS]}$		$0.35^{(N S)}$		$0.22^{[NS]}$	$0.76^{[NS]}$

<sup>\*</sup> Paired t-test for the statistical significance of mean change after plasmapheresis.

In both crystalloid and FFP groups the mean of acute change was reduction by -0.3gm/L versus -1.5gm/L while the mean of chronic change was reduction by - 4.4gm/L versus -4gm/L, these changes are statistically significant with a P value<0.001 however they are clinically not significant.

The mean of acute and chronic change in Hb concentration in the crystalloid group was not significant from that of FFP with P value of 0.35 and 0.76 respectively.

The change in PCV after plasmapheresis after first (acute change) and last session (chronic change) are shown in Table 12.

Table 12: The change in PCV after plasmapheresis after the first (acute change) and last session (chronic change).

			,	<b>3</b> /	Change after the last session compared to		
		After the	Acute	After the	First		
PCV L/ L	Baseline	first session	change	last session	session	Baseline	
Crystalloid							
group							
Range	(0.33 to 0.55)	(0.32 to 0.52)	(-0.03 to 0.04)	(0.28 to 0.48)	(-0.1 to 0.02)	(-0.13 to 0.01)	
Mean	0.404	0.4	-0.004	0.38	-0.019	-0.023	
SE	0.0113	0.0118	0.0034	0.0105	0.0056	0.0062	
*P (Paired t-test)			$0.25^{[NS]}$		0.002	0.001	
FFP group							
Range	(0.22 to 0.55)	(0.2 to 0.55)	(-0.06 to 0.02)	(0.18 to 0.5)	(-0.05 to 0.01)	(-0.09 to -0.01)	
Mean	0.429	0.416	-0.014	0.392	-0.024	-0.037	
SE	0.013	0.0137	0.0033	0.0131	0.0032	0.0036	
*P (Paired t-test)			< 0.001		< 0.001	< 0.001	
**P (Student's t-	DIGI		(1.6)		DIGI	raigi.	
test)=	$0.14^{[NS]}$		$0.9^{(N S)}$		$0.5^{[NS]}$	$0.06^{[NS]}$	

<sup>\*</sup> Paired t-test for the statistical significance of mean change after plasmapheresis.

<sup>\*\*</sup>Independent samples t-test for the statistical significance of difference in mean between the 2 groups

<sup>\*\*</sup>Independent samples t-test for the statistical significance of difference in mean between the 2 groups

In both crystalloid and FFP group the mean of acute change was reduction by -0.004L/L versus -0.014L/L while the mean of chronic changes was reduction by -0.023L/L versus -0.037L/L, these changes are statistically significant with P value≤0.001 however they are clinically not significant (except acute change in crystalloid group which is statistically and clinically not significant). The mean of acute and chronic change in PCV in the crystalloid group was not significant from that of FFP with P value of 0.9 and 0.06 respectively.

#### **Discussion**

In study, small volume plasmapheresis (about 500 – 1000 ml / session) was selected instead of a more extensive procedure (i.e. the volume of plasma to be exchanged is more than 1000 ml / session) because this involves minimal risks for patients. A 25 patients received fluids devoid from coagulation protein ((0.9 % Normal Saline(NS)) as in other studies <sup>(4-12)</sup> and the other 25 patients received diluted FFP (FFP/NS 1:1) as in many studies (13-17) TPE is generally associated with rapid and repeated removal of large quantities of plasma and its associated coagulant protein. When replacement fluid which is deficient in coagulation protein is used a fall in clotting factors activities can be observed immediately after the exchange. (1, 18) In this study, depletion of coagulation proteins was observed as a slight prolongation in PT, PTT, TT

The prolongation in coagulation screening tests in crystalloid group (e.g. Rh- isoimmunization) was higher but not significant from that in FFP. These minimal changes in coagulation screening tests are most likely due to small volume of PE which is the usual practice at the national blood transfusion center.

In this study, the range of exchanged plasma volume per a session of TPE in Rh isoimmunization – crystalloid group is from 500 to 560 ml). These findings agree with Mark E. Brecher study (1) as the prolongation is slight in the measured PT, PTT and TT, although such values frequently remain within normal range. The total amount of PE, spacing in days between sessions and number of sessions in the full course of exchange are the three factors directly related to the procedure of plasmapheresis & expected to affect the outcome of procedure especially the part related to derangement of coagulation parameters (19) In crystalloid group, significant correlation was found between total amount of PE in the full course of plasmapheresis and the coagulation screening tests. These findings are mostly due to increase loss of coagulant proteins with increasing total amount of PE, while spacing between sessions in days and number of sessions show significant correlation with TT only.

These findings could be due to the direct relation between TT and fibrinogen level <sup>(6)</sup>.In FFP group, all the three factors (total amount of plasma exchange, spacing between session and number of sessions) have no significant effect on coagulation screening tests (PT, PTT and TT) because there is partial replacement of coagulation material (20, 21) .In this study, it has been found that there is a significant reduction in fibrinogen level in the crystalloid and FFP group. This has been demonstrated by the study of Domen et al <sup>(6)</sup>, Flum et al <sup>(9)</sup> and Chrinside et al <sup>(11)</sup> as they demonstrated that fibrinogen was efficiently depleted by plasma exchange and its removal was maximal, where as less efficient removal was seen for other factors. In this study, the consequence of PE is a reduction in

circulating platelet but with no significant difference between crystalloid and FFP .This is consistent with the finding of Bracher et al <sup>(1)</sup> and Strobel <sup>(3)</sup>.

The current study shows a minimal reduction in PCV/Hb after a course of PE and this could be due to using of developed machines, small volume PE & replacement fluid. The vascular access was obtained from large veins, the free flow of blood from obstruction, the adequately needle bore diameter & the use of FFP & isotonic solution (0.9 % Normal Saline) as a replacement solution. All these measures diminished the risk of hemolysis & dilution. This is consistent with the findings of Foke et al (23) & Susan et al (24).

In addition to that, no hemorrhage was observed during or after PE in the patients of this study even after repeated plasmapheresis at short intervals.

It was concluded that Crystalloid, solution devoid from coagulation material can be used as a replacement fluid in the TPE if the volume of PE is small; in crystalloid group, significant correlation was observed between PT, PTT, TT and volume of PE/session, while spacing between sessions and the number of sessions was significantly correlated with TT.

Plasma fibrinogen concentration and platelets count were decreased acutely & over the period of the study, however it was clinically not significant.

There was a minimal reduction in PCV/Hb after a course of PE .

#### References

- **1.** Mark E. Brecher: Blood Banking and Transfusion Medicine, 1<sup>st</sup> Edition, Helen G. Jones, 2003; chapter 43:509-518.
- **2.** Anderson Ness: Scientific Basis of Transfusion Medicine, 2<sup>nd</sup> Edition, HarveyG.Klein,2000;chapter39:553-568.
- **3.** Academic press: Handbook of Transfusion medicine, 1<sup>st</sup> Edition, Frank J. Strobe, 2001; chapter 37: 315-322.

- **4.** Orlin J B, Barkman E M. Partial plasma exchange using albumin replacement: removal and recovery of normal plasma constituents. Blood, 1980; 56: 1055-1059.
- **5.** Sultan Y, Bussel A, Maisonneuve P. Potential danger of thrombosis after plasma exchange in the treatment of patient with immune disease. Transfusion, 1979; 19(5): 588-593.
- **6.** Domen R E, Kennedy MS, Jones L L. Hemostatic imbalance produced by plasma exchange. Transfusion, 1984; 24: 336-339.
- **7.** Lasky LC, Finnerty E P. Protein and colloid osmotic pressure changes with albumin and/or saline replacement during plasma exchange. Transfusion, 1984; 24: 256-259
- **8.** Al-Omari WR. Improved fetal survival with small volume plasmapheresis in Rhesus disease. Int. J. Gynecology and Obstetric., 1989; 30: 237-240.
- **9.** Morris A, Richard A, Frederick R. The hemostatic imbalance of plasma-exchange transfusion. Blood, 1979; 54: 694-702.
- **10.** Manno E, Bonadeo f, Parigi L, The hemostatic balance after plasma exchange transfusion in Myasthenia Gravis. Minerva Anestesiol, 2000; 66(6): 461-465.(Abstract)
- **11.** Ann Chirnside, Urbaniac S J, Prowse CV. Coagulation abnormalities following intensive plasma exchange on the cell separator. British Journal of Hematology, 1981; 48: 627-634.
- **12.** Ibrah: Tek. Effect of replacement fluid of plasma viscosity used for therapeutic plasma exchange. Therapeutic apheresis and dialysis 2004-April; 8(2): 144 (Abstract)
- **13.** Rosenkvist J, Berkowicz A, Holsoe E. Plasma exchange in Myasthenia gravis complicated with complement activation and urticurial reactions using fresh frozen plasma as replacement solutions. Vox Sang. 1984; 46: 13-18.
- **14.** Barclay GR, Ayoub Greiss M, Urbaniak S J. Adverse effect of plasma exchange on anti-D production in rhesus immunization owing to removal of inhibitory factors. Br. Med. J., 1980; 280: 1569-1571.
- **15.** French cooperative group on plasma exchange. Efficiency of plasma exchange in Guillian-Barre syndrome and role of replacement fluid, Ann Neurol, 1987; 22: 723-761.
- **16.** Fraser I D, M O, JE, Airth GR. Intensive antenatal plasmapheresis in severs Rhesus isoimmunization. Lancet, 1976; 3: 6-8.
- **17.** Jarl Eklund. Intensive plasma exchange as an adjunct to management to severe rhesus disease. Acta Obstet Gynecol, 1985; 64: 7-10.
- **18.** Mark E, Brecher: Blood Banking and Transfusion medicine, 1<sup>st</sup> Edition, Bruce C. McLeod, 2003; chapter 44: 519-543.

- **19.** Volkin RL, Starz TW, R K, Lewis JH. Change in coagulation factors, complement, immunoglobulins and immunocomplex concentrations with plasma exchange. Transfusion, 1982; 22: 54-58.
- **20.** Reimann P M and Mason P D. Plasmapheresis technique and complications. Intensive Care Medicine, 1990; 16:3-10.
- **21.** Jeffrey Mc Cullough, Micheal Chopek. Therapeutic plasma exchange.Laboratory Medicine, 1981; 12: 745-753.
- **22.** Bain B J, Laffan M A , Bradshhaw AE, Waters A H. Dacie and Lewis, Practical hematology, 1995;  $8^{th}$  edition; 49-82, 297-350, 445-463
- **23.** Lewis M S, Barbara J. Bain: Dacie and Lewis, Practical hematology, 2001; 9<sup>th</sup> edition; 339-390.
- **24.** Wing Wai Fok & Shiu-Chun Wu, Hemolysis in Plasmapheresis, Acta Nephrologica, 1996; 10: 16-21.
- **25.** Deanna M. Hines; Guidelines for Therapeutic Hemapheresis, 1993-1994 committee.

# Role of imprint cytology in breast lesions.

Hassanain H.Khudier<sup>1</sup> MBChB; FIBMSpath, Tahir A Hawramy<sup>2</sup> MBChB; DGS; CABS, Goran M Abdul-Qadir<sup>1</sup> MBChB.

#### Abstract

**Background:** Rapid cytological diagnosis of various tumors and especially that of breast was first introduced by Dudgeon and Patrick 1927. The accuracy of the imprint method has been increasing over the years both in breast pathology and in other body sites, indeed the average accuracy of (90-94%) in the past has reached (97-98%) in recent years.

*Objective:* A prospective study was performed to determine the value of this technique in the diagnosis of male and female patients with various breast lesions.

*Methods:* From June 2005 and February 2006 Imprint cytology was obtained from (110) specimens of (107) patients with various breast lesions in Sulaimania teaching hospital.

Cytological examinations of imprints stained by hematoxiline and eosin stain were examined and compared to histological results to detect its sensitivity, specificity and accuracy rate.

**Results:** The sensitivity and specificity of imprint cytology for both benign and malignant

breast lesions were 96.3% and 100% respectively; while over all accuracy was 98.9%. False negative diagnosis was seen in a single case of Paget's disease of the nipple (0.9%); however no false positive cases were found

The cytological diagnosis were malignant in 26 cases (23.7%), including (25) primary malignant tumors and (1) metastatic carcinoma, benign diagnoses were encountered in 71 cases (64.5%), suspicious in 4 cases (3.6%) and unsatisfactory in 9 cases (8.2%).

**Conclusion:** Imprint cytology is simple, rapid, inexpensive and accurate method for intraoperative diagnosis of breast lesions and can be used as adjunct to frozen section.

Key words: Imprint cytology, breast lesion

IRAQI J MED SCI, 2009; VOL.7 (4):61-66

#### Introduction

Rapid cytological diagnosis of various tumors and especially that of breast was first introduced by Dudgeon and Patrick 1927 through examination of fresh tissue by the wet film method <sup>(1)</sup>.Since that several series describing imprint cytology of the breast have been published; the acceptance and development of imprint cytology have been hampered because of concerns about its diagnostic accuracy in comparison to frozen section technique. Recent reviewed articles reveal that good correlation exists between imprint

<sup>1</sup>Dept. Pathology, College of medicine, University of Sulaimani, <sup>2</sup>Dept. Surgery, College of medicine, University of Sulaimani. Address Correspondence to: Dr. Hassanain H.Khudier

Institute: Sulaimania teaching hospital

E-mail: <a href="mailto:hhkpath@yahoo.com">hhkpath@yahoo.com</a>

Received: 20<sup>th</sup> July 2008, Accepted: 8<sup>th</sup>

December 2009

cytology (IC) diagnoses and corresponding final histology, moreover there are several reports on malignancy cases of correctly diagnosed by IC but falsely called negative on frozen section (FS) analysis (2). Intraoperative imprint smears like frozen sections helpin on diagnosis, wherein the fine needle (FNAC) aspiration cytology inconclusive or suspicious (3). accuracy of the imprint method has been increasing over the years both in breast pathology and in other body sites, indeed the average accuracy of (90-94%) in the past has reached (97-98%) in recent years <sup>(4)</sup>.

The technique of imprint cytology is accurate, simple, rapid,cost-effective and do not require any special instrument, in contrast to frozen section which is more time-consuming, required specialized equipment, need

well trained histopathologists, expensive and may not be always available (5-10).

The aim of this study is to assess the role of imprint cytology in the diagnosis of various breast lesions in comparison to the histopathological diagnoses.

#### Materials and methods

From June 2005 to Feb 2006, all patients who were admitted to the surgical ward of Teaching, Shorish and some private hospitals in Sulaimani city for excisional biopsy of a breast mass or for mastectomy were included in this study.

Imprint cytology was done on freshly removed tissue inside the theater; the suspected area was sliced into several pieces. For small mass, it was bisected, the freshly cut surface of tissue is then imprinted onto a clean glass slide. For large masses, the portion of tissue used for imprinting was trimmed to approximately (1 cm) in diameter, and the same above procedure is repeated (11).

The slides were immediately fixed in 95% ethyl alcohol, and then divided into two groups. The first group was withdrawn from the fixative after 10-20 seconds and preceded for rapid H&E stain. The second group remained in fixative (20-30 minutes) for traditional H&E cytological staining. The same piece of tissue used for imprinting was then fixed in 10% formalin for paraffin sectioning and histopathological examination. The first group was stained by the rapid method described by Scopa et al (12) while the second was stained by the routine H&E procedure described by Gubin (13).

The lesions were cytologically classified into four groups:1) Unsatisfactory: contained 4-6 clusters on the entire slide 2) Benign: If they contained cohesive clusters of epithelial cells without atypia 3)Suspicious: presence of atypical cells in a three

dimensional clusters 4) Malignant: when there is sufficient number of malignant cells<sup>(14-16)</sup>.

Statistical analysis: The clinical efficacy and diagnostic accuracy of imprint cytology was done by using sensitivity, specificity, positive value predictive (PPV), negative predictive value (NPP), and accuracy rate were measured. For statistical analysis all unsatisfactory and suspicious cases were excluded.

#### Results

One hundred and ten specimens, surgically removed from (107) patients including (96) specimens from females and (14) from males. The specimens were (93) exicional biopsies and (17) mastectomy specimens (15 from females, and 2 from male patients). We have (6) masses from (3) female patients with bilateral lumpectomy. There were 14 males (12.7%), and 96 (87.3%) females, with female to male ratio of 6.9:1.

Table (1) shows age distribution for patients with histologically benign and malignant breast lesions. The age ranged from 15 to 75 years with a mean of 36 years .Most benign lesions were in the second decade while most of the malignant tumors were in the fourth decade of life.

Table (2) shows the histological and cytological results. Histological results were 81(73.6%) benign and 29 (26.4%) were malignant however cytological diagnosis was benign in 71(64.5%), malignant in 26 (23.6%), suspicious in 4 (3.6%), and 9 (8.2%) cases were unsatisfactory.

In comparison with histological diagnosis the sensitivity and specificity of imprint cytology were (96.3%) and (100%) respectively, PPV was 100% and NPV was 98.6% and accuracy was 98.9% as represented in Table (3). There was no difference found between the results of the rapid and the routine

haematoxylin and eosin staining methods.

previously reported studies are shown in table (4).

The comparison of the current study imprint cytology results with that of

Table 1: Age distribution of patients according to histological diagnoses.

A 22 2401142	Les	Total	
Age groups	Benign	Malignant	
1019	11	0	11(10%)
2029	28	1	29(26.4%)
3039	16	7	23(21%)
4049	19	8	27(24.5%)
5059	6	5	11(10%)
6069	1	6	7(6.7%)
7079	0	2	2(1.8%)
Total	81	29	110(100%)

Table 2: Correlation between cytology and histological diagnoses of breast lesions

Histological		Cytological diagnosis					
diagnosis			Positive	Un	Total		
uragnosis	Negative cases	Suspicious cases	cases	satisfactory			
	70	2	0	9	81		
Benign	(86.4%)	(2.5%)	(0%)	(11.1%)	(100%)		
	1	2	26	0	29		
Malignant	(3.4%)	(7%)	(89.6%)	(0%)	(100%)		
	71	4	26	9	110		
Total	(64.50%)	(3.60%)	(23.70%)	(8.20%)	(100%)		

Table 3: Statistical analysis of all breast lesions

	<b>y</b>
Statistical tests	No. of cases
Histological diagnoses	110
Imprint cytological diagnoses	97
Unsatisfactory cases	9
True positive (TP)	26
True negative (TN)	70
False positive (FP)	0
False negative (FN)	1
Deferrals (suspicious)	4
Predictive	e Values:
Sensitivity	96.3%
Specificity	100%
Positive predicted value (PPV)	100%
Negative predicted value (NPV)	98.6%
Accuracy	98.9%
•	

Table 4: Imprint cytology in literature compared to the present study

Authors	Patients No.	No. FP	No. FN	Sens (%)	Spec (%)	Accu (%)	Deferrals
Scopa <i>et</i> <i>al.</i> ,1990	82	0(0%)	0(0%)	100%	100%	100%	4(4.9%)
Khanna <i>et al.</i> ,1991	86	0(0%)	1(1.25%)	98.4 %	100%	98.8 %	6(6.9%)
Rosa <i>et al.</i> ,1993	407	0(0%)	7(1.72%)	97.6 %	100%	98.3 %	12(2.9%)
Veneti <i>et al.</i> ,1996	351	1(0.28%)	5(1.4%)	97.1%	99.4 %	98.3 %	7(1.99%)
Scucchi etal.,1997	1197	0(0%)	9(0.75%)	97.5%	100%	99.2 %	9(0.75%)
Albert <i>et al.</i> ,2000	173	3(1.7%)	4(2.3%)	96. 5%	90 %	95.4 %	12(6.9%)
Current study, 2006	107	0(0%)	1(0.99)	96.3%	100%	98.9%	4(3.9%)

#### Discussion

Unsatisfactory imprint cytology was found in (9) cases (8.2%), four of them were gynecomastia, two cases of lipoma, and the remaining three cases found in fibrocystic disease of the breast(two cases) and one case of fat necrosis. Since the (4) cases of gynecomastia composed entirely of fibroadipose tissue and the (3) cases of lipoma and fat necrosis composed of fat cells only they were considered as unsatisfactory, similar results were found in other studies (17-20).

While in two cases of fibrocystic disease there were no cells apart from inflammatory cells, similar findings described by Albert *et al* <sup>(14)</sup>.

There were no false positive cases in all imprints taking; similar finding was seen by other investigators (3,12,20). This is probably because of the clear morphological features, adequate samples or results enhanced by using intraoperative cytology coupled with examination of gross surgical specimens<sup>(2,10)</sup>. There was only one false negative case later appeared to be Paget's disease of the nipple, similar results found by Veneti et al (9) which explained by lack of clear cytological evidence of malignancy with either few cells that could be easily missed or small cells which difficult to distinguish from normal cells that need to be evaluated with other histological parameters.

The rate of false negative in present study was (0.99%) which is higher than Scopa *et al.* (12), and Scucchi *et al.* (2) (0% and 0.75% respectively), but lower than that reported by Khanna *et al.* (20), Veneti *et al.* (9) Rosa *et al.* (10), and Hiregoudar *et al.* (3) (1.25%, 1.4%, 1.72%, and 2.5%) respectively.

There were (4) deferral (suspicious) cases (3.9 %) in current study, which was high in comparison to Succhi *et al.* (2) (0.75 %), Veneti *et al.* (1.99%) and Rosa *et al.* (10) (2.9%) but lower than Scopa *et al.* (12), Albert *et al.* (14) and Khanna et al. (20) (4.9%, 6.9% and 6.9%) respectively.

This difference may be due to higher number of cases in the previous studies compared to the current study, this allows the pathologist to gain good experience and confidence in the technique, another reason is the use of scraping method with imprinting, this allows high number of cells to detached from the tumour hence more material to be studied, and lastly if the pathologist had any doubt in mind he always inclined to call the lesion suspicious rather than malignant <sup>(15)</sup>.

Two cases of fibroadenoma, were considered as suspicious because of florid cell population and considerable atypia, It has been found throughout this study that on imprinting surprisingly fibroadenoma a large amount of cellular materials transferred to the slide. this hypercellularity that usually associated malignant imprints was confusing element in the cytological diagnosis of these tumors, similar finding was mentioned by other authors (10, 12,21). The other two cases (invasive ductal carcinoma and Paget's disease) were considered as suspicious due to paucity of cellular material, and the lack of definite malignant characteristics (2).

The sensitivity of the method was (96.3%) which is lower than that reported by other studies (12, 20,22), this may be due to sample size as the malignant cases in current study was smaller than other studies. The high specificity(100%) which is similar to other comparative study (3,22) might be due to the preservation of cellular details, avoiding problems and artifacts of freezing in FS<sup>(23)</sup>.Creager concluded that the sensitivity&specificity of IC are similar to that of FS<sup>(24)</sup>.The accuracy of the method was (98.9%) which is slightly higher than Veneti et al. (9) (98.3%), Rosa et al. (10) (98.3) and Khanna et al. (20) (98.8%), but lower than that of Scopa et al. (12)(100%) and Scucchi et al. (2) (99.2%) .IC smears are being increasingly used to evaluate sentinel lymph node which is the first lymph node to receive lymphatic drainage from the site of the primary with high tumour sensitivity(93%)<sup>(25)</sup>and specificity

(99.2,100%)<sup>(26, 27)</sup>. Worldwide research is in progress even with regard to immunohistochemisry and DNA markers in IC. (26-28).

In conclusion Imprint cytology is simple, rapid, inexpensive and accurate method for intraoperative diagnoses of breast lesions and can be used as adjunct to frozen section. Further studies, on larger samples in comparion with FS are needed to establish the method in the common clinical practice.

#### References

- **1.** Dudgeon LC, Patrick CV. A new method for the rapid microscopical diagnosis of tumours: With an account of 200 cases so examined. Br J Surg 1927; 25:250.
- **2.** Scucchi LF, Stefano DD, Cosentino L, Vecchione A: Value of cytology as an adjunctive intraopertive diagnostic method. An audit of 2,250 consecutive cases. Acta Cytol, 1997; 41:1489-1496.
- **3.** Hiregoudar AD, Godhi AS, Malur PR, Gogeri BV, Metgud SC. Accuracy of intraoperative imprint smears in breast tumours: A study of 40 cases with review of literature. Indian Journal of Surgery; 2006; 68: 302-305.
- **4.** Liu Y, Silverman JF, Sturgis CD, Brown HG, Dabbs DJ, Raab SS: Utility of intraopertive consultation touch preparations. Diag Cytopathol, 2002; 26:329-333.
- **5.** Anstasiadis P, Koutlaki N, Liberis V: Cytomorphologic features of non specific granulomatous mastitis diagnosed by imprint cytology. Acta Cytol, 2001; 45:887-889.
- **6.** Creager AJ, Geisinger KR, Shiver SA, Perrier ND, Shen P, Shaw JA, Young PR, Levine EA: Intraoperative evaluation of sentinel lymph node for metastatic breast carcinoma by imprint cytology. Modren Pathol, 2002(a); 126:838-839.
- **7.** Creager AJ, Shaw JA, and Young PR, Geisinger KR: Intraoperative evaluation of lumpectomy margins by imprint cytology with histologic correlation .A community hospital experience .Archives of Pathology and Laboratory Medicine, 2002(b); 126 (7):846-848.
- **8.** Thomson CE, Griffiths IR.: Imprint as a rapid technique for assessing the morphology of the central nervous system by immunofluorescence. J of Neuroscience Methods, 2000; 100:85-91.
- **9.** Veneti S, Mouzaka LI, Toufexi H, Xenitides J, Anastasiadis P: Imprint cytology:

- A rapid, reliable method of diagnosing breast malignancy .Acta Cytol, 1996; 40:649-652.
- **10.** Rosa GD, Boschi R, Boscanio A, Petrrella G, Vetrani A, Palombini L, Pettinato G: Intropertive cytology in breast cancer diagnosis: Comparison between cytologic and frozen section technique .Diag Cytopathol, 1993; 9:623-631.
- **11.** Rosai J.,Rosia and Ackerman's surgical pathology,9<sup>th</sup> edition, 2004, pp(1773- 1839), Mosby, St.Louis.
- **12.** Scopa CD, Melachrinou M, Apessou D, Bonikos D: Tissue imprints in surgical pathology: A rapid intropertive diagnostic aid.Diagn Cytopathol, 1990; 6:5-7
- **13.** Gubin N: Haematoxylin and Eosin staining of FNA smears .Acta Cytol, 1985; 29:648-649.
- **14.** Albert US, Duda V., Hadji P, Goerke K, Hild F, Bock K, Ramaswamy A, Schulz KD: Imprint cytology of core needle biopsy specimens of the breast lesions. Rapid approach to detecting malignancies, with comparisons of cytologic and histopathologic analysis of 173 cases. Acta Cytol, 2000; 44:57-62.
- **15.** Blumenfeld W, Hashmi N, Sagerman P: Comparison of aspiration, touch and scrap preparation simultaneously obtained from surgical excised specimens. Acta Cytol, 1998; 42:1414-1418.
- **16.** Yeoh GP, Chan KW: Fine needle aspiration of the breast masses: An analysis of 1533 cases in private practice .HKMJ, 1998; 4:283-287.
- **17.** Tan PH, Lai LM, Caaington EV, Opaluwa AS, Ravikumar KH, Cheety N, Kaplan V, Kelley CJ, Babu ED: Fat necrosis of the breast-A review .The breast (2005).
- **18.** Lanng C, Erikesen BØ, and Hoffmann J: Lipoma of the breast: a diagnostic dilemma. The Breast, 2004; 13:408-411.
- **19.** Sneige N: Fine needle aspiration of the breast: a review of 1.995 cases with emphasis on diagnostic pitfall. Diagn Cytopathol. 1993, 9:106-112.
- **20.** Khanna AK, Singn MR, Khanna S, Khanna NN: Fine needle aspiration cytology, Imprint cytology and Tru-Cut needle biopsy in breast lumps: a comparative evaluation. J Indian Med Assoc, 1991; 89(7):192-195.
- **21.** Ergete W: Fine needle aspiration of palpable breast lesions with histopathologic correlation. Ethiop J Heath Dev 1999; 13(3):181-186
- **22.** Maria F, Vassiliki O, Flora Z, Theodoros N, Afroditi N, Pauline A, Theodora D, Efstratios A, Evagelia K, Geoge CZ.: Imprint

- cytology on microcalcification excised by Vacume-Assisted Breast Biopsy: A rapid preliminary diagnosis. World Journal of Surgical Oncology 2007; 5:40
- **23.** Tomohiko A., Satoru M, Hideo M, Yuichi T.: Comparison of Frozen Section and Touch Imprint Cytology for Evaluation of Sentinel Lymph Node Metastasis in Breast Cancer. Annals of Surgical Oncology 2004; 11(8):747-750
- **24.** Creager AJ, Geisinger KR, Perrier ND, Shen P, Show JA, Youg PR, et al.intraoperative imprint cytologic evaluation of sentinel lymph nodes for lobular carcinoma of the breast. Ann Surg 2004; 239:61-64.
- **25.** Limberis V, Romanidis C, Galazios G, Koutsougeras G, Paradopoulous N, Lambropoulou M, Simopoulos C. Intraoperative estimation of sentinel lymph nodes in breast cancer by imprint cytology. European Jornal of Gynaecological Oncology 2009; 30(1):85-87.
- **26.** Hamidian JA, Narayanan S, MacNeill F, Osin P, Neurukar A, Gui G. Testing the feasibility of intra-operative sentinel lymph node touch imprint cytology. Annals of the Royal College of Surgeons of England 2009; 91(4): 336-339.
- **27.** Tamiolakis D, Papadopoulos N, Venizelos J, et al. Intraoperative Touch Imprint Cytological Analysis of Sentinel Lymph Nodes for the Presence of Metastases in Breast Cancer. Onkologie(International Jornal for Cancer Research and Treatment) 2006; 29:372-375.
- **28.** Anita M, Annalisa C, Patrizia C, et al. Touch Imprint Cytology in Tumor Tissue Banks for the Confirmation of Neoplastic Cellularity and for DNA Extraction. Arch Path Lab Med 2008; 132:974-978.

#### Evaluation of inhibitory effect of honey on some bacterial isolates

#### Raied Taha Al- Naama Msc.

#### Abstract

**Background:** Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives.

**Objectives:** The aim of this study was to investigate the antimicrobial activity of honey sample from Basrah region against certain bacterial isolate.

**Method:** different concentrations (25.0%, 50.0%, 75.0% and 100.0%) of honey sample where checked for their antimicrobial activities, using some medically important microorganisms including *Escherichia coli*, pseudomonas spp. and *staphylococcus aureus*. The minimum inhibitory concentrations (MIC) of the honey sample were determined on the selected microorganisms by using broth dilution technique

**Result:** The sample of honey show inhibitory effect *in vitro* at 50%, 75% and 100%

concentration on the various investigated microorganism except at 50% concentration where no inhibition zone on *Staphylococcus aureus*. However, no effect was observed at concentration 25%. The MIC for *Escherichia coli*, pseudomonas spp. and *staphylococcus aureus* were 6.25mg/ml, 1.5mg/ml and 12.5 mg/ml respectively. *Conclusion:* The study shows that honey, like antibiotics, has certain organisms sensitive to it, and provide alternative therapy against cretin bacteria. And shown to have an antimicrobial action against a broad spectrum of bacteria (both gram positive and gram negative bacteria).

**Key words:** Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, honey, antibiotics, sensitivity, antimicrobial.

IRAQI J MED SCI, 2009; VOL.7 (4):67-72

#### **Introduction**

The antibacterial activity of honey was first recognized in 1892, by van Ketel (1). Honey is produced from many sources, and its antimicrobial activity varies greatly with origin and processing (2). Honey has been used as a medicine in many cultures for a long time <sup>(3)</sup>. It has been rediscovered by the medical profession and it is gaining acceptance as an antibacterial treatment of topical infections resulting from burns and wounds (4). Numerous demonstrate that possesses antimicrobial activity (1,2,5).

Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives <sup>(5)</sup>, it destroys and / or

Dept. Microbiology, College of Dentistry, University of Basrah.

Address Correspondence to: Dr.Raied Taha Al-Naama

E-mail: raiedtaha@yahoo.com

Received: 17th March 2009, Accepted: 21st

December 2009

inhibits the growth of some pathogenic vegetative microorganisms <sup>(6)</sup>. An antifungal action has also been observed for some yeasts and species of Aspergillus and Penicillium <sup>(3)</sup>, as well as all the common dermatophytes <sup>(7)</sup>

inherent possesses Honey antimicrobial properties, some which are due to high osmotic pressure/low water activity, in which the low water activity of honey is inhibitory to the growth of the majority of bacteria, and to many yeasts and moulds. When applied topically to wounds, osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissue and reduce bacterial growth. Even when diluted with absorbed from wounds, honeys would be likely to retain a water activity sufficiently low to inhibit growth of most bacteria. Honey is mildly acidic, with a pH between 3.2 and 4.5, Gluconic acid is formed in honey when bees secrete the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid, the low pH alone is inhibitory to many pathogenic bacteria and, in topical applications at least, could be sufficient to exert an inhibitory effect<sup>(8)</sup>.

Hydrogen peroxide, the product of the glucose oxidase system and tetracycline derivatives have The antibacterial properties pathogens (9). Low concentrations of this known antiseptic are effective against infectious bacteria and can play role in the wound healing mechanism<sup>(10)</sup> and in Stimulation and proliferation of peripheral lymphocytic and phagocytic activity<sup>(11)</sup>. Other factors, such as low protein content, high carbon nitrogen ratio, low redox potential due to the high content of reducing sugars, viscosity/anaerobic environment and other chemical agents/phytochemicals are also likely to play some role in defining antibacterial activity of honey (12), Furthermore, honey has been employed to shorten the duration of diarrhea in patients with bactericidal gastro-enteritis due to bacterial infection<sup>(13)</sup>. However, honey has other important beneficial characteristics that are less influenced by storage conditions (14). The aim of this study was to investigate the antibacterial activity of honey sample from Basrah region against certain bacterial isolate.

#### Material and methods

#### Honey samples

The honey sample used in this study was collected from Basrah province / Iraq, (Almuftia region); it was collected in sterile container and checked for purity on blood agar plate by streaked on blood agar plate, and incubated overnight. The honey sample was diluted by physiological saline to 25.0%, 50.0%, 75% and the non diluted honey (100.0%) referred to as neat. The study done in Al Sader

teaching hospital/ College of medicine, it was carried out during the period from January 2009 to April 2009.

#### Microorganisms

Staphylococcus aureus, Escherichia coli and Pseudomonas spp. were obtained from the Al Sader teaching hospital laboratory as clinical isolates and maintained in blood and macconkey's agar and sub cultured in Müller Hinton media.

#### Antimicrobial susceptibility testing

The disc diffusion technique was used as previously described by Kirby-Bauer <sup>(15)</sup>, using different types of antimicrobials. All isolates were inoculated into Müller-Hinton broth (in 10 ml) and incubated for 18 h to 24 h; the density was then adjusted to 0.5 McFarland standards at wave length 625 nm.

### Microbiological tests

## Preparation of honey suspensions for the disc diffusion test

The disc diffusion test was carried out as described by Mirsa and Helms *et al.* <sup>(16, 17)</sup>. Eight millimetre diameter-filter paper was saturated with 0.1 ml of each of the honey suspensions. The density of the isolates was the same as that used in the antimicrobial susceptibility testing of the various chemotherapeutic agents. All the tests were performed in triplicate.

# Minimum inhibitory concentration (Broth dilution method) against the isolated organisms

The broth dilution technique was used to calculate the minimum inhibitory concentration (MIC) of the honey samples. The test was carried out as described by Heuvelink *et al.* (18). A suspension of the organism was adjusted to 1.5x10 <sup>5</sup> organisms/ ml and further diluted to 1:200 in Müller Hinton broth. Five millilitres each of Müller Hinton broth was pipetted into ten sterile screws capped test tubes. A weight of 100

mg/ml of the honey was dissolved completely in the first tube. A serial dilution of honey, with a dilution factor of half was established. Tube number 10 served as a positive growth control containing Müller Hinton broth and bacterial inoculum only, and an additional tube containing broth only was used as a negative control. A volume of 0.1 ml of the bacterial suspension (7.5x10<sup>5</sup> organism/ml) was added to each tube. The tubes were incubated at 37°C for 18 h and visually examined for evidence of turbidity. The lowest concentration of honey in the series that inhibited the growth of the organism was taken to be the MIC, expressed in mg/ml.

#### Results

Honey sample showed marked inhibition of growth on Pseudomonas spp., the maximum inhibition zone was shown at concentration of 100% as 23mm, which reduce to 10mm at 75% and 8mm at 50% concentration (Table 1). Also the table showed that Escherichia coli grow with inhibition zone at concentration of 100% as 22mm, and the inhibition zone reduce to 12mm at 75% and 8mm at 50% concentration. Staphylococcus aureus showed a little less inhibition zone with honey sample. These were 20mm 100% and 11mm concentration, however, no effect was observed at concentrations 50% and 25% (Table 1).

Table 1: Antibacterial activities of different concentrations of honey against microbial isolate.

	inhibition zone (diameter in mm)		
Concentrations % (mg\ml)	Staphylococcus aureus	Escherichia coli	Pseudomonas spp.
100%	20	22	23
75%	11	12	10
50%	0	8	8
25%	0	0	0

X2=1.8 df= 2

Table 2 shows the zone of inhibition on the net concentration of honev that produced a inhibition than tetracycline gentamicin on pseudomonas (23mm, 16mm and 0mm respectively), and on Escherichia coli (22mm, 18mm, 20mm respectively), Except for staphylococcus aureus, where the tetracycline produced similar inhibition of honey 20mm and 18mm to gentamicin.

P> 0.05

Studies on the minimum inhibitory concentration (MIC) of the honey on the tested organisms showed that the MIC were demonstrated against pseudomonas spp. (1.5 mg/ml) and the MIC was exhibited against *Staphylococcus aureus* (12.5 mg/ml), while the MIC for *Escherichia coli* was equal to 6.25 mg/ml (Table 3).

Table 2: Antibacterial activities of net honey against certain bacterial isolate compared with Gentamicin and Tetracycline.

	inhibition zone (diameter in mm)		
Organisms	Honey 100%	Gentamicin	Tetracycline
Staphylococcus aureus	20	18	20
Escherichia coli	22	20	18
Pseudomonas spp.	23	16	0
770 - 04	10.0	TD 0	0.4

Table 3: The minimum inhibitory concentration (MIC) of the honey against tested organisms.

Organisms	Minimum inhibitory concentration (MIC)
	(mg/ml)
Staphylococcus aureus	12.5
Escherichia coli	6.25
Pseudomonas spp.	1.5

#### Discussion

This study was undertaken to investigate in vitro antimicrobial activity of honey against certain bacterial isolates. In the study, honey sample showed the antimicrobial activity, and our result were in agreement with Willix et al. (19) who found that honey inhibited the growth of Staphylococcus aureus, Escherichia coli, and Pseudomonas sp., and also in agreement with Bilal (20) who found honey exhibited fairly a antimicrobial activity against both Gram-negative and Gram-positive bacteria, and a remarkable activity was observed with Pseudomonas aeruginosa and Staphylococcus aureus.

The study showed antimicrobial activity against *Staphylococcus aureus*,

and this result agreed with Molan (21) who found the *Staphylococcus aureus*, is one of the bacterial species most susceptible to the antibacterial activity of honey. These might be due to the osmotic effect, the effect of pH and the sensitivity of these organisms to hydrogen peroxide, which represented an 'inhibine', factor in honey (22).

The potency of neat honey (100% concentration) was found to be superior against all bacteria tested, and the best antimicrobial activity of honey occurs with pseudomonas sp. followed by *Escherichia coli*, these results of the study were in agreement with Adeleke et al. (23) where it showed an evident of increase in the percentage levels of bacterial sensitivity - as high as 100% for *P. aeruginosa* and 96.4% for *E.* 

coli. Also of interest is the finding that the activity of gentamicin, both 4.0 and 8.0  $\mu$ g/ml, was found to be virtually lower than that of undiluted honey or any of its aqueous dilutions.

And these results were corresponding with Abd-El Aal (24) who showed that honey have a greater inhibitory effect on isolated gramnegative bacteria (*Pseudomonas aeruginosa*, Enterobacter spp., and Klebsiella). Also El-Sukhon et al. (25) showed that gram negative bacteria to be more sensitive to action of honey than Gram-positive bacteria.

Mundoi et al. (26) discovered that the antimicrobial activity of honey was more with Pseudomonas and Acinetobacter spp, both with resistance to some antibiotics like gentamicin, ceftriazone, amikacin and tobramicin than other bacteria tested.

The previous study of Subrahmanyam <sup>(27)</sup> showed that strains of *Pseudomonas aeruginosa* resistant to routinely used and higher antibiotics were sensitive to the antibacterial action of honey.

Taormina et al. <sup>(28)</sup> studied the antimicrobial effect of honey on gram negative bacteria and attributed it to the presence of factors as high content of tetracycline derivatives, hydrogen peroxide and powerful antioxidants, as also to a naturally low pH, which is unsuitable for bacterial growth, and to the presence of phenolic acids, lysozyme, and flavanoids.

The demonstration of MIC showed that the most susceptible microorganisms to the honey are pseudomonas spp. Cooper (29) has reported that manuka honey had MIC of less than 10% against 17 strains of *P. aeruginosa* from infected wounds, and honeys which have a MIC of 10% to 20%, can be expected to be effective in preventing growth of Pseudomonas, followed by *Escherichia coli* and *Staphylococcus aureus*. and these

results was accordance with Willix et al. (30) who found the MIC (minimum inhibitory concentration) of the honeys was found to ranged from 1.8% to 10.8% (v/v), indicating that the honeys had sufficient antibacterial potency to stop bacterial growth if diluted at least nine times, and up to 56 times in the presence of *Staphylococcus aureus*.

The high antibacterial effect of honey sample in the disc diffusion test and the low MIC may be attributable to the presence of glucose oxidase, which is activated by dilution in water resulting in the production of hydrogen peroxide which is toxic to bacteria (31).

It was concluded that the result, the study shows that honey, like antibiotics, has certain organisms sensitive to it, and provide alternative therapy against cretin bacteria. And shown to have an antimicrobial action against a broad spectrum of bacteria (both gram positive and gram negative bacteria). These antimicrobial properties would warrant further studies on the clinical applications of honey against bacteria and other microorganisms.

#### References

- **1.** Dustmann JH. Antibacterial effect of honey. Apiacta. 1979; 14 (1): 7-11.
- **2.** Molan PC. The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. Bee World. 1992; 73:59-76.
- **3.** Quinn PJ, Carter ME, Markey BK & Carter GR. Enterobactereaceae. In Clinical veterinary microbiology. Wolfe Publishing, an imprint of Mosby-Year Book Europe Ltd. London, 1994; 109-135.
- **4.** Abuharfeil N., Al-Oran R. & Abo-Dhehada M. The effect of bee honey on the proliferative activity of human B- and T-lymphocytes and the activity of phagocytosis. Food agric. Immunol, 1999; 11, 169-177.
- **5.** Molan PC. The antibacterial activity of honey. 1. The nature of the antibacterial activity. Bee World. 1992; 73(1): 5-28.
- **6.** Chick H, HS and Shin Z. Ustunol, Growth and acid production by lactic acid bacteria and bifidobacteria grown in skim milk containing honey., J. Food Sci.2001; 66: 478–481.

- **7.** Brady NF, Molan PC and Harfoot CG. The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. Pharm Sci. 1997; 2: 1-3.
- **8.** Molan PC. The antibacterial properties of honey. Chem in NZ, 1995; pp 10 14.
- **9.** Snowdon JA and Cliver DO. Microorganisms in honey. International Journal of Food Microbiology. 1996; 31: 1-26. **10.** Molan PC. Why honey is effective as a medicine. 2. The scientific explanation of its effects. Bee World. 2001; 82(1): 22-40.
- **11.** Tonks A, Cooper RA, Price AJ, Molan PC, Jones KP. Stimulation of TNF-alpha release in monocytes by honey. Cytokine. 2001; 14: 240-242.
- **12.** Honey Health and Therapeutic Qualities, The National Honey Board, Longmont, CO, USA (2002) (http://www.nhb.org)
- **13.** Haffejee IE and Moosa A. Honey in the treatment of infantile gastro-enteritis. Br Med J. 1985; 290: 1866-1867.
- **14.** Cooper R.A., Molan P.C. & Harding K.G. The sensitivity to honey of gram-positive cocci of clinical signifigance isolated from wounds. J. appl. Microbiol.2002; 93 (5), 857-863.
- **15.** Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. Apr. 1966; 45(4):493–496.
- **16.** Mirsa NB and wamota IA. Salmonilla typhimurum outbreak at kenyatta National Hospital (1985). East. Afr. Med. J. 1989: 66 (7), 453-457.
- **17.** Helms M, Vastrup P, Gerner-Smidt P. and Molbak K. Excess mortality associated with antimicrobial drug resisitant S. typhimurium. Emerg. infect. Dis. 2002; 8 (15), 490-495.
- **18.** Heunvelink AE, van den Biggelaar FL, Zwartkruis-Nahuis JTM, Herbes RG, Huyben R, Nagelkerke N, Melchers WJ, Monnens LA and de Boer E. Occurrence of verocytotoxin-producing E. coli O157 on Dutch dairy farms. J. clin. Microbiol. 1998; 36 (12): 3480-3487.
- **19.** Willix DJ, Molan PC, and Harfoot CG. A comparision of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. Journal of Applied Bacterial. 1992; 73 (5): 388-394.
- **20.** Bilal et al. Antimicrobial activity of honey on selected microorganisms: A preliminary study. Biomedical Research (India). 1998; 9: 51-54.
- **21.** Molan P. The antibacterial activity of honey 1. The nature of the antibacterial activity. Bee World. 1992a; 1: 5-28.

- **22.** Postmes T, Van den Bogaard AE and Hazen M. Honey for wounds, ulcers and skin graft preservation. Lancet. 1993; 341: 756-757. **23.** Adeleke OE, Olaitan JO and Okpekpe EI. Comparative antibacterial activity of honey and gentamicin against *E. coli* and *pseudomonas aeruginosa*. Annals of Burns and Fire Disasters. 2005; (ISSN 1592-9566).
- **24.** Abd-El Aal AM, El-Hadidy MR, El-Mashad NB and El-Sebaie AH. Antimicrobial effect of bee honey in comparasion to antibiotic on organisms isolated from infected burns. Annals of Burns and Fire Disasters 2. 2007.
- **25.** El-Sukhon SN, Abu-Harfeil N and Sallal AK. Effects of honey on Bacterial Growth and Spore Germination. J. Food Prot. 1994; 57(10): 918-920.
- **26.** Moundoi MA, Padila-Zakour OI and Worobo RW. Antimicrobial activity of honey against food pathogens and food spoilage microorganisms. New York State Agricultural Experiment Station. 2001; 1: 61–71.
- **27.** Subrahmanyam M, Hemmady A R. and Pawar SG. Antibacterial activity of honey on bacteria isolated from wounds. Annnals of Bums and Fire Disasters. 2001; 14 (1):198-201.
- **28.** Taormina PJ, Niemira BA and Beuchat LR. Inhibitory activity of honey against food-borne pathogens as influenced by the presence of hydrogen peroxide (H2O2) and level of antioxidant power. International J. Food Microbiology. 2001; 69: 217-25.
- **29.** Cooper R. The use of honey as an antiseptic in managing Pseudomonas infections. J Wound Care. 1999; 8:161–164.
- **30.** Willix DJ, Molan PC and Harfoot CG. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J. Appl Bacteriol. 1992; 73(5): 388-94.
- **31.** Stinson EE, Subers MH, Petty J and White JW. The composition of honey. V. Separation and identification of the organic acids. Arch. Biochem. Biophys. 1960; 89: 6-12.

#### Lifetime incontinence due to ectopic ureter with hypoplastc dysplastic kidney in 22 yr old lady, a Case Report

**Ula M.R.Al-Kawaz** *MBChB*; *FIBMS* (*Urology*).

#### Abstract

Continuous urine dribbling together with normal micturition is the classical picture of ectopic ureter in girls. We present our case which 22yr old lady presented to our department with life time incontinence. The diagnostic work-up included: Excretory Urography, ultrasonography (US), cystogram, MRI and Cystoscopy, as well as a thorough exam of the external genitalia under general anesthesia. Our case presented an ectopic ureter that ends the vagina with single dysplastic renal system and nephrectomy with ureterectomy was done as treatment of choice.

Urinary incontinence in girls due to ectopic ureter is an uncommon disease. Eighty-five% of the cases are associated to renal duplication. IVU is highly sensible to defect renal duplication; and only 15% of ectopic ureters come with single system.

Kevwords: incontinence, ectopic ureter, duplex renal system

IRAQI J MED SCI, 2009; VOL.7 (4):73-74

#### Introduction

Girls with no other signs or symptoms but continuous dribbling of urine, despite successful toilet training, should be considered to have a ureter with an extravesical, infrasphinctenic orifice. Usually, the ureter with an ectopic orifice drains the upper moiety of a double collecting system or less commonly drains it a single hypoplastic and dysplastic kidney. Excretory urography is helpful for confirmation. and it is usually diagnostic of both the condition and the affected side or sides. Urography and MRI are additional diagnostic tests to verify the problem.

Cystourethroscopy is helpful to locate the site of the ureteric orifice

#### The case

A 22 years old lady presented with life time continuous incontinence with normal urination pattern, general examination was normal, urinalysis

Dept. Surgery, College of Medicine, AL-Nahrain University.

Adress Correspondence to:Dr Ula M.R. Al-

E-mail: ulamhm@vahoo.com

Received: 5<sup>th</sup> November 2008, Accepted:16<sup>th</sup> June 2009.

revealed few pus cells, biochemistry was normal, the history and the other findings were highly suggestive of ectopic ureter.

Excretory urography was done to her that showed non visualization of the Lt. Kidney, with normal functioning Rt. kidney, MRI showed non visualization of the Lt. Kidney in its normal position, and showed a fluid filled cyst near Lt. Lateral wall of the urinary bladder.

Cystourethroscopy was done and showed normal Rt. Ureteric orifice but the Lt. ureteric orifice was not visualized and there was spillage of urine through the vagina.

According to the above findings, exploration through modified Gibson incision and a dilated and tortuous ureter was found that ends in the posterior wall of the vagina draining a dysplastic and hypoplastic kidnev situated at the pelvis. Nephrectomy with ureterectomy was done. Figures (1, 2)



Figure 1: the dysplastic kidney with the ectopic ureter

Discussion:

Wetting is an exceedingly common pediatric complaint, and most children with urinary incontinence underlying have no structural abnormality. Their physical examination is normal, and their wetting is usually nocturnal. It is apparently due to immaturity of bladder and urethral sphincter function, is commonly hereditary, and is typically outgrown <sup>(1, 2)</sup>. Imaging is not recommended.

Anatomic abnormalities causing incontinence of urine include spinal dysraphism, sacral agenesis, epispadias (3)

conditions Such are usually careful physical evident on examination of the back, perineum, and lower extremities. Plain films may anomalies or, spinal epispadias, an abnormal (>1 0 mm) interpubic distance.

In girls who are otherwise well and whose history is that of continuous day and night, despite wetting, successful toilet training, for as long as anyone can remember, an extravesical infrasphinctenic ectopic ureteral orifice should be strongly suspected and imaging should be vigorously pursued. Ureteral ectopia with incontinence is uniquely female related, because the most caudal location for an ectopic Ureteral orifice in a male is always above the urethral sphincter.



Figure 2

Ectopia of the ureteral orifice is often associated with dysplasia of the kidney on that portion of the kidney drained by the ectopic ureter. As a rule, the more ectopic the orifice the worse will be the dysplasia (4).

Grande Moreillo et al described<sup>(5)</sup> their eleven girls with the classical picture of ectopic ureter in girls with continuous urine dripping together with normal micturition Ten girls had a double renal system, one of them being bilateral; one girl had single kidneys with renal ectopia. Treatment was heminephrectomy with ureterectomy in 9 cases, ureteroureterostomy with preservation of the hemikidney in one nephrectomy and ureterectomy in the case with a simple system <sup>(5)</sup>.

#### References

- 1. Perlmutter AD. Enuresis. In: Kelalis PP. King LA, Belman AB, eds. Clinical pediatric urology, 2nd ed. Philadelphia: Saunders, 1985:311-325
- 2. Smith DE. Diagnosis and management of the child with wetting. Aust Paediatr J 1967:3:193-205
- 3. Stannard MW, Lebowitz AL. Urography in the child who wets. AJR 1978:130:959-962
- **4.** Mackie GG, Stephens FD. Duplex kidneys: a correlation of renal dysplasia with position of the ureteral orifice. *J Urol* 1975:114:274-280
- **5.** Grande Moreillo et al : Ectopic ureter as cause of urinary incontinence in girls Actas Urol. Esp. 2000 Apr;24(4):314-8

#### Retroperitoneal Perforation of the Appendix Presenting as Right Thigh Abscess, A Case Report.

Ali Jaliel Awad¹ CABS, Hassan Hadi Al-Sikafi², MD; FRCSI; FRCS Ed; MRCS; LRCP.

#### Abstract

A case of retroperitoneal perforation of the appendix presenting with a thigh abscess is described. *Keywords:* Complicated acute appendicitis, retroperitoneal perforation, retroperitoneal abscess, Thigh abscess

IRAQI J MED SCI, 2009; VOL.7 (4):75-77

#### Case Report

A 45-year man presented with a 7day history of right loin pain and a 5day history on the lateral aspect of right thigh pain. He was pyrexial at 38°C, with a pulse of 100 beats per minute and blood pressure of 110/70 mmHg, with slight tenderness in the right loin and normal examination findings of the right lower limb<sup>1</sup>. Plain X-ray showed gas in the soft tissue of the gluteal region and lateral aspect of the right thigh. There was a collection of fluid in the right side of the abdomen and pelvis with inflammatory changes Laparotomy revealed no free intraperitoneal pus. A loop of distal ileum was adherent to right side wall of the pelvis with complete obstruction at this point. This was relieved and there was a retroperitoneal abscess extending from above the upper pole of the right kidney down to the presacral space and to the right side of the pelvis at the site where the loop of the ileum was adherent<sup>1-2</sup>. The right side of the colon was mobilized to drain the pus. The appendix was found amputated spontaneously about 1 cm from its base and was secured by a tie.

E-mail: alshuhub2003@yahoo.com

Received: 1<sup>st</sup> June 2009, Accepted: 7<sup>th</sup> October 2009.

#### Discussion

The position of the appendix is influenced by the changes in position and shape of the cecum undergoes during development. The ascending colon is normally retroperitoneal in position and the cecum is usually intraperitoneal.<sup>3</sup> If the cecum does not descend, the appendix lies retroperitoneally behind the cecum in front of the right kidney. In the latter appendix cases, the may intraperitoneal, or retroperitoneal with or without a paracecal fossa formed by peritoneum<sup>4-5</sup>. The clinical diagnosis of retroperitoneal abscess is often delayed or missed because of the insidious onset of symptoms and the paucity of localizing signs<sup>6-7</sup>. The route of spread can be broadly separated into two groups. Direct soft tissue extension of infection down the thigh almost always originates from the rectum below the peritoneal reflection. The second route of extension of infection into the thigh is through naturally occurring defects in the abdominal wall<sup>8-9</sup>. The diagnosis of a thigh abscess as such is not difficult, because it usually presents with the typical signs and symptoms of inflammation and gas that are reliably noted on X-ray of the soft tissue 10-11. Both the intra-abdominal process and the thigh abscess must be treated. In literature, when cases of thigh abscesses, secondary to intrawere abdominal sepsis, managed without attention to the underlying

<sup>&</sup>lt;sup>1</sup>Dept. General surgery, Al-Karama Teaching Hospital, Dept surgery, Medical City.

Adress Correspondence to:Dr Ali Jaliel Awad

etiologic process, such as by local thigh drainage plus antibiotics, the mortality was 93%. If some form of definitive therapy was added to the local management of the thigh abscess, such as appendicectomy, stoma, or debridement, the mortality was 34%. However, the overall mortality rate was 53%. <sup>12-13</sup> A thigh incision is mandatory to allow direct drainage of the pus and examination of the

viability of the fascia and muscle. The location of the thigh incision is best determined by clinical examination assisted by CT scan findings that can accurately localize the collection and the gas distribution in the soft tissue. Pott's disease should be excluded and gluteal abscess in thigh below the inguinal ligament<sup>14</sup>.

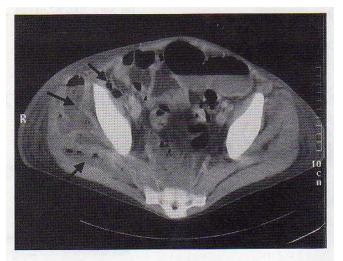


Fig. 1 CT scan showing free gas and collections on either side of the right iliac bone (arrows) and distended small bowel loops.

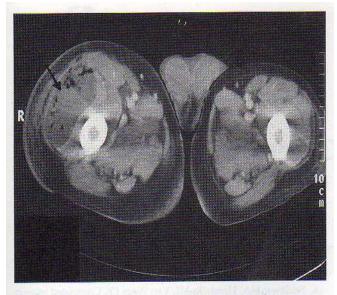


Fig. 2 CT scan showing gas and collection on the lateral aspect of the right thigh (arrow).

#### References

- **1.** Gatt D. Appendix sepsis tracking along the lateral cutaneous nerve of the thigh. *Postgrad Med J* 1985; 61:1015-1016.
- **2.** Sampson LH, Pegg SP. Appendicitis presenting as a swollen thigh. *Med] Aust* 1977; 1:406-408.
- **3.** Edwards JD, Eckhauser FE. Retroperitoneal perforation of the appendix presenting as subcutaneous emphysema of the thigh. *Dis Colon Rectum* 1986^9:456-458.
- **4.** Feldberg MA, Hendriks MJ, Van Waes PF. Computed tomography in complicated acute appendicitis. *Gastrointest Radial* 1985; 10:289-295.
- **5.** Stevenson EO, Ozeran RS. Retroperitoneal space abscesses. *Surg Gynecol Obstet* 1969; 128:1202-1208.
- **6.** Harris LF, Sparks JE. Retroperitoneal abscess: case report and review of the literature. *Dig Dis Sci* 1980; 25:392-395.
- **7.** William PL, Warrick R. *Gray's Anatomy*, 36th ed. Edinburgh, London, Melbourne, New York: Churchill Livingstone, 1980; p. 1353.
- **8.** Wakeley CPG. The position of the vermiform appendix as ascertained by an analysis of 10,000 cases. *Anat* 1933; 67:277-283.
- **9.** Collins DC. Acute retrocaecal appendicitis: based on seven hundred and fifty-one instances. *Arch Surg* 1938; 36:729-743.
- **10.** Meyers MA, Oliphant M. Ascending retrocecal appendicitis. *Radiology* 1974; 110:295-299.
- **11.** Fox TA, Gomez J, Bravo J. Subcutaneous emphysema of the lower extremity of gastrointestinal origin. Dis *Colon Rectum* 1978; 21:357-360.
- **12.** Rotstein OD, Pruett XL, Simmons RL. Thigh abscess: an uncommon presentation of intra-abdominal sepsis. *Am J Surg* 1986; 151:414-418?
- **13.** Pollock JH. A survey of the retroperitoneal space. / *Int Coll Surg* 1962;38:412-420.
- **14.** Meyers MA. Radiological features of the spread and localization of extraperitoneal gas and their relationship to its source: an anatomical approach. *Radiology* 1974; Ill: 17-26.

### المجلة العراقية للعلوم الطبية قائمة المحتويات

المقالات
<ul> <li>العلاقة بين حجم جريان الدم في وريد الحبل السري ووزن الجنين في الفصل الأخير</li> </ul>
من الحمل
مها البياتي ،وسن إسماعيل مجيد،عبير طالب مكي
<ul> <li>التحري عن الاصابة بفايروس الخلية العملاقة التنفسي في مجموعة من الاطفال في</li> </ul>
العراق
شوني ميخائيل اوديشو ، أنطوان صبري البنا ، ناهي يوسف ياسين
<ul> <li>مقارنة بين مستويات مصل البرو لاكتين مقاسا بتقنيتي الاشعاع المناعي والفلورة</li> </ul>
الرابط للانزيم
ريا سليمان بابان ، يحيى يحيى فريد زكي
<ul> <li>التنميط الوراثي لمستضدات الخلايا البيض البشرية-الصنف الثاني بواسطة تفاعل</li> </ul>
البلمرة المتسلسل - بتقنية الباديء المعين لسلسلة جينية معينة لمريضات سرطان الثدي
العراقيات
الحسن عباس الحسن ، نضال عبد المهيمن، علاء غني حسين ، أميرة النعمة، خليفة مهدي
❖ استخدامات البروبلاست في جراحة الفم والوجه والفكين
أياد عبد الخالق حسن
<ul> <li>االعلاقة بين الاصابه بالممرضاتTORCH و حصول الاجهاض التلقائي المتكرر</li> </ul>
نضال عبد المهيمن ، امال حسين،فاروق خالد حسن
<ul> <li>لمحة على التغيرات الحاصلة في نظام التخثر بسبب عملية فصل البلازما</li> </ul>
زينب محمد حسن ، ميادة سليم محمود ألنعيمي ، حيدر حسن جليل الشمري
<ul> <li>دورالفحص الخلوي للبصمات في تشخيص افات الثدي</li> <li>حسنين حافظ خضير ، طاهر هورامي، كوران محمد رؤوف</li></ul>
ين حـــــ مـــــر مـــرسي، ــــرسي، ــــرسي، ـــــــــ رووـــــــــــــــــــــــــ
<ul> <li>تقدير التأثير المثبط للعسل على بعض العزلات البكتيرية</li> </ul>
رائد طه
3

نفرير حاله:	
<ul> <li>دراسة سلس البول بسبب حالب منزاح بكلية الشاذة النمو في سيدة عمرها ٢٢ سنة </li> </ul>	سنة "
تقرير حالة "	
علا محمد	
الكواز	11
<ul> <li>إنفجار الزائدة الدودية خلف البريتون كحالة مرضية لخراج الفخذ الأيمن " تقر</li> </ul>	" تقرير
حاثة "	
على جليل عواد ، حسن هادي السكافي	Υ

## المجلة العراقية للعلوم الطبية

رئيس هيئة التحرير

## الأستاذ الدكتورة فائزة عفتان زغير

## هيئة التحرير التنفيذية

رئيــــس التحريـــــر	أ.د. فاخـــر سلمــــان شفيق
محـــــرر	أ.د. غسان عبد الامير الشماع
محـــــرر	أ.د. علاء غنـي حســـــين
محـــــررة	أ.د. نضـال عبـــد المهيمــــن
محـــــرر	أ.م.د.سمير مــحـمود جاســم
محـــــرر	أ. <sub>م</sub> .د. معتز عبد المجيد القزاز
محـــــرر	أ.م.د.حسام عبد الكريم أحمد
محـــــررة	أ.م.د.إيناس طالب عبـد الكريم
محـــــررة	أ.م.د. أثير جـواد عبـــد الأمير
محـــــرر	أ.م.د.حس عـزيز الحمـداني
محـــــررة	أ.م.د. هالة سامــح عـــارف
محـــــرر	م.د.وسيـــم فاضــل مـحمـــد
محـــــرر	م.د. علــــي فــؤاد هــادي
محـــــررة	م.د. سهـــاد محمد صــــالح

سكرتارية المجلة إسراء سامي ناجي ..

المحرر الفني

علياء نوري حاتم

## الهيئة الأستشارية

```
أ.م.د. أديب احمد كاظم الزبيدي (جامعة النهرين)
                     أ.د.أسامة سليمان الناصري (جامعة النهرين)
                 أ.د.اسامة نهاد رفعت (الأمارات العربية المتحدة)
      أ.د.امجد داود نبازي (المجلس العراقي للأختصاصات الطبية)
أ.د.أنعم رشيد الصالحي (معهد أبحاث الأجنة و العقم-جامعة النهرين)
                           أ.د.ثامر أحمد حمدان (جامعة البصرة)
                     أ.د.جاسم محمد عطبة المحنة (جامعة الكوفة)
                       أ.م.د.جليل إبراهيم صالح (جامعة الأنبار)
                     أ.د.حسام حسون علي (جامعة النهرين)
                  أ.م.د. حسن احمــد حسن باقي (جامعة النهرين)
                   أ.د. حكمت عبد الرسول حاتم (جامعة النهرين)
                 أ.م.د. حيدر جـــواد كاظم (جامعة النهرين)
                         م.د.خضير خلف إبراهيم (جامعة ديالي)
                     أ.د.رافع الراوي (الامارات العربية المتحدة)
                     أ.م.د.راهي كلف الياسري (جامعة القادسية)
                       أ.م.د.زهير عمران عيسى (جامعة كربلاء)
                    أ.د. سامي إسطيفان مطلوب (جامعة النهرين)
                                 أ.د.سر مد خوندة (جامعة بغداد)
                       أ.د. سوسن ساطع عباس (جامعة النهرين)
                  أ.د. عبد الحسين مهدي الهادي (جامعة النهرين)
                أ.م.د. عبد الرزاق حردان أحمـد (جامعة النهرين)
                         أ.م.د.عطا كطي علاوي (جامعة واسط)
                               أ.م.د.على خبر الله (جامعة بابل)
                           أ.م.د.فارس عبد الكريم (طب الكندي )
                    أ.م.د. فخر الدين نجم ناصر (جامعة كركوك)
                 أ.م.د. فرقــد بـــدر حمــــدان (جامعة النهرين)
                            أ.م.د.فرهاد سوليفان ( جامعة دهوك)
                أ.م.د. لمياء عبــد الكريم السعدي (جامعة النهرين)
```

أ.د.مؤيد ناجي مجيد (جامعة ذي قار )

أ.د.محمد حسن العلوان(الجامعة المستنصرية)

أ.د.محمود حياوي حماش (الأردن)

أ.م.د.مزاحم قاسم الخياط (جامعة الموصل)

أ.د. مها محمد جاسم البياتي (جامعة النهرين)

أ.د.نزار الحسني (المجلس العراقي للإختصاصات الطبية)

أ.د. هاشــم مهــدي الكاظمــي (جامعة النهرين)

أ.د. يعرب إدريس عبد القادر (جامعة النهرين)

## **IRAQI JOURNAL OF MEDICAL SCIENCES**

A MEDICAL JOURNAL ENCOMPASSING ALL MEDICAL SPECIALIZATIONS ISSUED QUARTERLY

CONTENTS
EDITORIAL
❖ ACCREDITATION Fakhir S. AL-Ani1- 3
ARTICLES
❖ THE RELATIONSHIP BETWEEN UMBILICAL VENOUS BLOOD FLOW & FETAL WEIGHT IN THE LAST TRIMESTER Maha M. Al-Bayati, Wasan I. Majeed, Abir T. Makki
❖ DETECTION OF RESPIRATORY SYNCYTIAL VIRUS INFECTION IN A SAMPLE OF INFANTS IN IRAQ Shony M. Odisho, Anton S. Al-Bana, Nahi Y. Yaassen
❖ COMPARISON BETWEEN SERUM PROLACTIN LEVELS DETERMINED BY VIDAS AND RIA TECHNIQUES Rayah S. Baban, Yahya Y.Farid
❖ GENOTYPING OF HLA-CLASS-II BY PCR-SSP OF IRAQI BREAST CANCER PATIENTS Ahmed A. Al-Hassan, Nidhal Abdul Muhymen, Ala'a Ghany Hussien, Ameera J. Al-Nnema, Khalifa Mehdi
❖ PROPLAST IN ORAL AND MAXILLOFACIAL SURGERY Ayad A. Hasan
❖ ASSOCIATION BETWEEN TORCH AGENTS AND RECURRENT SPONTANEOUS ABORTION Nidbal Abdul Mohymen Amal Hussien Farouk K Hassan 40-46

❖ GLIMPSE ON HEMOSTATIC CHANGES PRODUCED BY PLASMAPHERESIS Zainab Mohammad Hasan , Mayada Saleem Mahmood Al-Niami, Haider Hasan Jaleel AL-Shammari
❖ ROLE OF IMPRINT CYTOLOGY IN BREAST LESIONS. Hassanain H.Khudier, Tahir A Hawramy, Goran M Abdul-Qadir61-66
❖ EVALUATION OF INHIBITORY EFFECT OF HONEY ON SOME BACTERIAL ISOLATES
Raied Taha Al- Naama67-72
CASE REPORT
❖ LIFETIME INCONTINENCE DUE TO ECTOPIC URETER WITH HYPOPLASTC DYSPLASTIC KIDNEY IN 22 YR OLD LADY.
Ula M.R.Al-Kawaz
* RETROPERITONEAL PERFORATION OF THE APPENDIX PRESENTING AS RIGHT THIGH ABSCESS.
Ali Jaliel Awad , Hassan Hadi Al-Sikafi75-77

## العلاقة بين حجم جريان الدم في وريد الحبل السري ووزن الجنين في العلاقة بين حجم الفصل الأخير من الحمل

### مها ألبياتي '،وسن إسماعيل مجيد'،عبير طالب مكي"

#### الخلاصة

خلفية الدراسة: بدأ فحص الدوبلر بالتطور بسرعه في مجال فحوصات ألحمل وظهر أن موجات جريان ألدم توفر معلومات مهمة ابتداء من الاسبوع الثاني عشر للحمل وحتى نهايته من ألأو عيه الدموية للأم واوعية المشيمه والجنين. ومن أهم ألتطبيقات هو مقدار كمية الدم الجارية في ألأو عية ألدموية في الحبل السري

هدف الدراسة: تحديد العلاقة بين حجم جريان الدم في وريد الحبل السري من جهة و بين وزن الجنين ووزن المشيمة من جهه أخرى في الفصل ألأخير من الحمل في حالات ألولادات المبكرة والولادات الكاملة

تصميم ألدراسة: دراسه توقعية

مكان إجراء الدراسة: قسم النسائية والتوليد وقسم ألأشعة ألتشخيصية في مستشفى الكاظمية التعليمي

الأشخاص وطرائق العمل: ضمت ألدراسة خمسون سيده حامل خلال المرحله ألأولى من المولاده. تم تقسيم ألمرضى ألى مجموعتين ، مجموعة " أ" : ألأطفال المولودين أقل من ٣٧ أسبوع من ألحمل ، ومجموع " "ب": ألأطفال ألمولودين أكثر من ٣٧ أسبوع من ألحمل . تم قياس قطر وريد ألحبل السري باستخدام فحس الموجات فوق الصوتية التقليدي وأجري فحص ألدوبلر ألموجي لقياس سرعة وحجم جريان ألدم في وريد ألحبل ألسري لكل وحده من وزن ألمشيمة في مجموعتى ألدراسة .

ألنتائج: ظهر فرق احصائي مهم بين كل من : قطر وريد الحبل ألسري (  $^{1}$   $^{1}$   $^{1}$  ملم مقابل  $^{1}$   $^{1$ 

الإستنتاج: نسبة زيادة حجم جريان الدم في وريد الحبل السري هي اقل من ألزيادة ألحاصلة في وزن الجنين و ألمشيمة. يتحقق أنخفاض مهم في حجم جريان ألدم بألتناسب مع وزن الجنين مع تقدم مدة الحمل

مفتاح الكلمات: جريان الدم في وريد الحبل السري ، وزن الجنين ، وزن ألمشيمة

'فرع الأمراض النسائية و التوليد [كلية الطب - جامعة النهرين] تشعبه الاشعه التشخيصيه - فرع الجراحه[ كلية الطب - جامعة النهرين] فرع الأمراض النسائية و التوليد - مستشفى الكاظمية التعليمي

المجلة العراقية للعلوم الطبية ٢٠٠٩ م المجلد ٧ العدد ٤ ص٤ - ١٠

## التحري عن الاصابة بفايروس الخلية العملاقة التنفسي في مجموعة من التحري عن الاطفال في العراق

شوني ميخائيل اوديشو ' ، أنطوان صبري البنا ' ، ناهي يوسف ياسين '

#### الخلاصة

خلفية الدراسة: فايروس الخلية العلاقة التنفسي من المسببات الرئيسة لالتهاب القصبات الحاد والتهابات الرئوية في الاطفال الرضع.

هدف الدراسة: الكشف عن اضداد فايروس الخلية العملاقة في الاطفال باستعمال اختبار الاليزا العير مباشر وكذالك الكشف عن المستضد الفايروسي باستعمال التشخيص السريع -RSV . Respi

المواد وطرق العمل: تضمنت الدراسة الكشف عن اضداد الفايروس في ١٨٤ نموذج مصل الاطفال يعانون من اعراض تنفسية شديدة واطفال يعانون من عدة انواع من السرطان كما شملت الدراسة ١٠٠ نموذج مسحات من الأنف والحنجرة للكشف عن المستضد الفايروسي تم الحصول عليها من مستشفى الطفل المركزي في بغداد.

النتائج: شملت الدراسة ١٨٤ نموذج مصل جمعت من (١٠٤ لاطفال يعانون من اصابات الجهاز التنفسي، ١٠٤ لاطفال لا تظهر عليهم اصابات تنفسية و ٢٦ نموذج لاطفال مصابون بالسرطان) حيث تم الكشف عن اضداد فايروس الخلية العملاقة التنفسي واستعمال فحص الاليزا في أطفال لا يعانون من اعراض تنفسية حيث سجلت في ٢٦% من النماذج وبمعدل معيار ٤٩٤. كما كشفت اضداد فايروس الخلية العملاقة التنفسي في ٩٦% من النماذج لأطفال يعانون من مختلف انواع السرطان وبمعدل معيار ٥٨٠. كما ان اضداد فايروس الخلية العملاقة التنفسي البشري سجل في ٩٩% من النماذج لأطفال يعانون من اعراض تنفسية شديدة وبمعدل 1٤١١ والذي تفوق على المجموعتين السابقتين.

تم تشخيص مستضد فايروس الخلية العملاقة التنفسي البشري باستعمال Respi-RSV للتشخيص السريع حيث سجلت في 50% من النماذج المرضية في افرازات الانف والحنجرة لأطفال يعانون من اصابات تنفسي شديدة ، حيث كان تشخيص مستضد فايروس الخلية العملاقة التنفسي في النماذج التي تم فحصها متزامناً مع تشخيص الاضداد وبنسبة ٥٠% من نماذج المصول المفحوصة.

الاستنتاج: الكشف عن الاضداد لفايروس الخلية المعلاقة لاطفال يعانون اعراض تنفسية شديدة وبمعيار عال مقارنة مع المجاميع التي لم تظهر عليهم اصابات تنفسية.

الكشف عن المستضد الفايروسي في افرازات الانف والحنجرة لاطفال يعانون من التهاب القصبات والتهابات رئوية.

مفتاح الكلمات: فايروس الخلية العملاقة التنفسي ،التهاب القصبات الحاد ،Respi-RSV .

' فرع الاحياء المجهريه[ كلية الطب البيطري - جامعة بغداد] ' المركز العراقي لبحوث السرطان والوراثة الطبية / الجامعة المستنصرية

## مقارنة بين مستويات مصل البرولاكتين مقاسا بتقنيتي الاشعاع المناعي والفلورة الرابط للانزيم

#### ريا سليمان بابان ، يحيى يحيى فريد زكى

#### الخلاصة

خلفية الدراسة: من الممكن تقدير مستوى هرمون البرولاكتين في مصل او بلازما الانسان كميا بواسطة العديد من التقنيات المختبرية كالتشخيص بالامتصاص المناعي الرابط للانزيم ELFA والتشخيص بالفلورة الرابط للانزيم (miniVIDAS) .

هدف الدراسة: لتخمين مقدار قوة الارتباط او الجمع لقيم البرولاكتين الكلي والحر في مصل الدم والتي تم قياسهم بطريقتين مختلفتين VIDAS و RIA .وكذلك لتوقع قيمة البرولاكتين المراد قياسها بطريقة الـRIA بمقابل قيمة معطى بطريقة الـVIDAS .

طريقة العمل: تم استخدام تقنيتا الـ VIDAS و RIA في قياس مستوى البرولاكتين في مصل دم خمس وعشرين امراة تعاني من العقد الليفية الرحمية في مختبرين كانوا قد قدموا لمستشفى الكاظمية التعليمي خلال الفترة من كانون الثاني ٢٠٠٨ ولغاية نيسان ٢٠٠٩. قيس كل من البرولاكتين الكلي والحر في مصل دم المرضى بجهازي الـ VIDAS و الـ RIA باستخدام لوازم الطريقتين الخاصة بذاك الجهاز. كما استخدمت الطرق الاحصائية لعلاقتي الارتباط والارتداد للمقارنة بين هاتين الطريقتين.

النتائيسية : كشفت الدراسة عن وجود علاقة موجبة ذات إرتباطات إيجابية هامّة جداً بين مستوى البرولاكتين في المصل الكلي عند قياسه بكلتا التقنيتين كما هو في حالة مستوى البرولاكتين في المصل الحر عندما يقاس بنفس التقنيتين، P<0.001 0.997 R2 و P<0.001 0.997 R2 على التوالي. كما وجد ان هناك علاقة خطية ايجابية عالية بين مستوى البرولاكتين في المصل الكلي عند قياسه بكلتا التقنيتين كما هو في حالة مستوى البرولاكتين في المصل الحر عندما يقاس بنفس التقنيتين ، PO.358x+0.57 هو المحل الحر عندما يقاس بنفس التقنيتين ، PO.358x+0.57 هو المحل الحر عندما يقاس بنفس التقنيتين ، PO.358x+0.49 و PO.358x+0.49 و PO.998 هلى التوالي. وعند حساب نسبة البرولاكتين المتبقية المئوية بكلتا الطريقتين كل على حدة وجد ان النسبتين تقريبا مقاربة لبعضهمها البعض PO.50x+0.89% هلى التوالي.

الاستنتاج: وجد ت علاقة موجبة ذات إرتباطات إيجابية هامّة جداً بين مستوى البرولاكتين الكلي والحر في مصل الدم عند قياسهما بطريقة الـ VIDAS و الـ RIA . كما وجدت علاقة خطية ايجابية عالية بين كلتا الطريقتين لتوقع قيم البرولاكتيت بطريقة ال RIA مقابل قيم معروفة مقاسة بطريقة ال VIDAS .

مفتاح الكلمات: برولاكتين الاشعاع المناعي، برولاكتين الفلورة الرابط للانزيم، العقد الرحمية، ترسيب البرولاكتين بمتعدد الاثلين الكلايكول

### فرع الكيمياء و الكيمياء الحياتية [كلية الطب - جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٩ م المجلد ٧ العدد ٤ ص٢٠ - ٢٦

التنميط الوراثي لمستضدات الخلايا البيض البشرية-الصنف الثاني بواسطة تفاعل البلمرة المتسلسل – بتقنية الباديء المعين لسلسلة جينية معينة لمريضات سرطان الثدي العراقيات

احمد عبد الحسن عباس الحسن ، نضال عبد المهيمن ، علاء غني حسين ، احمد عبد الحسن عباس العمة ، خليفة مهدي  $^{"}$ 

#### الخلاصة

**خلفية الدراسة**: إن نسبة حدوث سرطان الثدي في النساء يختلف باختلاف العرق او القومية. العديد من اليلات مستضدات الخلايا البيض البشرية لها علاقة بالاستعداد او الحماية من سرطان الثدي، خصوصية الاليل تختلف حسب العرق.

هدف الدراسة: نظمت هذه الدراسه لتسلط الضوء على احتمالية وجود مصاحبة بين اليلات مستضدات خلايا الدم البيض البشرية)الصنف الثاني (مع سرطان الثدي في المريضات العراقيات.

الاشخاص وطرق العمل: تضمنت الدراسة ٦٠ شخص: ٣٠ مريضة مصابة بسرطان الثدي، ١٢ مريضة مصابة باورام الثدي الحميدة كمجموعة ضابطه اولى و ١٨ امراة سليمة ظاهريا كمجموعة ضابطه ثانية. استخدم فحص تفاعل البلمرة المتسلسل – بتقنية الباديء المعين لسلسلة جينية معينة لتقييم تنميط اليلات مستضدات الخلايا البيض البشرية.

النتائيج: أظهرت معاينة التوزيع التكراري الليلات مستضدات الخلايا البيض البشرية الصنف الثاني عدم وجود مصاحبة موجبة بين هذه الاليلات ومرض سرطان الثدي عند المقارنة مع مجموعتى السيطرة، لكن هناك نقصان مهم معنويا في تكرارية الاليلين:

DR\*010101, 0102, 0201-0204, 04-13 and DQB1\*0401, 02

في مرضى سرطان الثدي مقارنة بالسيطرة الاصحاء

الاستنتاجات: أظهرت النتائج بان الأليلين: 13-010101,0102,0201-0204,04 DR\*

and DQB1\*0401,02

ربما تمنح الحماية من المرض.

مفتاح الكلمات: سرطان الثدي، مستضدات الخلايا البيض البشرية ، تفاعل البلمرة المتسلسل.

'فرع الأحياء المجهرية [كلية الطب - جامعة النهرين] 'فرع علم الأمراض [كلية الطب - جامعة النهرين] "معهد الطب العدلي

المجلة العراقية للعلوم الطبية ٢٠٠٩ م المجلد ٧ العدد ٤ ص٧٧ - ٣٣

#### استخدامات البروبلاست في جراحة الفم والوجه والفكين

#### أياد عبد الخالق حسن

#### الخلاصة

خلفية الدراسة: البروبلاست عباره عن ماده مصممه لزرعها في الانسجه وهي موجوده تجاريا عن طريق شركة دو DOW. ويكون لونها اسود رصاصي على شكل قطع او شرائح ممزوجه بمادة التفلون. اول من حضر البروبلاست هو العالم هومسي Homsy سنة ١٩٧٠ ولكنه اخترع سنة ١٩٦٨ ليلبي متطلبات الزرع الجراحي.

هدف الدرآسة: لتقييم البروبلاست في هندسة الشكل الخارجي لعظام الوجه.

طريقة العمل: استعملت مادة البروبلاست على ١٨ مريضا. معدل الاعمار كانت ٢٧,٩% تراوحت مابين ١٨-٣٥ سنه. تم متابعة المرضى بستة اعوام في شعبة جراحة الوجه والفكين في مستشفى الكاظميه التعليمي.

النتائيج: أظهرت النتائج فائده مادة البروبلاست في بناء عظام الوجه وكانت نسبة النجاح النتائيج: أظهرت النتائج فائده مادة البروبلاست في بناء عظام الوجه وكانت نسبة النجام ، ٢٨ زرعه كانت مستقره ، تم رفعها نتيجة الاصابه بالخمج infection. وفي كلتا الزرعتين كان التثبيت قد تم باستخدام الكلاليب المعدنيه wire fixation وعن طريق الفم. ومن هذه ال ١٦ زرعه (٣) منها زحفت قليلا و (١٣) زرعه كانت مستقره تماما ولم تتحرك من مكانها.

الاستنتاج: البروبلاست مفيد لبناء عظام الوجه ولكن هنالك بعض الصعوبات الفنيه عندما يوضع فوق المكانات المحدبه مثل عظم الوجنه وحافة محجر العين وذلك لصعوبة ازالة حافات الزرعه edge effect ولكن ممكن تجاوز هذا عن طريق التنعيم الجيد لحافات الزرعه بواسطة مشرط حاد.

مفتاح الكلمات: بروبلاست-مسامات- زرعه-ماده تجميليه

### فرع الجراحة - مستشفى الكاظمية التعليمي

## العلاقة بين الاصابه بالممرضات TORCHو حصول الاجهاض التلقائي المتكرر

### نضال عبد المهيمن ، امال حسين ،فاروق خالد حسن "

#### الخلاصة

خلفية الدراسة: إن الإصابه بداء القطط ،فايروس الحصبه الالمانيه، الفايروس المضخم و فايروس هربس والتي يطلق عليها مجتمعة بـ TORCH، من الممكن ان تتسبب باحهاض المرأه الحامل او اعتلال الاطفال حديثي الولاده. كما ان هذه الممرضات تتسبب بتحييد الاستجابة المناعية للحوامل من Th2 الى Th1 اضافة الى الزياده بما يسمى ب apoptosis الى من الممكن ملاحظته سريريا باجهاض الجنين للحامل المصابه.

هدف الدراسة: التحرى عن وجود علاقه بين الاصابه بهذه الممرضات و حلات الاجهاض التلقائي المتكرر.

المواد و طرائق العمل: تضمنت الدراسه الحاليه مائة وتسع عشرة إمرأة، تراوحت متوسط اعمار هن بين ( $^{\circ}$  ۲۳.9)، تم تقسيمهن الى ثلاثة مجاميع: مجموعة (أ) إجهاض تلقائي متكرّر (RSA) وعدد هن ۲۲ إمرأة و متوسط اعمار هن بينَ ( $^{\circ}$  ۲۸. + ۲۸. )، مجموعة) ب( $^{\circ}$  به القائي غير متكرّر (RSA) وعدد هن ۳۶ إمرأة و متوسط اعمار هن بينَ ( $^{\circ}$  ۲۲. و الجهاض تلقائي غير متكرّر ( $^{\circ}$  د ميطرة (حمل ناجح): وعدد هن ۲۳ إمرأة ومتوسط اعمار هن بينَ ( $^{\circ}$  ۲۳. + ۸۸. ) و مجموعة ( $^{\circ}$  سيطرة (حمل ناجح): وعدد هن ۲۳ إمرأة ومتوسط اعمار هن بينَ ( $^{\circ}$  ۲۳. + ۸۸. ). تم جمع نماذج دم وريدي من كل المرضى وكذلك مجموعه السيطره تم التحرى عن وجود الاضداد النوعيه IgM/IgG، الخاصه بـ IgM/IgG، الخاصه و Cytomegalovirus CMV/IgG IgM Rubella/IgM/IgG , HSV/IgM and ) و ، Toxoplasma

النتائيج: إستناداً الى نتائج فحص ELISA ، أظهرت الدراسة الحالية ان هناك اختلافا معنويا (p<0.05) بين المجموعة (أ) و المجموعة (ج) في نسبة الاصاية (cytomegalovirus ) . ذات المضاد المناعى نوع (م) .

الاستنتاجات: وجد ان هنالك ارتباط بين الاصابة Toxoplasma gondii و الاصابه الاوليه بدكستنتاجات الاجهاض التلقائي المتكرر.

مفتاح الكلمات: تورج ، الاجهاض المتكرر التلقائي، اختبار الاليزا

فرع الأحياء المجهرية [كلية الطب - جامعة النهرين] فرع الأحياء المجهرية [كلية الطب - الجامعة المستنصرية]

#### لمحة على التغيرات الحاصلة في نظام التخثر بسبب عملية فصل البلازما

## زينب محمد حسن ، ميادة سليم محمود ألنعيمي ، حيدر حسن جليل الشمري "

#### الخلاصة

خلفية الدراسة: الفكرة الأساسية لعملية الفصل موضوع البحث هي الإزالة العالية التنظيم والكفوءة لمكونات الدم الدائرة في الجسم إما الخلايا (فصل الخلايا) أو فصل مكون البلازما (فصل البلازما / تبديل البلازما). إن هدف العلاج بعملية الفصل هو إزالة الخلايا الدائرة أو المواد المسؤولة مباشرة عن إحداث عملية المرض باستعمال أجهزة فصل الخلايا الذاتية في والتي تؤمن وتضمن فصل أو عزل مكون واحد أو أكثر من مكونات الدم وإعادة باقي المكونات إلى الجسم. عملية فصل البلازما عن خلايا الدم وإعادة الأخيرة إلى الجسم. تصاحب هذه العملية عدة تغيرات في نظام التخثر عند استعمال نوعين من سوائل التعويض.

هدف الدراس : هدف هذه الدراسه إلى تحديد تأثير: ١. فصل البلازما العلاجي على بعض فحوصات التخثر في حالة استعمال السوائل البلورانية أو سائل البلازما الحديث التجميد المخفف كسوائل معوضة والمقارنة بين هذين النوعين من السوائل. ٢. الحجم الكلي للبلازما المستبدلة خلال عملية الفصل، المسافات بين الجلسات بالأيام وعدد الجلسات على الفحوص المسحبة للتخثر

المرضى وطرائق العمل: ضمت الدراسة ٥٠ مريضاً مصابين بأمراض مختلفة خضعوا لجلسات فصل البلازما العلاجي لأمراض متعددة الذين أحيلوا إلى المركز الوطني لنقل الدم خلال فترة الدراسة الممتدة من شهر شباط ٢٠٠٤ إلى شهر تموز ٢٠٠٤. طريقة غسل البلازما العلاجي تضم ٢-١٢ جلسة بأجهزة فصل خلايا الدم الذاتية بنوعيها (جهاز نوع هيمونتكس أم سي اس + وجهاز نوع فرزينيس اس. تك ٢٠٤ نم استعمال سائلين تعويضيين. عينات الدم جمعت من المرضى قبل وبعد الجلسة الأولى وبعد الجلسة الأخيرة وأجريت عدد من الفحوصات الخاصة بالتخثر مباشرة بعد أخذ العينات. ضمت الدراسة ٢٠ شخص كمجموعة سبطرة.

النتائيج: في مجموعة السائل البلوراني عدد المرضى الذين حدث تغيير حيث أصبح أوقات البروثرومبين والثرمبوبلاستين و

الثرومبين عالي بشكل غير طبيعي بعد فصل البلازما العلاجي ولا يوجد تغيير في اوقات الثرمبوبلاستين والثرومبين في

مجموعة سائل البلازما حديث. في مجموعة السائل البلوراني لوحظ أن هناك علاقة معنوية بين اوقات البروثرومبين و

الثر مبوبلاستين والثرومبين مع حجم البلازما المستبدل خلال الجلسة على التوالي في حين المسافة بين الجلسات وعدد

الجلسات تربطها علاقة معنوية مع وقت الثرومبين فقط. تركيز الفيبرينوجين في البلازما وعدد الصفائح الدموية تنقص بشكل

حاد ومدة عمل هذه الدراسة ومع ذلك فان العلاقة غير معنوية من الناحية السريرية .

الاستنتاج: السائل البلوراني:سائل خالي من مواد التخثر بالاستطاعة استعماله كسائل معوض بفصل البلازما العلاجي إذا كان حجم البلازما المبدلة قليل. لا يوجد اختلاف معنوي في التغيرات

الخاصة في نظام التخثر في حالة استعمال السائل البلوراني أوسائل البلازما حديث التجميد المخفف كسوائل معوضة. لم تحدث مضاعفات مهمة معنوياً خلال هذه الدراسة وذلك لصغر حجم البلازما المبدلة في فصل البلازما العلاجي.

مفتاح الكلمات: البلازما المجمدة النضرة / تبديل البلازما العلاجي

فرع علم الأمراض / أمراض الدم - مستشفى اليرموك التعليمي فرع علم الأمراض [كلية الطب - الجامعة المستنصرية] فرع علم الأمراض / أمراض الدم [كلية الطب - جامعة بغداد]

## المجلة العراقية للعلوم الطبية ٢٠٠٩ م المجلد ٧ العدد ٤ ص ٤٠ ـ ٦٠ دور الفحص الخلوي للبصمات في تشخيص افات الثدي

### حسنین حافظ خضیر ۱، طاهر هورامی۲، کُوران محمد رؤوف۱

#### الخلاصة

خلفية الدراسة: التشخيص الخلوي السريع باستعمال الطبعات للاورام المختلفة وخصوصا اورام الثدي قد استخدم للمرة الاولى عام ١٩٢٧ من قبل Dudgeon and Patrick . ان الدقة التشخيصية لهذا الفحص قد اخذت بالتزايد وخصوصا تلك المستعملة لامراض الثدي وكذلك لبقية اجزاء الجسم وبمعدل يتراوح بين (٩٠ -٩٤ %) وفي السنوات الاخيرة وصل الى) ٩٠-٩٨ %).

هدف الدراسة: اجريت هذه الدراسة للتعرف على دور هذه الطريقة في تشخيص المرضى من الذكور والاناث و المصابين بافات الثدي .

**طريقة العمل:** اجري الفحص الخلوي للطبعات من حزيران ٢٠٠٥ الى كانون الثاني ٢٠٠٦ والمأخوذة من ١٠٤ مريضا والمصابين بمختلف افات الثدي بعد صبغها بصبغة الهيماتوكسلين والايوسين وبالطريقتين السريعة والروتينية.

النتائيج: كانت الحساسية والدقة النوعية للفحص الخلوي باستخدام الطبعات (٩٣.٦ %) و (١٠٠ %) على التوالي بينما كانت الدقة التشخيصية الكلية (٩٨.٩ %).

الأستنتاج: أن الفحص الخلوي باستعمال الطبعات هو فحص بسيط وسريع ودقيق وغير مكلف ويمكن استعماله لتشخيص افات الثدى داخل غرفة العمليات .

مفتاح الكلمات: الفحص الخلوي ، الطبعات، افات الثدي

فرع الامراض [ كلية الطب - جامعة السليمانية ] فرع الجراحة [كلية الطب - جامعة السليمانية]

## المجلة العراقية للعلوم الطبية ٢٠٠٩ م المجلد ٧ العدد؛ ص٦٦- ٦٦ تقدير التأثير المثبط للعسل على بعض العزلات البكتيرية

#### رائد طه النعمة

#### الخلاصة

خلفية الدراسة: لقد اثبت ان للعسل تأثير مثبط على ما يقارب ٦٠ نوع من البكتريه والتي تشمل البكتريا الهوائية، البكتريا اللاهوائية، البكتريا السالبة لصبغة جرام.

**هدف الدراسة**: أجريت هذه الدراسة بهدف التحري عن فعالية المضاد الجرثومي لنموذج العسل في منطقة البصره ضد بعض العزلات البكتيرية.

طريقة العمل :استخدمت أربعة تراكيز ( ٢٥%و ٥٠%و ٥٧%و ١٠٠%) من عينات العسل لمعرفة فعالية المضاد البكتيري لها وذلك باستخدام بعض الجراثيم المهمة طبيا مثل بكتريا القولون المعوية، عصيات السيدوموناس والمكورات العنقودية الذهبية. وتم تحديد اقل تركيز مثبط من عينات العسل باستخدام طريقة الوسط السائل المخفف.

النتائيج: أظهرت الدراسة تأثير مثبط لنموذج العسل خارج الجسم بتركيز ٥٠% و ٧٥% و ٢٠٠% على البكتريا المستخدمة في الدراسة، كما أظهرت الدراسة أن بكتريا المكورات العنقودية الذهبية لا تتأثر بتركيز ٥٠% في نموذج العسل. وبينت الدراسة أن جميع البكتريا المستخدمة لا تتأثر بتركيز ٢٠% من نموذج العسل. كما أظهرت الدراسة بأنه اقل تركيز مثبط لنمو بكتريا القولون المعوية، عصيات السيدوموناس والمكورات العنقودية الذهبية كان مرح ٢٠٪ ملغ/مل و ٢٠٠٥ ملغ/مل و ٢٠٠٥ ملغ/مل على التوالى.

الاستنتاج: بينت الدراسة أن العسل يشابه المضادات الحيوية من حيث تأثيره على الإحياء المجهريه و يمثل العلاج البديل لبعض أنواع البكتريا. حيث يمثل مضاد بكتيري واسع الطيف ضد أنواع البكتريا ( البكتريا السالبة لصبغة جرام و البكتريا الموجبة لصبغة جرام).

مفتاح الكلمات: المكورات العنقودية الذهبية، بكتريا القولون المعوية، عصيات السيدوموناس، عسل، مضادات حيوية، حساسية، مضاد بكتيري.

#### فرع الاحياء المجهرية [ كلية طب الاسنان - جامعة البصرة ]

## دراسة سلَسِ البولِ بسبب حالب منزاح بكلية الشاذّة النمو في سيدة عمرها ٢٢ سنة " تقرير حالة "

#### علا محمد الكواز

#### الخلاصة

ان البول مستمر الثقطير الذي يحدث سوية مع عملية التبوّل الطبيعي هي الصورة الكلاسبكية التي يأتي بها حالات الحالب المنزاح في البنات. نُقدّمُ حالتنا لسيدة تبلغ من العمر ٢٢ سنة قدّمت إلى قسمنا بسلس بولي مستمر مدى الحياق. العمل التشخيصي مُتضمّن: الاشعة الملونة الوريدية السونار ، اشعة تلوين المثانة، تصوير بالرّنين المغناطيسي وتنظير المثانة، بالإضافة إلى فحص شامل للأعضاء التناسلية الخارجية تحت التخدير العام حالتنا مثلت حالب منزاح الذي ينتهي بالمهبل صادرا من نظام الكلوي شاد النمو الوحيد وكان الخيار العلاجي المتاح هواستئصال الكلية الضامرة مع الحالب المنحاز . السلس البولي في البنات بسبب حالب منزاح مرض غير شائع . خمس وثمانون بالمئة من الحالات ترتبط بالمضاعفة الكلوية . ؟ وفقط ١٥ % مِنْ الحالب المنزاح يَجيء بالنظام الوحيد.

مفتاح الكلمات: سلس البول ، حالب منزاح ، مضاعف الجهاز البولي .

#### فرع الجراحة [ كلية الطب - جامعة النهرين ]

## إنفجار الزائدة الدودية خلف البريتون كحالة مرضية لخراج الفخذ الأيمن " تقرير حالة "

### علي جليل عواد '، حسن هادي السكافي'

#### الخلاصة

حالة مرضية لإنفجار الزائدة الدودية خلف البريتون تظهر كخراج للفخذ الأيمن كما هي موصوفة في هذه الحالة. موصوفة في هذه الحالة. مفتاح الكلمات: إلتهاب الزائدة الدودية المعقد ، إنفجار خلف البريتون ، خراج خلف البريتون ، خراج الفخذ.

فرع الجراحة ـ مستشفى الكرامة التعليمي أفرع الجراحة ـ مدينة الطب