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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.

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# Iraqi Journal of Medical Sciences

A Medical Journal Encompassing All Medical Specializations

Issued Quarterly

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## Communication Skill

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Emeritus Professor of Surgery, Head of Accreditation Committee of Iraqi Medical Colleges

### Introduction

A large and compelling evidence base in communication science shows that communication is vitally important to doctors and patients. However, changes are needed in the attitudes and skills that underlie the way doctors communicate. For this reason, training in communication skills has become an increasingly prominent part of undergraduate and postgraduate medical training.

It has been found that the communication skills of medical students who have not had this training actually get worse as they progress through medical school. So, whether you are a specialty trainee, Rotator doctor, or

medical student, it is never too soon to start fine tuning your skills. Doing this will give you a head start in enhancing your personal development and in progressing your professional career.

### What is effective communication all about?

Nowadays, we're getting good at making the most of what we have—we are all mindful of delivering efficient services with scarce resources. However, we are not so good at making the most of what we are. In terms of communication, this means being able to give people the information they need in a clear and concise manner and with the right attitude. Good communication leads to

more satisfying interaction with colleagues, helps you to manage your time better, and makes you a more effective team member and leader.

Learning to communicate effectively means making the most of every opportunity to interact with others: to be positive and encouraging to your team, to show empathy and concern to your patients, and to be able to deal with demands and difficult emotions. Having an understanding of what type of communicator you are and being able to identify the ways in which better communication can lead to better outcomes will help you to maximise your personal effectiveness in many different situations, giving you the advantage in interviews, assessments, and in the day to day work.

### **What is needed?**

At no stage in our careers should we stop developing and learning about communication. Research has shown that poor communication can contribute to, dissatisfaction among patients, lack of

compliance, and medicolegal problems. Improved communication skills could have a positive effect on all these.

Curriculum changes at medical school have led to a much earlier focus on the teaching and assessment of communication skills. Throughout your medical career, your interactions with others will be observed and measured through exams, supervision, and appraisals. In the rotation years you will be expected to develop generic communication skills

At interview, your leadership skills, initiative, empathy, and team playing might be tested—how you motivate others, negotiate, and deal with conflict.

How does patient feedback influence your practice? How do you manage stress? These are questions about communication skills. Knowing some of the theories and research in the field will help you to become more confident in discussing the underlying issues. In this way, improving your communication skills raises the profile of other areas of your portfolio.

At all stages of your medical training there is an expectation that you can identify your weaknesses and discuss plans for improvement.

Knowing your own strengths and weaknesses, it entails realizing the effect of your behavior on others and the influence of your own emotions and prejudices on your judgments and behavior. The aim of increasing self-awareness is to be able to manage the impact of your emotions in your day to day practice—and to improve your relationships overall.

### **Advise for effective communication**

- **Use clear language:** tailor your language to your patients' understanding and information needs.

- **Be conscious of your non-verbal communication:** It is important to maintain eye contact—reading notes or looking at the computer screen may convey negative messages.

- **Negotiate an agenda:** Ask patients what they need from the consultation, and explain what can be covered.

- **Establish a dialogue:** Determine whether your patient agrees with the diagnosis and management plan. Patients who disagree with the diagnosis probably won't adhere to the treatment.

- **Be flexible in your consultation style:** Tailor your approach to the individual patient. A more directive style may be appropriate for patients who want less involvement in decision making. A supportive style—listening attentively and asking questions about psychosocial issues—helps facilitate the disclosure of sensitive information.

- **Provide the information that patients want:** Doctors tend to talk too much about drug treatment, whereas patients want to know about causes and the likely diagnosis and prognosis. They want more openness about side effects and advice on how to relieve pain and emotional

distress and what they can do for themselves. Providing this information helps their symptoms, reduces distress, improves physiological status, reduces hospital stay and use of analgesia, and improves quality of life.

- **Reflect on the outcomes of your interactions with others:** Why do some doctors work well and others not so well? Communication difficulties are one of the main reasons that patients complain about doctors. The most common criticism is not about the doctors' competence but that they have failed to listen or to offer sufficient explanation.

- **Apologize when mistakes occur:** Apologizing and expressing regret at the suffering experienced by a patient is not an admission of liability. Ineffective communication is the single largest factor behind litigation by patients. Good communication, including effective apology, It never does any harm to

apologize—for yourself or on behalf of colleagues.

- **Empathies and listen:** Your relationship with the patient is vitally important. It facilitates therapeutic space in which patients can express their concerns and receive support and advice. Empathy is the ability to understand what another person is experiencing and to communicate that understanding to the person. As the patient begins to relate his or her story, it is necessary to silence our own internal talk, including the diagnostic reasoning process, which can interfere with our ability to listen.

- **Mindful practice:** This is your ability to observe not only the patient but your own performance during the consultation. Mindful doctors can easily be identified by patients and colleagues—they are present, attentive, curious, and unhindered by preconception.

- **Establish rapport:** Recognition and explicit acknowledgment of the emotional content in your patient's story is particularly important in establishing rapport. Doctors often respond to emotional cues by offering premature reassurance, explaining away distress as normal, attending to physical aspects only.

## Conclusion

Communication is important in all aspects of your training, and learning more about communication skills will help you perform better in exams, assessments, interviews, and appraisals—as well as in your day to day practice. Maximising your effectiveness in communication not only enhances your personal performance in many different spheres but also improves your relationships with patients and facilitates career progression.

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## Semi-automated Computational Method for Skeletal Muscle Fiber Typing with Lectins: Correlation with Morphometric Studies

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### Abstract

- Background** Muscle fiber typing has been an extensive field of study for many years. Though, limited researches applied lectin histochemistry in the clinical diagnosis of muscle disorders; attention was directed mainly towards enzyme histochemistry.
- Objective** The use of lectins as recognition systems based on specific protein-carbohydrate interactions in correlation with muscle fibers morphometric standards and optical density features to favor the diagnostic procedures of muscle disorders.
- Methods** Cross-sections of tibialis anterior muscle from 15 adult rats were stained with Con A, PNA, SBA, WGA, SWGA, LFA, UEA-I, and UEA-II lectins. Photographs of stained sections were analyzed with ImageJ 1.44 software for muscle fiber area, perimeter, optical density, and integrated density.
- Results** There were statistically significant differences between the parameters of muscle fiber types under study ( $P < 0.05$ ) concerning Con A, LFA and UEA-II lectins, but not for the remaining lectins, regarding the optical density and integrated density of muscle fibers.
- Conclusions** Lectins make accurate recognition of muscle fiber types on fixed paraffin sections when combined with computerized methods to quantify the features seen in muscle biopsies destined for pathological investigations.
- Key words** Lectins, Muscle fiber typing, Quantitative, Optical density, Morphometry

### Introduction

Skeletal muscles are composed of a large number of muscle fibers which differ in their histochemical and physiological properties. Although overlap exists between different fiber types, they can be grouped into Type I fibers (oxidative, slow contracting, and fatigue resistant), Type IIA, or intermediate fibers (oxidative-glycolytic, fast contracting, and fatigue resistant) and Type IIB fibers (glycolytic, fast contracting, and susceptible to fatigue)<sup>(1)</sup>. Muscles, in man, are all of the mixed variety; different types of fibers are not readily distinguished in sections stained by conventional histological techniques, but they are seen quite clearly with the use of more specialized

histochemical methods<sup>(2)</sup>. In animals, the histochemical-type fiber designated intermediate resembles, to some extent, human Type IIA fibers<sup>(3)</sup>.

Many studies have employed enzyme histochemistry in detecting the metabolic activities of muscle fiber types<sup>(4)</sup>. In addition, some immunocytochemical techniques use fluorescent or enzymatic-labeled antibodies to distinguish different types of fibers; these techniques take the advantage of the ability to probe the myosin composition of fibers in which more than one isoform is present as in human limb muscle with features intermediate between Type IIA and Type IIB<sup>(5)</sup>. Thus, expanding the classification of Type II fibers into more than A

and B subclasses results in the necessity to re-designate human Type IIB fiber as Type IIX<sup>(6,7)</sup>.

Histochemical assessment of the different fiber types in muscle tissue by morphological investigation of muscle biopsy specimens is essential for diagnosis of certain neuromuscular disorders<sup>(8)</sup>. Determination of muscle fiber types is routinely performed on cryostat-sectioned, unfixed muscle biopsy specimens, using enzyme histochemical reactions; because fixation and embedding destroy this enzyme activity, alternative procedures are preferable for routinely processed specimens<sup>(9)</sup>.

Lectins, on the other hand, have come a long way since their first detection in plants as hemagglutinins to their present status as ubiquitous recognition molecules with myriad exciting functions and applications<sup>(10)</sup>. Lectins are proteins that bind different carbohydrate motifs. They are important reagents used for studies of changes in the carbohydrate composition of glycoproteins and proteoglycans. Lectins are of non-immune basis; they agglutinate cells and/or precipitate glycoconjugates. These substances bear at least two sugar binding sites. Lectins have no enzymatic activity, may be soluble or membrane bound, and are of bacterial, animal, or plant origin<sup>(11)</sup>. Such recognition systems based on specific protein-carbohydrate interactions, using carbohydrate-specific tools, can be favorable contributes to diagnostic procedures and to investigations of cell biological processes<sup>(9)</sup>.

There is considerable evidence that lectins are involved in many physiological and pathological events so that cellular protein glycosylation pattern is influenced by several changes, such as the occurrence of disease. Thus, the altered glycoform population of a given glycoprotein may be diagnostic of the disease responsible for the alteration itself<sup>(12)</sup>. Such abnormal glycosylation has been detected in significant diseases including cancer development in different tissues<sup>(13)</sup>. For example, the quantitative precipitation method of Con A - carbohydrate interaction was used by Basu and coworkers<sup>(14)</sup> for differentiation between

prostate cancer and benign prostatic hyperplasia, a fact that highlights the growing importance of the use of lectins in the medical field.

These findings are related to the ability of lectins to stain selectively specific structures, especially membranes. In addition, lectins have the capacity to probe specifically the glycoconjugate composition and distribution in cells of the biopsied human muscle, making the application of lectin histochemistry a useful tool in the investigation of muscle disorders<sup>(15)</sup>.

This work aims at investigating the plausibility of using lectin stains for quantitative muscle fiber typing on fixed paraffin sections in correlation with the morphometric standards and optical density features of these fibers. Some clinical implications of such use of lectins in muscle fiber typing are also discussed.

### Methods

A sample of 15 adult rats (8 males and 7 females) *Rattus rattus norvegicus albinus* aged 3 months was selected on the basis of being apparently active and healthy, with 300±50 g body weight. Animals were housed 2 per cage and fed standard diet pellets. Cages were 60 cm length by 30 cm width. Animals were anaesthetized with chloroform-impregnated cotton-wool in airtight jars for 2-3 minutes prior to decapitation. Muscle samples were obtained from the freshly sacrificed rats by open excision biopsy. The right and left counterparts of tibialis anterior muscle were chosen in this study for sectioning.

Tissue samples were prepared by fixation with Bouin's solution for 16 hours at room temperature (22 °C) followed by impregnation in paraffin. This fixation protocol is recommended by Allison<sup>(16)</sup> in order not to affect the tissue binding sites of lectins. Cross sections of 10 µm thickness were made ready for staining.

Eight types of lectins, Concanavalin A (Con A) from *Canavalia ensiformis* (jack bean) – a mannose binding lectin, peanut agglutinin (PNA) from *Arachis hypogaea* (peanut) and soybean agglutinin (SBA) from *Glycine max* (soya bean) –

galactose binding lectins, wheat germ agglutinin (WGA) and succinylated wheat germ agglutinin (SWGA) from *Triticum vulgare* (wheat) – glucose binding lectins, limax flavus agglutinin (LFA) from the slug *Limax flavus* with sialic acid affinity, and *Ulex europaeus* agglutinin I and II (UEA-I and UEA-II) from *Ulex europaeus* – fucose binding lectins, all from Sigma™ USA were used for staining as follows:

1. Dewaxing the paraffin sections in xylene for 20 minutes then hydrating them through descending concentrations of ethanol alcohol (99%, 90%, 70%, 50%, 30%) each for 3 minutes.
2. Hydrated paraffin sections were washed in normal saline solution (0.9% NaCl) for 10 minutes.
3. In order to keep the slides flooded by the lectin-normal saline solution, the slides were cleaned at the margins of each section by cotton wool to isolate the sections. Spillage of the lectin-normal saline solution was prevented through fitting each slide in a prepared pit on a sheet of softened dental wax.
4. Sections were flooded by lectin-normal saline solution and kept for 90 minutes in a humid chamber, protected from light.
5. Lectin-stained slides were washed in normal saline solution for 20 minutes and mounted in non-fluorescent fractoil (BDH) mountant.

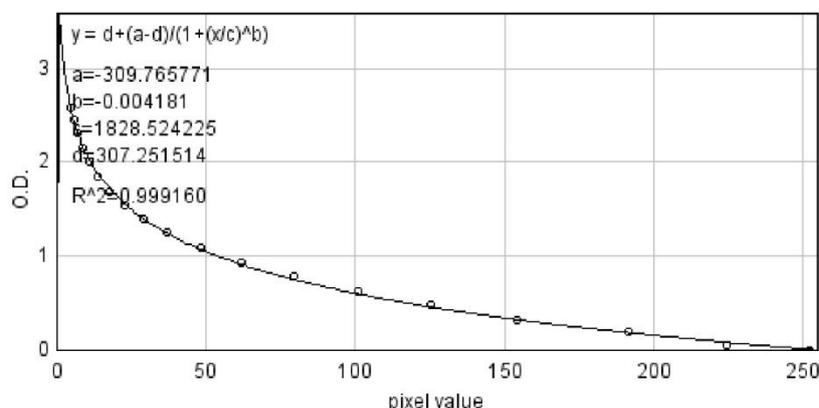
Examination of sections was done with Olympus fluorescent microscope (400X) using a systemic

random selection of 5 fields per section. Digital photographs with a camera factor of 3 were taken for the selected fields; these photographs then were transferred to a PC with installed Image 1.44 software. In each field, 10 muscle fibers from each type (I, II, and Intermediate) were identified qualitatively depending on their eye-observed fluorescence intensity<sup>(17)</sup>, and their margins were traced with an optical mouse.

Muscle fibers were segregated into three groups according to their area (area of selection in calibrated square micrometers) and perimeter (length of the outside boundary of the selection in calibrated micrometers) to be given the designation Type I, Type II, and Intermediate, which is in agreement with the previous results of Al-Kaabi work<sup>(18)</sup>.

While in order to measure the optical density of muscle fibers lectin staining, the gray scale level was calibrated according to a standard optical density step tablet<sup>(19)</sup>. A calibration curve is displayed in figure 1. With tracing of each muscle fiber, the Mean Gray Value within the selection (the sum of the gray values of all the pixels in the selection divided by the number of pixels) was reported in calibrated units (optical density). For red-green-blue (RGB) colored images, the mean was calculated by converting each pixel to gray scale using the formula:

$$\text{Gray} = (\text{Red} + \text{Green} + \text{Blue}) / 3$$

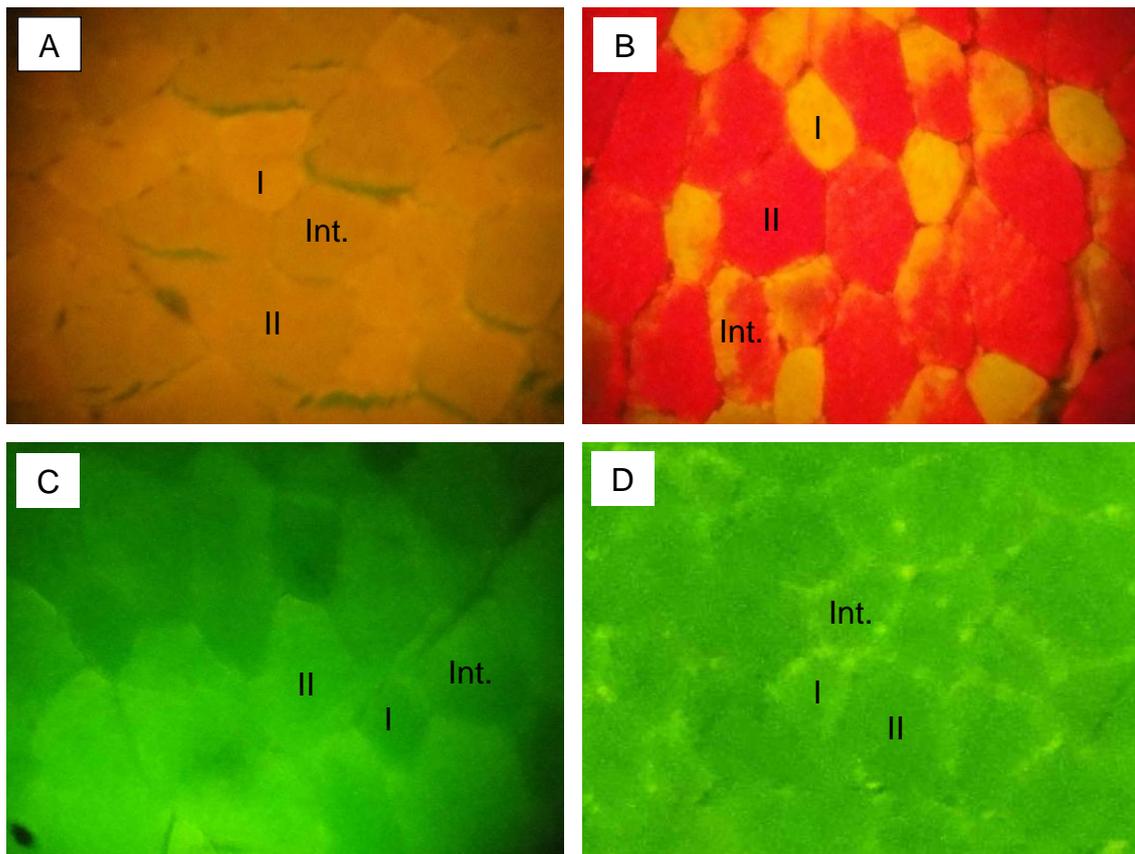


**Figure 1. Calibration curve of a standard optical density step tablet. The Mean Gray Value within the selection is displayed in calibrated units representing the optical density of muscle fibers**

The calibrated Mean Gray Value of the pixels in the selection was thus calculated, representing the optical density of that muscle fiber, and the Integrated Density (the sum of the values of the pixels in the image or selection, which is equivalent to the product of Area and Mean Gray Value) was measured. Statistical analysis was performed with Microsoft Office Excel® 2010 tool.

**Results**

The mosaic pattern of muscle fibers was demonstrated with sections stained with Con A, LFA, and UEA-II lectins as shown in figure 2. However, PNA, SBA, WGA, SWGA, and UEA-I lectins did not express apparent muscle fiber typing. Such qualitative typing was more easily observed in sections stained with LFA lectin than those stained with the lectins Con A and UEA-II.



**Figure 2. Cross sections of rat tibialis anterior muscle stained with lectins. (A) Con A, (B) LFA, and (C) UEA-II demonstrate fluorescent mosaic pattern of muscle fiber types (I, II, and Intermediate (Int.)). (D) SBA labeled section reveals absent mosaic pattern of muscle fiber types. 400 X. Bar = 50 μm**

Table 1 shows the averages of the muscle fiber area, perimeter, optical density, and integrated density for each muscle fiber type stained with Con A, LFA, and UEA-II lectins. Comparison between the optical density and integrated density of muscle fiber types was done with a single factor ANOVA. The results revealed statistically significant differences

between the various muscle fiber types ( $P < 0.05$ ) in regard to the studied parameters with Con A, LFA and UEA-II lectins. On the other hand, PNA, SBA, WGA, SWGA, and UEA-I lectins gave statistically insignificant results in relation to the optical density and integrated density of muscle fibers.

**Table 1. Averages of muscle fiber area, perimeter, optical density, and integrated density for Type I, Type II and Intermediate muscle fibers stained with Con A, LFA, and UEA-II lectins**

Lectin	Fiber Type	Area ( $\mu\text{m}^2$ )	Perimeter ( $\mu\text{m}$ )	OD <sup>†</sup>	ID <sup>††</sup>
Con A	I	2437.0 $\pm$ 469.6 <sup>‡</sup>	199.6 $\pm$ 21.1	97.9 $\pm$ 9.3	239019.4 $\pm$ 48339.8
	II	7123.2 $\pm$ 1079.8	338.9 $\pm$ 58.9	83.0 $\pm$ 11.1	593741.2 $\pm$ 141408.2
	Intermediate	3350.6 $\pm$ 583.8	228.3 $\pm$ 51.5	76.8 $\pm$ 20.9	256623.9 $\pm$ 81923
LFA	I	2711.0 $\pm$ 251.7	209.6 $\pm$ 15.1	122.6 $\pm$ 10.9	333457.0 $\pm$ 51420.0
	II	7018.7 $\pm$ 788.6	332.4 $\pm$ 46.5	97.2 $\pm$ 8.1	681643.3 $\pm$ 89489.9
	Intermediate	3975.5 $\pm$ 910.5	255.1 $\pm$ 48.3	111.7 $\pm$ 5.8	442741.6 $\pm$ 95177.0
UEA-II	I	2854.6 $\pm$ 514.0	212.4 $\pm$ 16.4	40.7 $\pm$ 11.6	116973.1 $\pm$ 42491.2
	II	8641.7 $\pm$ 1598.2	368.1 $\pm$ 44.3	65.7 $\pm$ 15.6	562837.7 $\pm$ 154479.5
	Intermediate	4665.6 $\pm$ 1230.6	269.9 $\pm$ 47.3	32.9 $\pm$ 7.8	156830.6 $\pm$ 65460.7

<sup>†</sup> OD = Optical Density, <sup>††</sup> ID = Integrated Density, <sup>‡</sup>  $\pm$  Standard Deviation

## Discussion

In our results, three lectins (Con A, LFA, and UEA-II) showed the mosaic pattern of muscle fibers in concordance with previous works<sup>(17)</sup>. Though these three lectins gave statistically significant difference between the optical density of the three types of muscle fibers, LFA stain was qualitatively better for faster eye recognition of the chess-board appearance of muscle sections as the red-yellow discrimination of colors was easier for the eye to catch, a point in favor of using the computerized methods for the detection of this typing as qualitative examination may be subjective and subtle in the differentiation between these types of muscle fibers. Such quantitative computerized methods have been used for fiber parameters other than the optical density where muscle fiber size was analyzed automatically<sup>(20)</sup>.

Five of the lectins used in this study (PNA, SBA, WGA, SWGA, and UEA-I) revealed weak, homogeneously distributed sarcoplasmic reaction. It is interesting that these lectins have different sugar preferences, even though, they failed to stain muscle fibers with different intensities; it could be due to the narrower pattern of sugar agglutination in comparison with Con A, LFA, and UEA-II lectins.

Since complex glycoconjugates are of importance for a number of biological events such as interaction between cells, growth,

development, and changes in function of cells, the lectin-staining pattern in skeletal muscle is considered to be very complex; it might be related to development, specialization, and function of the individual muscles<sup>(17)</sup>.

Con A has an affinity for cell surface  $\alpha$ -D-mannosyl and  $\alpha$ -D-glucosyl glycoproteins<sup>(21)</sup>, LFA lectin reacts with any sialic acid linkage<sup>(22)</sup>, and UEA-II has an affinity for N,N'-diacetylchitobiose<sup>(23)</sup>. In our results, the optical density of Con A stain was more in type I muscle fibers followed by type II then the intermediate, LFA stain optical density was maximum in type I followed by the intermediate then type II, and the optical density of UEA-II stain was highest in type II followed by type I then the intermediate. In other words, mannose, glucose and sialic acid residues were expressed in higher concentrations in type I slow contracting, fatigue resistant muscle fibers, while fucose glycoconjugates were abundant in type II fast contracting, fatigue susceptible fibers. It indicates that muscle fiber types express different levels of glycoconjugates according to their functional specialization, reflected by the different intensities of lectin stains in terms of optical density.

This use of quantitative methods paves the road for the integration of computer programs into more sophisticated analyses regarding glycoconjugate expression detected by lectin

stains; the expanding importance of the ability of lectins to distinguish between delicate variations of oligosaccharide structure makes them perfectly suitable as decoders for such carbohydrate-encoded information. Thus, whilst sugars are able to carry the biological information, lectins are capable of interpreting this "glycocode"<sup>(12)</sup>.

Lectins with binding sites for sialic acid are known to stain the sarcolemma, connective tissues, and blood vessels in both normal and dystrophic muscles, though no difference was reported between the two<sup>(8)</sup>. Recent methodology, in addition, has characterized a fast, reliable, and inexpensive method of fluorescent lectin staining that can be used for skeletal muscle fiber and general connective tissue visualization during immunofluorescent analysis<sup>(11)</sup>. This indicates that the sialic acid lectins such as LFA can be used to delineate muscle fiber types with their surrounding connective tissue and capillary network in normal and diseased muscles with the advantage of ease of fiber typing and accurate demarcation of morphometric features.

Latest studies have incorporated different methods in order to reach new approaches for the diagnosis of muscle disorders. These methods include combined immunofluorescence techniques<sup>(24)</sup> or individualized lectin staining<sup>(8)</sup>. The use of UEA-II, for example, forms the first model of a legume lectin with an unrestrained binding site and illustrates the importance of hydrophobic interactions in protein-carbohydrate complexes. Together with other known legume lectin crystal structures, it shows how different specificities can be grafted upon a conserved structural framework, so that the different isoforms of myosin may be investigated to yield fiber typing not readily distinguished by currently used histochemical methods<sup>(25)</sup>. Thus, fucose or chitobiose specific lectins can be now considered under investigation for their significance in muscle fibers, especially during physiological or pathological changes.

In conclusion, lectins are "decoding" the way that how cells are recognizing and interacting

with other cells in addition to revealing the exceptional features at the cell membrane and cytosol. The unique glycoconjugates present at the different types of skeletal muscle fibers make the recognition of these types accurate when combined with computerized methods that quantify the percentage of muscle fiber types in larger muscle biopsies destined for pathological investigations. The use of various lectins with overlapping specificity is recommended for staining fixed paraffin sections in order to reach exact identification of muscle fiber types and to make a precise diagnosis when combined with quantitative computerized methods. The role of individual lectins in distinguishing subtle changes in disease conditions of muscle fibers is yet to be studied.

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## Exocrine Pancreas under the Effect of Glucocorticoids: Histological and Morphometry Study

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### Abstract

- Background** The histological changes obtained from the effect of glucocorticoids on exocrine pancreas is well known but little is known about the morphometrical changes on the acinar cells and ducts which are associated with the histological findings.
- Objective** To reveal these morphometrical changes which are associated with the histological changes occurred on the exocrine pancreas by the use of different doses and durations of dexamethasone.
- Methods** Healthy female rabbits were used and divided to six groups. The first four groups regarded as the treated groups, they received different doses and different durations of dexamethasone sodium phosphate. The fifth and sixth group considered as the control group. The pancreas obtained from these animals were processed to make a paraffin blocks, the slides obtained were stained by H&E stain, and Masson's acid Fuchin Aniline blue Trichrome stain (MT). Diameter of serous acini and intercalated ducts were measured, height of acinar cells and intercalated duct cells and counting of serous acini number in a unit area were estimated.
- Results** Histologically, by H&E, degenerative changes of serous acinar cells were evident which were dose and duration related. By MT stain there was decrease in collagen fibers in the interlobular spaces. Morphometrically, the diameter of serous acini, height of acinar cell and glandular density were decreased when the dose and duration of treatment were increased.
- Conclusion** There was increase in exhausted acinar cells as compared to metabolically active cells when the dose and duration of dexamethasone sodium phosphate treatment were increased.
- Key words** Exocrine pancreas, glucocorticoids and morphometry.

### Introduction

Many previous studies showed that the glucocorticoid affects the cells of various organs of body<sup>(1)</sup>. Glucocorticoids mediate their effects through a specific intracellular receptors present in almost all cell types including exocrine pancreatic tissues<sup>(2)</sup>. So the glucocorticoids can induce cell maturation, cell differentiation or even cell death (apoptosis)<sup>(3)</sup>. Therefore glucocorticoid acts directly on pancreatic acinar cells<sup>(4)</sup>. These are accomplished by their effects on increasing

amylase and trypsin activities which depend on the dose and duration of steroid treatment<sup>(5-7)</sup>. The histological effects of glucocorticoids on exocrine pancreas were demonstrated by some workers<sup>(8)</sup> as enlarged lumina of the acini, zymogen depletion of acinar cells, and abundance of cytoplasmic vacuoles with deformation and distortion of the nucleus and cytoplasm. But others suggested that hydrocortisone- treated animals showed a higher density of zymogen granules in the acinar cells and an increased number of autophagic vacuoles in the pancreatic acinar cells<sup>(9)</sup>.

The glucocorticoids had a dual effects on exocrine pancreas so that glucocorticoids in appropriate doses has a stimulating effect upon the acinar cell function, whereas large doses of these drugs reduce the exocrine tissue secretory function and affecting its protein-synthesizing capacity, also the corticosteroid deficiency is accompanied with morphofunctional atrophy of the exocrine region of the pancreas<sup>(10)</sup>. In contrast; Other workers demonstrated this dual effects of glucocorticoids on the rat pancreas: inhibition and potentiation by that chronic treatment with large doses of glucocorticoid may sensitize the acinar cells and induce hypersecretion of trypsin and lipase, whereas acute treatment inhibit the secretory function of exocrine pancreas<sup>(11)</sup>. Puccio *et al.*<sup>(12)</sup> observed that hydrocortisone induced pancreatic hypertrophy and significantly increased enzymatic activities independent to the dose used, but with high dose, there was a diminution of both cell size and number of zymogen granules associated with increased excretion of secretory products, in contrast to that, the low dose did not modify acinar cell size and they caused a significant increase of the number of granules per cell. They concluded that hydrocortisone was an important modulator of pancreatic development in the rat by inducing stimulation of pancreatic activities associated with modifications in cell structure and components and this varied according to the dose.

The glucocorticoids are required for the maintenance of the structural integrity of the exocrine pancreas, in addition to estrogens and other factors from adrenals or testes. So that the exocrine pancreas of the castrated-adrenalectomized rats showed changes in the shape of the acini with widening of intralobular and interlobular spaces, partial depletion of zymogen granules and reduction of acinar lumen size. However, by giving glucocorticoids, the histological findings were changed and they concluded there was enlargement of lumen of the acini, increasing in zymogen granules content of acinar cells, so the glucocorticoids are

one of the important factors for the structural integrity of the exocrine pancreas<sup>(13)</sup>.

This study aims to evaluate the effect of dexamethasone on the rabbit's exocrine pancreas histologically and morphometrically.

## **Methods**

### **Histology:**

Healthy White New Zealand female rabbits weighing between 1000-1250 g were used and kept in separated plastic cages and fed *ad-libitum*. The animals were divided into five groups, seven animals in each. The first group was treated daily for 10 days with (0.5 mg/kg b.w.) intramuscular injection of dexamethasone sodium phosphate (ZMC import- export GmbH Germany) in the thigh muscle. The second group treated with (1.5 mg/kg b.w.) of the same reagent for 10 days, third group received (0.5 mg/kg b.w.) of dexamthasone for 15 days, the fourth one treated with (1.5 mg/kg b.w.) of dexamethasone sodium phosphate for 15 days, the fifth group considered as control animals (1), they received equal amounts of 0.9% saline solution as intramuscular injections for 10 days. Sixth group of control animals (2) also were received intramuscular injections of 0.9% saline solution for 15 days.

Twenty-four hours after the last injection, the animals were anaesthetized with chloroform. After dissection of the abdomen, the pancreas were removed and the glands were fixed in 10% formaline solution for 24 hrs., dehydrated, cleared, embedded in paraffin and the blocks obtained were sectioned and stained by Haematoxylline and Eosin stain (H&E), and Masson's acid Fuchin Aniline blue Trichrome stain (MT).

Staining methods and techniques were done on the basis of Luna<sup>(14)</sup>.

### **Morphometry:**

On the stained sections, the mean diameter of the serous acini and intercalated ducts with the height of both acinar cells and intercalated duct cells, and the number of the serous acini in a unit area were determined using the Visopan

Projection Microscope. In determination of these parameters a 500 X magnification, which is achieved with the 40/0.65 objective, was used, each division of the measuring ruler of the microscope corresponds to a length of  $2 \mu\text{m} = 0.002 \text{ mm}$ . the unit area in counting of cells was an area of  $0.0144 \text{ mm}^2$ . After completion of measurement operation, statistical analysis was done between treated groups and the control one using unpaired T-test <sup>(15)</sup>.

## Results

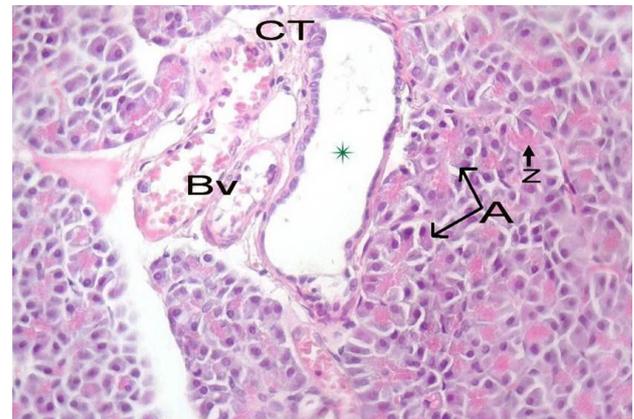
### Histological changes:

The histological findings of all the treated animals showed preservation of the exocrine pancreatic architecture, however the histological changes which were detected differed from one animal to another and even from one pancreatic lobule to another within the same animal.

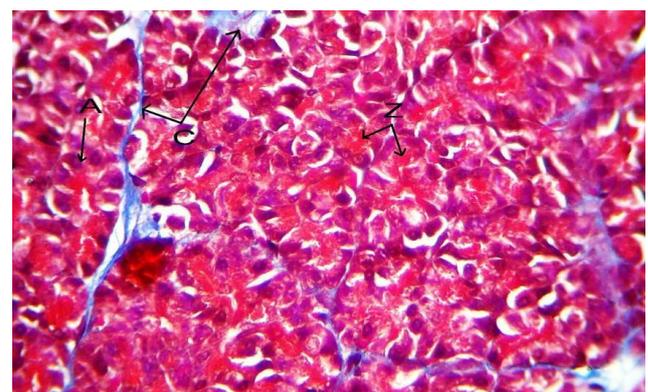
The following histological changes were observed in both H&E and Masson's Trichrome (MT) sections in the treated groups as compared to control one (Figures 1 and 2).

1. Distortion in the arrangement of the acinar cells and ill defined cellular outlines in many areas of the gland, although they were preserved in other areas (Figure 3).
2. There were vacuoles in the cytoplasm of the acinar cells in many areas which were sometimes very large and differ in their size and location (Figure 3).
3. Deformed nucleus and cytoplasm of acinar cells were seen (Figure 3).
4. Evidence of zymogen depletion was observed in many areas, although some areas still preserved little zymogen granules (Figure 3).
5. In most acinar cells, there were irregular nuclei, but in some cells a rounded nuclei with prominent nucleoli were also observed (Figure 3).
6. There were proliferated duct cells in many ductal structures (Figure 4). These changes were seen in all treated groups and started to become to more extent as the dose and duration of treatment were increased starting from the first to the fourth group.

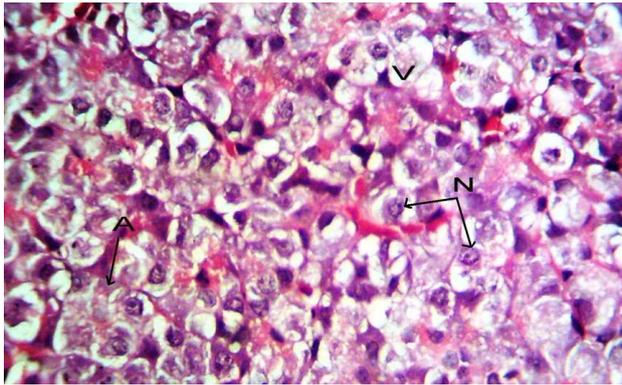
7. Shrinkage of the acinar cells with deformed and pyknotic nuclei (Figure 5). These features start to appear from the second group and become more evident in the third and fourth group i.e. as the dose and duration of treatment were increased.
8. Masson's Trichrome sections shows that the collagen fibers in the interlobular stroma of exocrine pancreas of treated animals were much less than that of control group (Figures 2 and 6).



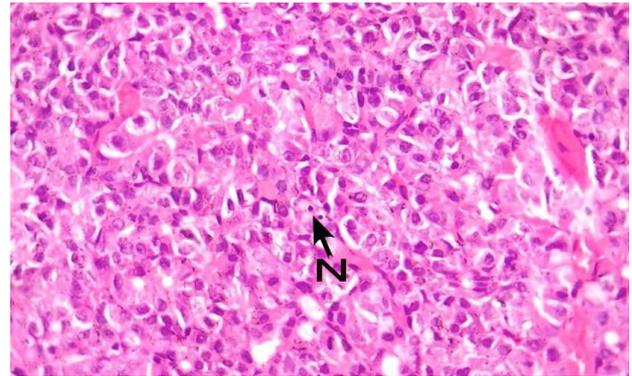
**Figure 1. Light micrograph of the control rabbit exocrine pancreas showing the serous acini (A) containing zymogen granules (Z) and the interlobular duct (\*), blood vessels (Bv) and connective tissue (CT). (H&E X 400)**



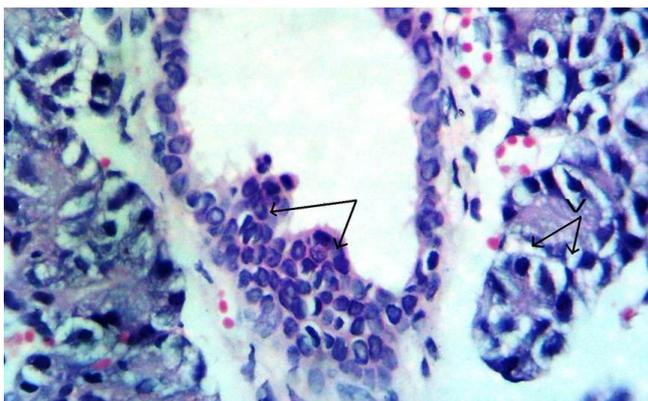
**Figure 2. Light micrograph of the control rabbit exocrine pancreas showing the serous acini (A) containing zymogen granules (Z) and the collagen fibers separating the lobules (C). (MT X 400)**



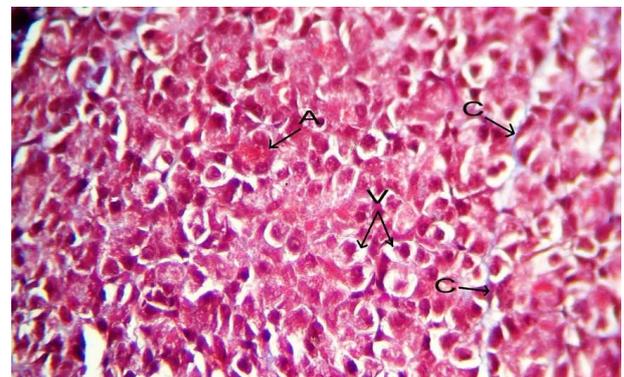
**Figure 3.** Light micrograph of the treated rabbit exocrine pancreas (group 1, 2, 3 and 4) showing distortion in the arrangement of acinar cells (A) with vacuolation (V) and depleted zymogen, deformed nuclei with appearance of prominent nucleolus in some areas (N). (H & E X 600)



**Figure 5.** Light micrograph of treated rabbit exocrine pancreas (group 2, 3 and 4) showing pyknotic nuclei (N) with shrinkage of their acinar cell. (H & E X 400)



**Figure 4.** Light micrograph of treated rabbit excretory duct (group 1, 2, 3 and 4) showing proliferated duct cells (→), serous acini with vacuolation (V). (H & E X 600)



**Figure 6.** Light micrograph of the treated rabbit exocrine pancreas (group 1, 2, 3 and 4) showing distortion in the arrangement of acinar cells (A), vacuolation (V), deformed cytoplasm and nuclei, depleted zymogen associated with decrease in collagen fibers in between the lobules (C). (MT X 400)

#### **Morphometrical changes:**

The following changes were seen and presented in table 1 as numerical data:

1. The diameter of serous acini started to decrease as the dose and duration of dexamethasone treatment were increased and, that change appeared statistically significant in all treated groups.
2. The height of acinar cells also decreased starting from the first treated group to the

fourth one (as the dose and duration of treatment increased).

3. The diameter of intercalated ducts remained near the control value.
4. The height of intercalated duct cells also was not changed.
5. The number of serous acini in a unit area (glandular density) was decreased as the dose and duration of dexamethasone treatment were increased.

Table 1. Morphometrical analysis of exocrine pancreas

Groups	Serous acini		Intercalated ducts		Glandular density (no. of acini)
	Diameter	Height	Diameter	Height	
first group (10 low)	25.23 ± 0.9*	15.5 ± 0.7*	20.3 ± 1.3	5.1 ± 0.3	22.6 ± 0.6
Second group (10 high)	19.45 ± 0.9**	12.0 ± 0.7**	20.1 ± 0.9	5.1 ± 0.3	19.3 ± 0.5**
Third group (15 low)	21.46 ± 0.6**	13.44 ± 0.8**	20.2 ± 1.2	5.2 ± 0.3	20.0 ± 0.5**
Fourth group (15 high)	16.71 ± 0.7**	9.63 ± 0.6**	20.1 ± 0.6	5.1 ± 0.3	17.2 ± 0.7**
Fifth group (Control(1))	29.25 ± 1.2	18.38 ± 1.1	20.5 ± 1.6	5.2 ± 0.3	23.6 ± 0.8
Sixth group (control(2))	29.20 ± 1.1	18.30 ± 1.1	20.4 ± 1.7	5.3 ± 0.4	23.5 ± 0.9

\* Significant difference from control at  $P \leq 0.05$ , \*\* at  $P \leq 0.001$

### Discussion

Two types of cellular responses to glucocorticoid were observed. Some cells showed an increase in their metabolic activity as judged by the rounded nuclei with enlarged prominent nucleoli and zymogen depletion. At the same time in these metabolically active cells, there was a beginning of degenerative processes at the cellular level. These degenerative changes seen, indicated that, the cells ceased the protein synthesis and are in the process of becoming exhausted or non functional and therefore led to increase cellular turn over, which improved morphometrically by decrease in the diameter of serous acini and decrease in the height of acinar cells since the reduction in the zymogen production (decrease protein synthesis and loss of zymogen granules) which occurred made the apical area of acinar cells depleted from zymogen and shrinkage occur, therefore, decreased the height of cells and in turn the diameter of acini<sup>(16-18)</sup>. These findings were in agreement with the results obtained by Finkelbrand *et al*<sup>(8)</sup> who divided these cells into two types, dark cells mean the metabolically active cells and the light cells, which are the cells, ceased their protein synthesis and become exhausted.

The results were dose and time dependent, i.e. when the period of treatment was elongated and the dose administered was increased, the exhausted cells or non-functional cells were increased and the metabolically active cells decreased and even rarely seen, as well as morphometrically there was a decrease in the glandular density (number of serous acini in unit area) which confirmed that the exhausted cells

were increased and the active cells decreased as the dose and duration of dexamethasone given increased.

The depleted zymogen granules observed in this study were due to excretion of the secretory products of the cells by the effect of glucocorticoids<sup>(12)</sup>. These findings would emphasize the explanation that the glucocorticoid increases the secretion of enzymes in the pancreatic acinar cells<sup>(6,19)</sup>. However, the chronic use of glucocorticoid produces blockade on enzyme excretion<sup>(20)</sup>. The results also suggested that the zymogen depletion as well as the other signs of acinar cell stimulation might be accompanied by an increased amylase activity. However, when the acinar cells became exhausted and ceased protein synthesis, a decrease in zymogen granule contents of acinar cells may be occurred associated with a decrease in the height of these cells<sup>(8,16,17,21)</sup>.

The appearance of nuclei with prominent nucleoli was indicative of increased metabolic activity of acinar cells<sup>(8)</sup>. But in other cells of group 2, 3 and 4, the pyknotic nuclei appeared with shrinkage of acinar cells occurred associated with decrease diameter of serous acini and low height of acinar cells. These changes in the nuclei were indicative of degenerative changes<sup>(22)</sup>.

The presence of vacuoles that differed in their size and locations in the cytoplasm of the treated acinar cells was due to local cellular degeneration<sup>(23,24)</sup>.

The irregular cell outlines observed were indicated the degenerative changes occurred in the acinar cells which would end by cell lysis<sup>(8)</sup>.

In general, the degenerated cytoplasm of acinar cells was seen in this study also described by many workers<sup>(22,25)</sup>.

Glucocorticoids stimulate the synthesis of pancreas-specific proteins, most probably via a cytoplasmic-nuclear receptor system<sup>(26,27)</sup>. These corticosteroids appear to either promote specific RNA accumulation or enhance the transcription of RNAs from gene loci intimately related with pancreas-specific proteins<sup>(4,28-32)</sup>. Thus, glucocorticoids seem to play an important role in the maintenance of a proper level of exocrine enzymes in the differentiating embryonic pancreas<sup>(33-35)</sup> as well as in the mature gland<sup>(13)</sup>. It should, however, be emphasized that only very low concentrations of corticosteroids ( $10^{-8}$  -  $10^{-6}$  M) are needed for their regulatory effect in the intact animal<sup>(8)</sup>. In contrast, higher concentrations more than that level of these hormones appear to adversely affect the synthetic activity of the pancreatic acinar cells which occurred in this study.

Wellmann and Volk<sup>(25)</sup> describe the proliferated duct cells observed in this study, it is one of the mechanisms involved in the neogenesis of B cells of pancreas<sup>(36)</sup>. Non significant changes were obtained by the measuring of the diameter of intercalated ducts and the height of duct cells since these structures have nothing to do with the synthesis of proteins which was suppressed in the acinar cells of this study<sup>(18)</sup>.

In the present study, there were changes in the intensity of staining with Masson Trichrome of the connective tissue because there was a decrease in collagen fibers in the interlobular spaces. This is because the glucocorticoids are regarded as a fibroblast growth inhibitor factor (anti-fibrotic agent)<sup>(37,38)</sup>.

So the glucocorticoids are important hormones for maintaining the structural integrity of the exocrine pancreas<sup>(13)</sup>, however, the effects of these glucocorticoids on the exocrine pancreatic structure and function are dose and duration dependent<sup>(39)</sup>. Therefore, it is important to notice that an appropriate dose of glucocorticoid renders a stimulating effect upon the acinar cell function, whereas large doses of the glucocorticoid reduce the exocrine tissue secretory function and affect its protein-synthesizing capacity<sup>(10,40)</sup>.

In conclusion, acinar cells were shifted from metabolically active cells to exhausted cells (non-functional) and degenerative changes appeared as the dose and duration of treatment of glucocorticoid increased more than that needed by the body.

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## Role of Visfatin in the Pathogenesis of Gestational Diabetes Mellitus

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### Abstract

- Background** The recently discovered adipocytokine visfatin has insulin-like properties. It lowers blood glucose and improves insulin sensitivity; however, clinical data on visfatin are limited.
- Objective** To evaluate the role of visfatin in GDM (gestational diabetes mellitus), we determined visfatin levels in women with GDM and healthy pregnant women.
- Methods** A total of 60 women were evaluated: 30 women with gestational diabetes mellitus and 30 healthy pregnant women to serve as control subjects. Serum visfatin concentrations were analyzed using an enzyme-linked immunosorbent assay the study was done in Al-Yarmouk Teaching Hospital during the period from November 2010 to March 2011.
- Results** Serum visfatin concentrations were significantly lower in the gestational diabetes mellitus group ( $0.27 \pm 0.1$  ng/ml) than in the healthy control group ( $1.37 \pm 0.25$  ng/ml) ( $P=0.0001$ ).
- Conclusions** Our results show that there are decreased concentrations of serum visfatin in gestational diabetes mellitus subjects and this may indicate that visfatin plays a role in the pathogenesis of gestational diabetes mellitus. However; further experiments are needed to clarify this role.
- Key Words** Visfatin, Gestational Diabetes Mellitus

### Introduction

Gestational Diabetes mellitus is defined as Carbohydrate intolerance that begins or is first recognized during pregnancy<sup>(1)</sup>. It occurs in 3% to 5% of pregnant women and is associated with adverse effects for both mother and fetus<sup>(1)</sup>. Gestational diabetes mellitus share a number of epidemiologic, physiological, and genetic characteristics with diabetes mellitus type two and seems to be a significant risk factor for the development of diabetes mellitus type 2 in later life<sup>(2)</sup>.

A variety of polypeptides secreted from adipose tissue, such as TNF- $\alpha$  (tumour necrosis factor- $\alpha$ )<sup>(3)</sup>, resistin<sup>(4)</sup> and leptin<sup>(5)</sup>, might play an important role in metabolic homeostasis and the development of Type II diabetes,

dyslipidaemia and arteriosclerosis<sup>(6)</sup>. Recently reported, the novel adipocytokine visfatin (52 kDa cytokine with 491 amino acids), which was previously known as PBEF (pre-B-cell colony-enhancing factor). It was originally isolated as a secreted factor that synergizes with interleukine-7 and stem cell factor to promote the growth of B-cell precursors<sup>(7)</sup>.

Visfatin is a peptide that is predominantly expressed in, and secreted from, visceral adipose tissue<sup>(8,9)</sup> and exerts insulin-mimicking effects through activation of an insulin receptor, although in a manner distinct from that of insulin<sup>(9)</sup>. The role of visfatin in human physiology and pathophysiology remains to be elucidated, while, according to some authors, plasma concentrations of visfatin are elevated in

obesity <sup>(10)</sup> and type 2 diabetes <sup>(11)</sup>, which are states characterized by insulin resistance (IR) and typically also observed in gestational diabetes mellitus (GDM).

Acute administration of recombinant visfatin to mice leads to a reduction of plasma glucose independent of changes in plasma levels of insulin. Thus it works synergistically with insulin to lower blood glucose concentrations <sup>(9)</sup>. Chronic elevation of visfatin in mice reduces insulin plasma concentrations <sup>(9)</sup>, and it was suggested that visfatin improves insulin sensitivity <sup>(12)</sup>. Visfatin affects the insulin signal transduction pathway by inducing tyrosine phosphorylation of the insulin receptor and IRS1 and 2 (insulin receptor substrate 1 and 2) in the liver. Furthermore, an autocrine/paracrine function on visceral adipose tissue as well as an endocrine role modulating insulin sensitivity in peripheral organs might be modes of action <sup>(12)</sup>. To evaluate the role of visfatin in GDM we determined this novel adipocytokine in women with GDM and healthy pregnant controls.

**Methods**

All subjects were carefully instructed about the aims of the study and written informed consent was given. Thirty women with GDM (mean age, 36±2 years) diagnosed during pregnancy weeks 24-28 and 30 healthy pregnant controls (mean age, 29±2 years) were included in the study. All subjects were non-smokers.

Women were diagnosed as GDM if two or more of the four glucose levels in the tolerance test exceeded the National Diabetes Data Group Criteria as follows:

- Fasting more than ≥5.3 mmol/l
  - 1-hour postload 75-g glucose value ≥10.0 mmol/l
  - 2-hour postload glucose value ≥8.6 mmol/l
  - 3-hour postload glucose value ≥7.5 mmol/l
- The (OGTT) was performed between 24<sup>th</sup> and 28<sup>th</sup> weeks of gestation.

Blood samples were obtained directly from a cannulated vein for the purpose of a routine glucose challenge test at 24-28 weeks of gestation. The serum was separated by

centrifugation, and stored at -20 °C until further analysis. Serum visfatin was analyzed using kit manufactured by (BIO VISION).

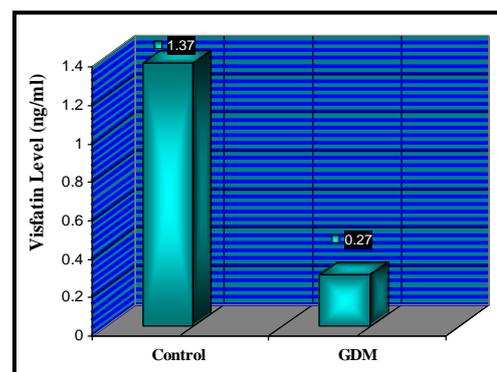
Inclusion criteria were signed informed consent, absence of a clinically relevant illness, normal findings in the medical history and physical examination except for GDM, and normal laboratory values. Subjects were excluded if any clinically relevant abnormality was found as part of the screening or in any of the laboratory tests including circulating anti-insulin antibodies and anti-islet cell antibodies. No subject was on a special diet or reported to have any medication, including “over-the-counter” drugs, at the time of blood sampling.

**Results**

Table 1 shows the clinical results of our subjects, both those with GDM and those with healthy control groups. Serum visfatin concentration was significantly lower in the GDM group (Mean= 0.27±0.1) than in the healthy control group (Mean= 1.37±0.25) (p-value= 0.0001) as shown in and Figure 1.

**Table 1. Clinical Data**

Parameters	Controls	GDM	P-Value
<b>Visfatin Level (ng/ml)</b>	1.37±0.25	0.27±0.1	0.0001



**Figure 1. Serum Visfatin Level plotted for both GDM subjects and those who were in the healthy group**

## Discussion

This study describes the recently identified adipocytokine visfatin in women with GDM and healthy pregnant controls.

The results of this study suggest an inverse association of plasma visfatin concentrations with gestational diabetes mellitus.

The cause of reduced fasting visfatin and mitigated response to glucose challenge in women with GDM is not directly accessible from this study. It has been demonstrated that plasma visfatin concentrations are inversely correlated to progressive-cell deterioration in patients with type 1 or type 2 diabetes<sup>(13)</sup>.

The present data argue against an assumption that altered pancreatic insulin secretion has contributed to reduced plasma visfatin in GDM because insulin plasma concentrations were comparable in fasting conditions. This is important, as insulin is known to suppress glucose induced visfatin release in vitro and in vivo<sup>(14)</sup>.

On the contrary, as it is known that glucose induces visfatin release, which is also a consistent finding in this study, one might have expected higher visfatin concentrations in women with GDM. Thus, factors other than glucose and insulin alone seem to influence the regulation of visfatin in pregnancy, such as proinflammatory cytokines<sup>(15)</sup>. Indeed, an association between plasma tumor necrosis factor- $\alpha$  and visfatin mRNA in subcutaneous adipose tissue has recently been reported<sup>(16)</sup>.

The finding in this study that gestational diabetes mellitus subjects have lower plasma visfatin concentrations suggests that an insufficiency of visfatin may play a role in the pathogenesis of gestational diabetes mellitus, these finding are in agreement with study done by chan et al 2006 and haider et al 2007.

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## Molecular Assessment of Staphylococcal Bacteria in Highly Educated Communities

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### Abstract

- Background** Emergence of resistant strains of *staphylococcus aureus*, namely methicillin-resistant *S. Aureus* (mrsa) in all levels of urban and rural societies has become a haunting problem for the recent world.
- Objectives** This study assesses and explores the transfer of resistant *Staphylococcus aureus* bacteria in certain high social class of community focusing on nail as reservoir for transmitting the infection.
- Methods** One hundred swabs taken from nails were collected from college students in Malaysia. Assays for identification and differentiating *Staphylococcus aureus* were conducted to identify target bacteria. Moreover, this study compared the efficacy of the different identification tests with gold standard, PCR assay. The tests used were tube coagulase, DNase agar test, antibiogram, several routine biochemical identification tests and PCR assays. PCR assay used specific primers for resistance or species-related genes: *mecA*, *ermA*, *ermB*, *ermC*, *msrA*, *linA*, *femA*, and *nuc* genes.
- Results** A total of 155 bacterial isolates were isolated from college students' nails, non-PCR assays of identification and resistance detection revealed presence and spread of MRSA in nails of 3 college students. PCR-amplification of the *nuc* gene was used as a baseline test to detect *Staphylococcus aureus*. 20 isolates were detected as *Staphylococcus aureus* using traditional tests while PCR showed only 4 isolates are *S. aureus*, only 3 of them are MRSA. Sensitivity of antibiogram ranged from 88.9 to 100% but its specificity was very low (0-100%). For tube coagulase, sensitivity was 36.4-100% while specificity was also not so high (66.7-100%).
- Conclusion** Collectively, nails proved to have potential for the transfer of MRSA in community of college students in South East Asia. Moreover, PCR assay for identification of *S. aureus* resistance proved to be superior on other methods.
- Keywords** *Staphylococcus aureus*, MRSA, PCR, antibiotic resistance

### Introduction

*Staphylococcus aureus* is a normal colonizer in human and various animal species<sup>(1)</sup>. It normally colonizes the nares, hands, the rectum or vagina in human and causes infections. However, in the past 50 years, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) which confer resistance toward  $\beta$ -lactam antibiotics had become prevalent in many parts of the world and in turns causes infections that were extremely difficult to treat<sup>(1,2)</sup>. The resistance in MRSA is due to the presence of the

resistance gene, *mec* gene which encodes for the low affinity penicillin-binding protein 2a (PBP2a), or in rare cases, the hyperproduction of  $\beta$ -lactamase<sup>(3-7)</sup>.

Recently, another concern about MRSA is the spread of MRSA to the community, community-acquired MRSA (CA-MRSA) that have caused infections and death in normal people without any healthcare-associated risk factors<sup>(8-10)</sup>. CA-MRSA infections commonly occur as skin and soft tissue infections such as impetigo, furuncles, or abscesses in healthy individuals<sup>(2,11-13)</sup>.

However, it also can lead to the rare but life threatening disease like necrotizing pneumonia, necrotizing fasciitis or septicemia<sup>(13-26)</sup>.

The CA-MRSA infections raised the concern of the public. These infections are transmitted among healthy persons. The recent data shows that the skin-skin contact and the skin-formite contact are important routes of transmission when compared to the nasal colonization<sup>(9)</sup>. The repeated close physical contact especially between broken skins during sport and games along with poor personal hygiene is the main contribute to the outbreak of MRSA<sup>(10)</sup>. However, environmental hygiene is also very important. Formites and commonly touched household objects such as soaps, doorknobs, toilet handles and kitchen sinks can serve as the reservoir for the MRSA and involved in the recurrent infections<sup>(9)</sup>.

A person may accidentally acquire MRSA through scratches into their nail areas. In the case of poor personal hygiene, MRSA may reside under the nail as this place provides a suitable niche for their survival. Due to severe shortage in researching MRSA survival in and/or under nail, the current study aimed at investigating the presence of MRSA under the nail of college students; moreover, this study explored the circulation of MRSA with nails using different identification techniques. In addition, the identification techniques in the current study were also evaluated and compared with each other regarding their capability and efficiency in detecting and diagnosing MRSA from nails.

## Methods

Subjects of the current study were 100 college students of University Putra Malaysia Kuala Lumpur, Malaysia except those who were involved in healthcare assignments such as medical and veterinary students. The research project was approved by the Research Ethics Committee of their institution, according to the Declaration of Helsinki. One hundred swabs of students' nail were collected in the period between October and December 2010; age,

gender, field of study and history of cold/flu for each student were recorded during sample collection. The collected swabs were incubated in nutrient broth (Merck, KGaA, Germany) for 4 to 6 hours at 37°C for pre-enrichment. Then, the cultures were streaked onto the 5% human defibrinated blood agar plates (Merck, KGaA, Germany) and incubated at 37°C for 18-24 hours. The isolates obtained were identified by standard procedure using colony morphology, Gram's stain characteristic, catalase test and glucose oxidation and fermentation test. Of the one hundred students, 27 were males and 73 were female (71 Malay, 26 Chinese, and 3 Indian), which age between 19 to 34 years old.

## Media

All Gram-positive cocci isolates were cultured on mannitol salt agar (MSA) (Oxoid, Cambridge, UK) and incubated at 37 °C for 18 to 24 hours. The ability to ferment mannitol was confirmed by the growth of yellow colonies on MSA surrounding by yellow zone after incubation, which indicates a positive result<sup>(27)</sup>. All of the mannitol- positive isolates were the presumptive of *Staphylococcus aureus*.

## DNase Test

Single colonies of mannitol positive isolates were streaked on DNase agar (Oxoid, Cambridge, UK) and incubated at 37 °C for 18 to 24 hours. The agar was flooded with 1 N HCl after incubation and the presence of clearing zone indicates a positive result<sup>(27)</sup>.

## Tube Coagulase Test

Bacterial suspensions of a 0.5-2.0 McFarland standard of mannitol-positive isolates were prepared by suspending the isolates in 3mL Phosphate-Buffer Solution (PBS). Then, 350 µL of bacterial suspension was added to 150 µL of citrated human and rabbit plasma in sterile glass test-tubes. The tubes were incubated at 37 °C for 4 hours and observed for clot formation in every 30 minutes interval. If clotting did not occur, all coagulase-negative tubes containing citrated rabbit plasma, were further incubated at room temperature for 18 hours for citrated rabbit plasma while citrated human plasma coagulase-

negative tubes were further incubated at 37 °C for 18 hours. Agitation of the tubes was avoided when observation was made to prevent disruption of partially formed clots. The formation of clots indicates a positive result <sup>(27)</sup>.

#### Antibiogram Typing

The susceptibility of all mannitol-positive isolates to antimicrobial agents (Oxoid, Cambridge, UK) was determined by using Mueller-Hinton agar (Merck, KGaA, Germany) disk diffusion method according to the guidelines of the CLSI. The bacteria suspension turbidity was adjusted to a 0.5 McFarland standard. The plates were incubated at 37 °C for 24 hours. Eleven antibiotic discs at the specific absolute concentration were as follow: cefoxitin (30 µg), chloramphenicol (30 µg), oxacillin (1 µg), vancomycin (30 µg), erythromycin (30 µg), trimethoprim (1.25 µg), penicillin G (1 µg), ampicillin (10 µg), methicillin (10 µg), tetracycline (30 µg), and gentamycin (10 µg) <sup>(28,29)</sup>.

#### DNA Extraction

Eleven presumptive *Staphylococcus aureus* isolates which shows resistant toward cefoxitin, oxacillin, erythromycin, and methicillin were cultured in Luria-Bertani broth (Merck, KGaA, Germany) at 37 °C for 24 hours. DNA extraction was done by using the GeneJET™ Genomic DNA Purification Kit (Fermentas, #K0721). The purified DNA was stored at -20°C for further DNA typing.

#### Genomic Determination of the Resistant Genes

The specific genes responsible for antimicrobial resistance were determined by using polymerase chain reaction (PCR). In this study, the genes to be amplified are *mecA*, *ermA*, *ermB*, *ermC*, *msrA*, *linA*, *nuc*, and *femA*. The sequences, primers, PCR conditions for each gene are shown in table 1 (30-36). The amplified PCR products were analyzed and detected by ethidium bromide staining following 1.0% agarose gel electrophoresis at 80V for 45 minutes.

**Table 1: Sequences primers and PCR conditions used in amplification of *mecA* gene *ermA* gene *ermB* gene *ermC* gene *msrA* gene *linA* gene *nuc* gene and *femA* gene**

Target gene	Primer sequences	PCR Condition	Size (bp)	Thermocycler	Reference
<i>mecA</i>	5'-TCCAGATTACAACCTTCACCAGG-3' 5'-CCACTTCATATCTTGTAACG-3'	32 cycles of 94 °C for 30 s 53 °C for 30 s and 72 °C for 50 s	162	Techne	30
<i>ermA</i>	5'-GTTCAAGAAC AATCAATACA GAG-3' 5'-GGATCAGGAA AAGGACATTT TAC-3'	32 cycles of 94 °C for 30 s 52 °C for 30 s and 72 °C for 60 s	421	Eppendorf	31
<i>ermB</i>	5'-CCGTTTACGAAATTGGAACAGGTAAAGGGC-3' 5'-GAATCGAGAC TTGAGTGTGC-3'	32 cycles of 94 °C for 30 s 55 °C for 30 s and 72 °C for 60 s	359	Bio Rad	32
<i>ermC</i>	5'-GCTAATATTG TTAAATCGT CAATTCC-3' 5'-GGATCAGGAA AAGGACATTT TAC-3'	32 cycles of 94 °C for 30 s 52 °C for 30 s and 72 °C for 60 s	572	Bio Rad	31
<i>msrA</i>	5'-GGCACAATAA GAGTGTTTAA AGG-3' 5'-AAGTTATATC ATGAATAGAT TGCCTGTT-3'	30 cycles of 94 °C for 60 s 50 °C for 60 s 72 °C for 90 s	940	Bio Rad	33
<i>linA</i>	5'-GGTGGCTGGG GGGTAGATGT ATTAAGTGG-3' 5'-GCTTCTTTTGAATACATGGTATTTTCGATC-3'	32 cycles of 94 °C for 30 s 57 °C for 30 s and 72 °C for 60 s	323	Bio Rad	34
<i>nuc</i>	5'-GCGATTGATGGTGATACGGTT-3' 5'-AGCCAAGCCTTGACGAACTAAAGC-3'	32 cycles of 94 °C for 35 s 52 °C for 35 s and 72 °C for 50 s	276	Eppendorf	36
<i>femA</i>	5'-CTTACTTACTGCTGTACTCG-3' 5'-ATCTCGCTTGTATGTGC-3'	32 cycles of 94 °C for 40 s 48 °C for 40 s and 72 °C for 50 s	684	Techne	35

### Statistical analysis

The data of the current study was processed using Microsoft EXCEL 2007 (Microsoft, Corp., USA). P value less than 0.05 was considered significant.

### Results

From 100 nail swabs, 155 bacterial isolates were obtained. Out of 155 bacterial isolates, 132 (85.2%) were Gram-positive cocci and 23 isolates (14.8%) were non Gram-positive cocci. Only Gram-positive isolates were subjected to other tests. For catalase test, 129 out of 132 Gram-positive isolates (97.7%) were catalase-positive. In glucose oxidation and fermentation test, 113 isolates (85.6%) can utilize glucose in both aerobic and anaerobic conditions, 19 isolates (14.4%) cannot utilize glucose or utilize in one condition only (aerobically or anaerobically). Acid production from the fermentation of mannitol in the mannitol salt agar (MSA) produces yellow colonies and changes the surrounding media into yellow color which was indicated as a positive result. The high salt content in the MSA inhibit the growth of most,

but not all, bacteria other than staphylococci (59). A total of 70 isolates (53.0%) showed a positive result in mannitol salt agar test. The mannitol- positive isolates acted as presumptive of *Staphylococcus aureus* (*S. aureus*). Based on the result of DNase test, 20 isolates (28.6%) out of the 70 presumptive *S. aureus* isolates gave positive test results as indicated by the presence of clearing zone on the surrounding of the colonies when flooded with 1 N HCl. In tube coagulase test, the positive results were determined by the formation of clot within the glass tube. Both citrated human plasma and citrated rabbit plasma gave the same number of isolates showing positive results, which were 13 isolates (18.6%) out of the 70 isolates tested. These 13 positive isolates were considered as *S. aureus* while the rest were considered as coagulase-negative staphylococci (CoNS). However, the activity of fibrinolysin was absent in this test. The details of each test result are shown in Table 2.

**Table 2. Identification of *S. aureus* with various tests**

Results	MSA (N=132)	Catalase (N=132)	GO & FT (N=132)	DNase (N=70)	Tube Coagulase (N=70)	
					CHP	CRP
<b>Positive</b>	70 (53.0%)	129 (97.73%)	113 (85.6%)	20 (28.6%)	13 (18.6%)	57 (81.4%)
<b>Negative</b>	62 (47.0%)	3 (2.3%)	19 (14.4%)	50 (71.4%)	13 (18.6%)	57 (81.4%)

GO = glucose oxidation, FT = fermentation test, CHP = citrated human plasma, CRP = citrated rabbit plasma

### Antibiogram Typing

The susceptibility data of all presumptive *S. aureus* was presented in Table 3. From the results, all isolates (100%) were susceptible toward vancomycin (30 µg) and gentamycin (10 µg). There was one isolate (1.4%) showing resistance and 1 isolate (1.4%) had intermediate resistance toward methicillin (10 µg). The number of the isolates which were resistant toward other antimicrobial agents were: Cefoxitin (30 µg) 1 (1.4%), Chloramphenicol (30 µg) 1(1.4%), Oxacillin (1 µg), 1(1.4%),

Erythromcin (30 µg) 10(14.3%), Trimethoprim (1.25 µg) 5(7.2%), Tetracyclin (30 µg) 2(2.9%), PenicillinG (1 µg) 58(82.9%), and Ampicillin (10 µg) 59(84.3%).

Based on the result, all coagulase-positive isolates (100%) were resistant toward penicillin G (1 µg) and ampicillin (10 µg), while all were susceptible toward vancomycin (30 µg) and gentamycin (10 µg). There was only one isolate (7.7%) resistant toward tetracycline and one isolate (7.7%) was intermediate toward erythromycin.

**Genomic Determination of Resistant Genes**

By using PCR amplification, only four isolates (36.4%) were found to be positive for the presence of *nuc* gene while the other seven isolates (63.6%) were negative for the presence of *nuc* gene. *mecA* gene was present in 9 isolates (81.8%), including the coagulase isolates and the CoNS. Ten isolates (90.9%) possess the *ermC* gene, *femA* gene, and *msrA* gene. All of the isolates tested (100%) did not possess *ermA* and *ermB* gene. In contrast, all of the tested isolates (100%) conferred resistance to lincosamides as they possess *linA* gene. None of the isolates possessed all the three combinations of the *erm* genes. The *erm* gene is mostly found in the

isolates that possess the *mecA* gene. The prevalence of the resistant genes among the 11 isolates is shown in table 4. Moreover, the resistant genes within each isolate were shown in table 5.

In comparison with the coagulase test (Table 3), only one coagulase isolate tested showed the presence of the *nuc* gene. However, three CoNS isolates possess the *nuc* gene. The *mecA* gene which confers resistance to methicillin was detected in nine isolates (81.8%) whereas only two isolates showed resistance to methicillin in antibiogram typing.

**Table 3. Antibiogram typing among coagulase-positive isolates and coagulase-negative isolates**

Antimicrobial Agents	Coagulase Positive (N=13)			Coagulase Negative (N=57)			General For All Isolates (N=70)		
	S	I	R	S	I	R	S	I	R
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Cefoxiton	13(100)	-	-	56(98.2)	-	1 (1.8)	69(98.6)	-	1(1.4)
Chloramphenicol	13(100)	-	-	56(98.2)	1(1.8)	-	69(98.6)	1(1.4)	-
Oxacillin	13(100)	-	-	56(98.2)	-	1(1.8)	69(98.6)	-	1(1.4)
Vancomycin	13(100)	1(7.7)	-	57(100)	-	-	70(100)	-	-
Erythromycin	12(92.3)	-	-	48(84.2)	5(8.8)	4(7.0)	60(85.7)	6(8.6)	4(5.7)
Trimethoprim	13(100)	-	-	52(91.2)	2(3.5)	3(5.3)	65(92.8)	2(2.9)	3(4.3)
Penicillin G	-	-	13(100)	12(21.1)	-	45(78.9)	12(17.1)	-	58(82.9)
Ampicillin	-	-	13(100)	11(19.3)	-	46(80.7)	11(15.7)	-	59(84.3)
Methicillin	13(100)	-	-	55(96.4)	1(1.8)	1(1.8)	68(97.2)	1(1.4)	1(1.4)
Tetracycline	12(92.3)	-	1(7.7)	56(98.2)	-	1(1.8)	68(97.1)	-	2(2.9)
Gentamycin	13(100)	-	-	57(100)	-	-	70(100)	-	-

**Table 4. Prevalence of resistant genes in 11 isolates**

Resistant genes	Coagulase-positive isolates (n=1)		Coagulase-negative isolates (n=10)		General for all isolates (n=11)	
	Positive	Negative	Positive	Negative	Positive	Negative
<i>nuc</i>	1 (100%)	-	3 (30%)	7 (70%)	4 (36.4%)	7 (63.6%)
<i>mecA</i>	-	1 (100%)	9 (90%)	1 (10%)	9 (81.8%)	2 (18.2%)
<i>ermA</i>	-	1 (100%)	-	10 (100%)	0	11 (100%)
<i>ermB</i>	-	1 (100%)	-	10 (100%)	0	11 (100%)
<i>ermC</i>	1 (100%)	-	9 (90%)	1 (10%)	10 (90.9%)	1 (9.1%)
<i>femA</i>	1 (100%)	-	9 (90%)	1 (10%)	10 (90.9%)	1 (9.1%)
<i>msrA</i>	1 (100%)	-	9 (90%)	1 (10%)	10 (90.9%)	1 (9.1%)
<i>linA</i>	1 (100%)	-	10 (100%)	-	11 (100%)	0

Table 5. The distribution of resistant genes among each isolate

Isolates No.	Resistant Genes							
	<i>nuc</i>	<i>mecA</i>	<i>ermA</i>	<i>ermB</i>	<i>ermC</i>	<i>femA</i>	<i>msrA</i>	<i>linA</i>
4	-	+	-	-	+	+	+	+
17b	-	+	-	-	+	+	+	+
22b	-	-	-	-	-	+	+	+
24	+	-	-	-	+	+	+	+
27a	-	+	-	-	+	+	+	+
39a	+	+	-	-	+	+	+	+
47a	+	+	-	-	+	+	+	+
50	+	+	-	-	+	+	+	+
81a	-	+	-	-	+	+	+	+
91b	-	+	-	-	+	+	+	+
95b	-	+	-	-	+	-	-	+

Based on the molecular results, four isolates were confirmed as *S. aureus*, as they were positive for *nuc* gene. However, only one of them was coagulase positive isolate. From 100 nail swab samples collected, these four *S. aureus* were isolated from 4 students. Out of these four isolates, three were confirmed as MRSA as they were positive for *mecA* gene, indicating that MRSA was isolated from 3 students. One of the isolates that possesses *nuc* gene did not contain *mecA* gene but contained other resistant genes (*ermC*, *msrA*, *linA* and *femA*). Therefore, it was categorized as multi-resistant *Staphylococcus aureus*. Six of the CoNS also possessed *mecA* gene; thus, they were categorized as methicillin-resistant coagulase-negative staphylococci. One of the CoNS was

with neither *erm* genes nor *mecA* gene detected. Thus, it was non methicillin-resistant CoNS.

#### Comparison of antibiogram and other tests with PCR assay

Discrepant results were observed in our study. The antibiogram typing failed to detect methicillin resistance in seven isolates (77.8%), eight isolates (88.9%) for oxacillin resistance, one isolate (10% with *erm* genes and 11.1 with *msrA* gene) for erythromycin resistance. The sensitivity and specificity of the antibiogram typing compared with the PCR identification is shown in table 6 while the findings of comparison (sensitivity and specificity) other test compared to PCR assay (Golden standard) are shown in table 7.

Table 6. Sensitivity and specificity of the antibiogram typing compared to PCR detection of resistant genes in the isolates

Antibiogram Typing Result	PCR results		% Sensitivity	% Specificity
	Positive	Negative		
Methicillin	Positive	2	100	22.2
	Negative	7		
Oxacillin <sup>a</sup>	Positive	1	100	20
	Negative	8		
Erythromycin <sup>b</sup>	Positive	9	90	0
	Negative	1		
Erythromycin <sup>c</sup>	Positive	8	88.9	50
	Negative	1		

<sup>a</sup>: molecular detection of oxacillin resistant is based on the detection of *mecA* gene, <sup>b</sup>: comparison of erythromycin-resistant with the *erm* genes, <sup>c</sup>: comparison of erythromycin-resistant with the *msrA* gene

**Table 7. Identification of *S. aureus* with the common tests compared with the PCR detection of *nuc* gene.**

Tube Coagulase Test		PCR detection of <i>nuc</i> Gene		% Sensitivity	% Specificity
		Positive	Negative		
Human Plasma	Positive	1	0	100	70
	Negative	3	7		
Rabbit Plasma	Positive	1	0	50	66.7
	Negative	3	7		
DNase	Positive	1	1	36.4	100
	Negative	3	6		
MSA	Positive	4	7	50	66.7
	Negative	0	0		
MSA/DNase/rabbit plasma	Positive	1	1	50	66.7
	Negative	3	6		

### Discussion

Recently, the emergence of MRSA infections in the community among healthy individuals without any risk factors had increased steadily and become a great concern of the community<sup>(10,37)</sup>. In this study, all the three MRSA isolates originated from female students. In addition, all of these three did not have history of cold/flu. The resistant *mecA* gene was predominantly present in the female isolates (8 persons, 88.9%) and seldom present in male isolate (1 person, 11.1%). All of the multiresistant CoNS and *S. aureus* were mainly isolated from female, 13.7% (10 out of 73 females) and 3.7% (1 out of 27 males) from male. Figure 9 shows the distribution of *mecA* gene among the students with different gender.

It was mentioned that the presence of *mec A* gene does not always mean that the *Staphylococcus spp.* show resistance to methicillin as shown in the susceptibility test of E-test and disk diffusion test. This may be due to the presence of the incomplete regulator genes (*mecI* and/or *mecRI*) or maybe because of the inability to express the *mecA* gene<sup>(38)</sup>. The current study proposed that MRSA can colonize nail just like skin and anterior nares (unpublished data). During physical contact, the MRSA may attach to the skin but they also retain underneath nail of the person. In addition, MRSA

may transmit from our daily use item such as cell phones, coins, keys, doorknobs and others (unpublished data).

The resistance to erythromycin was mainly due to the presence of either, *ermA*, *ermB*, *ermC* or combination of the *erm* genes. It was indicated that *ermA* and *ermC* genes are responsible for most of the erythromycin resistance in *S. aureus* which is similar to the result obtained from the current study. All of the isolates contain only *ermC* gene. The *ermA* is part of the transposon Tn554 in the chromosome, while the *ermC* is located on the plasmid<sup>(39)</sup>. *ermC* gene is the most prevalent form where it was found in all eight isolates. None of the isolates contained *ermA* and *ermB* genes. As described previously, *ermB* gene is commonly found on animal strains<sup>(39)</sup>. The results also coincide with the research done by Eady *et al.*; who documented that *ermC* is predominant in clinical and commensal coagulase-negative staphylococci<sup>(39)</sup>.

Resistance towards macrolides is due to the presence of *msr* gene. In this study, *msrA* instead of *msrB* was chosen. Ten of 11 isolates (90.9%) tested contain *msr A* gene. Previous studies reported that no *S. aureus* contains both *erm* and *msrA* genes<sup>(40)</sup>. However, all of the *S. aureus* (100%) in the current study possessed combination of these two genes. Most of the CoNS also contain both *erm* and *msrA* genes.

There were only two isolates showing either *msrA* or *erm* gene. *lin A/linA'* gene is responsible to confer resistance to lincosamides only. It was found in the current study that all of the isolates (100%) contained this *linA* gene. It is uncommon for staphylococci to confer resistance only to lincosamides<sup>(41)</sup>. Although the incidence of *linA* gene to appear alone is low, there was one isolate (9.1%) with this condition, which contains *linA* gene alone without *msr* genes or *erm* genes. Most of the isolates contained *linA* gene in conjugation with *mrsA* gene, *erm* genes or both. The *S. aureus* specific gene, *femA* which does not cross react with other bacteria such as *S. epidemidis* was used to identify pure *S. aureus*. Although *femA* sequences are phylogenetically conserved to staphylococci; however, *femA* for *S. aureus* is 78% homologous to the *femA* of *S. epidemidis*<sup>(42)</sup>. Therefore, there is a possibility of giving false positive *S. aureus*. In order to confirm that isolates were *S. aureus*, another gene, *nuc* gene was used together with the *femA* gene. In our study, ten of 11 isolates (90.9%) contained *femA* gene. However, only four of 11 isolates contained *nuc* gene.

Discrepant results were observed in our study. The antibiogram typing failed to detect methicillin resistance in 7 isolates (77.8%), 8 isolates (88.9%) for oxacillin resistance, 1 isolate (10% with *erm* genes and 11.1% with *msrA* gene) for erythromycin resistance. The sensitivity and specificity of the antibiogram typing compared with the PCR identification is shown in table 6. The methicillin resistance is attributed to the expression of *mecA* gene which produces low affinity penicillin binding protein 2a (PBP2a), or in rare cases, attributed to the hyperproduction of the  $\beta$ -lactamase enzymes or production of altered binding capacity proteins<sup>(3,5-7)</sup>. However, the presence of *mecA* gene does not always mean that *S. aureus* confer resistance to methicillin, as it can be explained by the incomplete regulator genes (*mecl* and/or *mecRI*) or inability to express *mecA* gene. Therefore, many isolates in the current study were susceptible to methicillin in antibiogram typing but possessed *mecA* gene. The discrepant results

in our study could be explained by this mechanism as well. Therefore, the sensitivity of methicillin was 100% with specificity of 22.2%. The oxacillin resistance also expressed *mecA* gene. In this case, the sensitivity of oxacillin disk was 100% but with 20% only specificity. Erythromycin resistance in the isolates was encoded by *erm* and *msrA* genes. The erythromycin disk diffusion method showed sensitivity of 90% and specificity of 0% when compared with the PCR results for *erm* genes and sensitivity of 88.9% and specificity of 50% when compared with *msrA* gene.

Tube coagulase is one of the most reliable methods to identify *S. aureus*. There are 2 types of methods to detect the production of coagulase from *S. aureus*, tube coagulase test (TCT) and slide coagulase test (SCT). The SCT works by detecting the bound coagulase, which is also known as "clumping factor" that react directly to the fibrinogen in plasma, causing rapid cell agglutination. Negative SCT should reconfirm with TCT because they might produce extracellular coagulase. The extracellular coagulase detects a substance in the plasma known as coagulase reacting factor (CRF) to form a complex, which later reacts (clot formation) with the fibrinogen to form fibrin (form clot)<sup>(27)</sup>. In the current study, both human and rabbit plasma were used in the TCT. A previous study had showed that human plasma gives discordant results<sup>(43)</sup>. Rabbit plasma was the standard in performing the coagulase test. On the other hand, the current study reports that human and rabbit plasma give the same results. There were no difference in the TCT result using human and rabbit plasma. This might due to the fact that fresh human plasma was used. Both TCT using human and rabbit plasma gave the similar sensitivity and specificity, namely, 100% and 70%, respectively, as shown in Table 7. Three of the coagulase-negative isolates (30%) had *nuc* gene, indicating that some of the isolates were misidentified as CoNS. It was reported that these coagulase-negative *S. aureus* may probably react weakly or negatively with the TCT<sup>(27)</sup>. In our

study, all the methicillin-resistant *S. aureus* were negative for coagulase test.

DNase test was also used to identify *S. aureus*. In our study, DNase test gave a sensitivity of 50% and specificity of 66.7%, which have a lower value compared to other studies<sup>(27)</sup>. Two of the 11 isolates were positive for DNase test. However, 3 of the 4 *S. aureus* isolates were DNase negative, which were also methicillin resistant.

MSA test also aids in the identification of *S. aureus*. The MSA gave a sensitivity of 36.4% only and specificity of 100%. Four of 11 MSA positive isolates (36.4%) were confirmed as *S. aureus* with the presence of *nuc* gene. The other 71 isolates gave a false positive by the absence of *nuc* gene.

After comparing individual test to identify the *S. aureus*, none of the single phenotypic tests can accurately identify *S. aureus*. Detecting *S. aureus* using the TCT gave the highest sensitivity of 100%, followed by the DNase 50% and lastly the MSA with 36.4%. Meanwhile, MSA test showed the highest specificity, 100%, followed by TCT with 70% and lastly the DNase test with 66.7%. Data of the current study was same as the previous studies in which the sensitivity of TCT reached 94-100%<sup>(27)</sup>. In contrast to the other studies, the sensitivity and specificity in our study were much lower<sup>(44)</sup>. Among the 3 phenotypic tests, TCT was shown to be more suitable to identify *S. aureus* (100% sensitivity and 70% specificity). Our finding was different from another study where DNase test was superior to TCT<sup>(44)</sup>. Due to the low sensitivity and specificity, MSA and DNase were used in routine identification of *S. aureus* at the initial stage<sup>(43)</sup>.

The current study showed that there were three MRSA isolates (3%) from 100 nail swab samples. This also indicates that MRSA can be found in and colonize the nail of the healthy individuals. This provided evidence that MRSA do exist actively in population of high social rank such as university students. The level of MRSA existence in university students might be much lower than other sectors of the community as known that

university students maintain better personal hygiene than others, which is an important precaution step to be away from the dangerous MRSA. Therefore, detection of MRSA in university students can be considered as dangerous indicator for the given community. Furthermore, methicillin-resistant coagulase-negative staphylococci (MRCoNS) were also isolated from the student's nail swabs. Six MRCoNS (6%) were isolated from swabs of 100 student's nail. There was one isolate (1%) showing multi-resistance but susceptible to methicillin. All of these 11 isolates were also resistant to other antimicrobial agents such as erythromycin and lincosamides.

Taken together, it was found that MRSA do not only found in mails of highly educated people but can circulate between nails and other parts of the body, Besides, we have proven the existence of MRSA and methicillin-resistant CoNS among college students' nail. Furthermore, screening of resistance genes was shown to be superior on all other modes of detection/identification of MRSA. Attention should be raised for MRSA identified in community. Good personal hygiene and environmental hygiene are important to prevent the colonization of MRSA. Besides, there is no single phenotypic test can be used to adequately identify *S. aureus*. Initially tested isolates with MSA and DNase, followed by TCT can improve the efficiency and accuracy. However, final confirmation with the "golden standard", PCR should be performed to identify *S. aureus* and their antibiotics resistance. Hence, it is recommended doing larger study on the circulation of MRSA among university students in other regions of the world. Moreover, it is recommended that MRSA containing protocols should cover all sectors of the community even these composed of high socioeconomic individuals.

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## Immunohistochemical Expression of Epstein Barr Virus Antigen Latent Membrane Protein-1 and Bcl-2 in Classical Hodgkin Lymphoma

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### Abstract

- Background** Different genetic and environmental factors appear to be involved in the pathogenesis of classical Hodgkin's lymphoma (HL) and among them is Epstein Barr virus.
- Objective** To evaluate the immunohistochemical expression of the Latent Membrane Protein-1 of Epstein-Barr virus and Bcl-2 in Classical Hodgkin Lymphoma and to correlates this expression with some clinicopathological parameters and finally to find if there is any relation between LMP-1 and Bcl-2 in classical Hodgkin's lymphoma.
- Method** Retrospective study of fifty paraffin-embedded blocks of lymph nodes biopsies from patients diagnosed as Classical Hodgkin's Lymphoma. Three representative sections were prepared for each case. The first stained with H&E and the other two sections stained immunohistochemically for LMP-1 and Bcl-2 .
- Results** Immunohistochemical expression of LMP-1 and Bcl-2 was detected in 90% and 66% of Hodgkin's lymphoma cases and in 60% and 80% of control subjects, respectively. The patients (9/50) who were less than 16 years were positive for LMP-1 antigen. 21/45 (46.6%) of positive cases for LMP-1 & 14/33 (42%) of positive cases for Bcl-2 were of mixed cellularity subtype. Intensity of LMP-1 but not Bcl-2 expression was significantly high in mixed cellularity compared to other subtypes. No statistically significant relation between LMP-1 and Bcl-2 expression in HRS cells of Hodgkin's lymphoma cases.
- Conclusion** The high prevalence and high expression of LMP-1 confirms the association of the virus with HL. Although Bcl-2 is highly expressed in HL, the two markers were not related to each other.
- Keywords** Classical Hodgkin's Lymphoma, Epstein Barr virus, Immunohistochemistry, Epstein Barr virus latent membrane protein-1, Bcl-2.

### Introduction

Hodgkin's lymphoma (HL) is a lymphoproliferative malignancy of B-cell origin <sup>(1)</sup> According to the WHO classification, Hodgkin's lymphoma (HL) is divided into a classical variant and a nodular lymphocyte predominant variant which are characterized by the presence of Hodgkin's and Reed-Sternberg (H-RS) cells or lymphocytic and histiocytic (L&H) cells, respectively <sup>(2)</sup>.

According to National Cancer institute in 2008, the worldwide incidence rate of HL is 3.1 per 100 000 population/year. Its incidence varies from 3.5 per 100,000 population/year <sup>(3)</sup> in Europe, 3.5 per 100,000 population/year in united state <sup>(4)</sup>, >5.5 per 100,000/year in Yemen and Lebanon, to <1 per 100,000 population/year <sup>(5)</sup> in Bangladesh, Japan and China. In Iraq, the incidence of HL from

1995 to 2006 ranged between 1.49 and 1.01/100,000 population/year<sup>(6)</sup>.

Viruses are etiologically associated with significant types of human leukaemia and lymphomas<sup>(7)</sup>. The Epstein-Barr virus (EBV) plays an important role and individuals with a history of infectious mononucleosis have an increased incidence of Hodgkin's lymphoma<sup>(8)</sup>. Approximately 30% to 40% of patients with Hodgkin lymphoma (HL) in the Western world and in some developing regions carry the EBV in the malignant Hodgkin Reed Sternberg (HRS) cells<sup>(9)</sup>.

EBV infection is an early event in the development of HL as the viral genomes are found in a monoclonal form, indicating that infection of tumor cells has occurred before their clonal expansion<sup>(9)</sup>. Clonal viral genomes are found in the Hodgkin Reed-Sternberg cells (HRS). The latent infection results in expression of the viral oncogenes LMP-1 and LMP-2A. LMP-1 is the major transforming protein of EBV. It is a member of the tumor necrosis factor receptor (TNFR) superfamily and most closely resembles CD40. However, in contrast to CD40, LMP-1 signaling is constitutively active and requires no ligand. LMP-1 upregulates cellular Bcl-2 and other proteins that inhibit apoptosis and also stimulates cytokine production (interleukin IL-6 and IL-8)<sup>(10,11)</sup>.

B cell lymphoma-2 (Bcl-2) family proteins are key regulators of the apoptotic process. Bcl-2 blocks the induction of apoptosis by inhibiting the activation of pro-apoptotic family members such as BAX and preventing mitochondrial membrane depolarization<sup>(12)</sup>. Dysregulation of Bcl-2 expression, which results in abnormal cell growth, certainly contributes to the development of some tumors<sup>(13)</sup>. Over expression of Bcl-2 may result in accumulation of cells in the G0 phase of cell cycle division<sup>(14)</sup>; causing resistance to chemotherapeutic drugs and radiation therapy, while decreasing Bcl-2 expression may promote apoptotic responses to anticancer drugs<sup>(12,15)</sup>. Consequently, Bcl-2 has become a very attractive target for the design of new anticancer drugs<sup>(16)</sup>.

The study intended to evaluate the immunohistochemical expression of the Latent Membrane Protein-1 of Epstein - Barr virus and the anti-apoptotic protein Bcl-2 in Classical Hodgkin Lymphoma using a specified automated cellular image analysis system (Digimizer software analysis) then correlates their expression with clinicopathological parameters including: age of the patients and histological subtypes of the disease and to find if there is any relation between LMP-1 and Bcl-2 in classical Hodgkin's lymphoma.

### Methods

This retrospective study was conducted on fifty paraffin-embedded blocks of lymph nodes biopsies from patients diagnosed as Classical Hodgkin's Lymphoma. The cases were selected from archive files of the Department of Pathology of the Teaching Laboratories, Specialized Surgical Hospital in Baghdad Medical City and Al-Kadhimiya Teaching Hospital from November 2010 to June 2011. The control group consist of 20 age matched subjects having reactive lymph nodes biopsies which were obtained from Al-Kadhimiya Teaching Hospital Laboratories. Clinicopathological parameters including the age and histological subtypes of the tumor were obtained from the available histopathological reports. Ethical approval for the use of all specimens was obtained.

For each case, three representative sections were prepared. One section stained with Hematoxylin and Eosin and the histopathological diagnosis was revised by a pathologist, while other two sections were stained immunohistochemically for LMP-1 and Bcl-2 with horseradish peroxidase (HRP)-labelled-streptavidin-biotin method. The work was done at Al-Kadhimiya Teaching Hospital Laboratories.

This technique basically uses an unlabeled primary antibody, which was mouse monoclonal antibody purchased from DAKO (code no. of the kits were IS 753 for LMP-1 and M 0887 for Bcl-2), it binds to its corresponding antigen, followed by a

biotinylated secondary antibody to which the avidin-biotin complex (one avidin molecule, three biotin-labeled peroxidase molecules) attaches. If the sought-after antigen is present in the section, there will be an antibody-antigen interaction and an enzymatic reaction that can be detected by the chromogen, diaminobenzidine (DAB), which can be visualized by light microscopy.

Negative technical controls were obtained by replacing the primary antibody with buffer saline and positive tissue controls for each antibody were included with the samples using follicular lymphoma for Bcl-2 and Nasopharyngeal carcinoma for LMP-1.

Using a Noval light microscope (Noval, 320M), representative areas of IHC spots were selected and captured through the 40×objective, with a Sony digital camera (digital still camera DSC-W210). Each picture was analyzed by Digimizer (Version 3.7.0) software image analysis. The analysis of micro images by Digimizer software will give rise to three main digital parameters which are the number of objects stained, the average intensity and the mean area stained. For purpose of statistical analysis we use the following variables:

**A. Color Intensity:** which means the average intensity of the brown color for the selected objects depending on the expression of antigens in the cells. Digimizer color scale range from 0.000 representing black intense color to 0.999 for white color, taking into consideration that the higher the digital number, the lesser the staining intensity is, (i.e. the digital number that represents color intensity is inversely proportional to the actual intensity).

**B. Fractional area stained:** which equals to  $[(\text{mean area} * \text{Number of objects}) / \text{area of a single image field}] * 100$

**C. Digital Labeling Index:** for better estimation of the immunohistochemical expression of the LMP-1 and Bcl-2 molecular markers we used an arithmetic tool named as Digital Labeling Index.

This tool is calculated according to the following formula: (Fractional area \* reverse Intensity).

The digital intensity obtained by digimizer image analysis software was converted into three categories (weak, moderate and strong) by referring to NordiQC laboratories participating in schemes, institute of pathology. The NordiQC classified the color intensity of DAB positive cells into three categories: strong +3(0.292-0.521) for dark brown immunostained slides, moderate +2(0.522-0.668) for brown immunostained slides, weak +1(0.669-0.72) for light brown to yellow color immunostained slides and negative for non-stained slides.

Data were analyzed using SPSS program (Statistical Package for Social Sciences) version 16 and Microsoft Office Excel 2007. A *p* value of less than 0.05 was considered a statistically significant.

**Results**

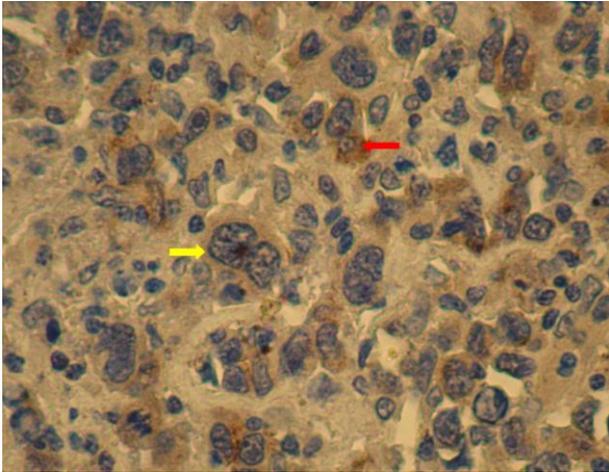
Most of HD cases were in childhood and early adult age, between 10-39 years with peak in 20-29 years and there was male predominance with M: F ratio of 1.27:1 (Table 1).

**Table 1. Distribution of HD cases according to age groups**

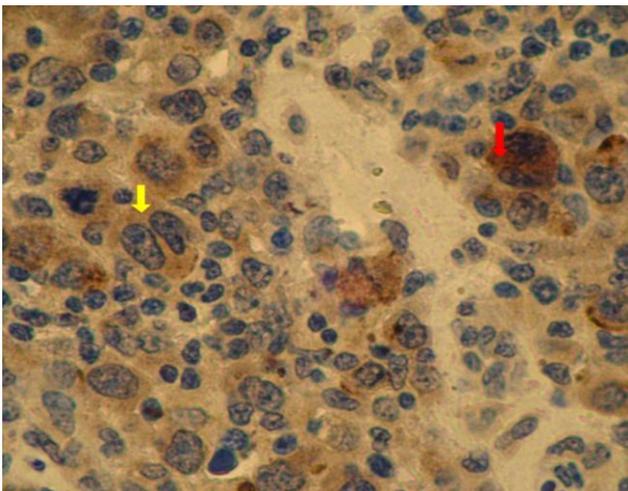
Age group (years)	Control	Hodgkin Lymphoma	Total
<16	6	9	15
16-34	10	22	32
35-49	3	11	13
≥50	1	8	9
<b>Total</b>	20	50	70

Regarding the histopathological subtype distribution of HD, the mixed cellularity was the commonest histological subtype (42%) followed by nodular sclerosis (22%) then lymphocyte depleted (24%) and only (12%) proved to be lymphocyte rich subtype. Moreover 74% of the cases presented with cervical lymphadenopathy.

Positive sections for both LMP-1 and Bcl-2 showed brown diffuse cytoplasmic stain of HRS cells of HD (Figure 1 & 2).



**Figure 1. Moderate, brown, diffuse cytoplasmic LMP-1 expression in HRS cells in lymph node from patient with Hodgkin's lymphoma (40X)**



**Figure 2. Moderate, brown, diffuse cytoplasmic Bcl-2 expression in HRS cells in lymph node from patient with Hodgkin's lymphoma (40X)**

LMP-1 was detected in 90% of Hodgkin's lymphoma cases versus 60% of control, ( $p$  value =0.007). However when applying T-test for immunohistochemical expression in control and lymphoma cases, the three digital analysis

parameters of the Digimizer were significantly higher in lymphoma than control group as shown in table 2.

**Table 2. Comparison of the digital parameters of LMP-1 between control and Hodgkin's lymphoma cases**

Parameter	Control	Lymphoma	P value
Intensity	0.91±0.14	0.32±0.23	<0.001
Fractional area	4.76±0.54	10.1±2.50	0.041
DLI	5.91±1.26	38.32±41.66	0.001

DLI = Digital labeling index

By using Digimizer software analysis for grading the intensity of the stained sections, all positive lymphoma cases showed high and moderate expression in contrast to control where all the positive cases showed weak expression as shown in table 3.

**Table 3. Distribution of the control and Hodgkin's lymphoma cases into different grades of intensity of LMP-1 expression\***

Intensity grade	Control	Lymphoma	Total
Negative	8(40%)	5(10%)	13(19%)
Weak	12(60%)	0	12(17%)
Moderate	0	9(18%)	9(13%)
Strong	0	36(72%)	36(51%)
<b>Total</b>	<b>20(100%)</b>	<b>50(100%)</b>	<b>70(100%)</b>

Chi square test was valid although the cases show different pattern of expression

By applying spearman rank linear correlation, there was significant inverse correlation between the age and LMP-1 in all three digital parameters of Digimizer. Figure 3 showed the positive correlation between the age and Digital labeling index of LMP-1 expression. Furthermore all children who were less than 16 years (9/50) were positive for LMP-1 expression as shown in table 4.

**Table 4. Age dependent expression of LMP-1 in control and lymphoma cases**

Age groups (years)	LMP-1	Control	Hodgkin Lymphoma	Total	P
<16	Negative	3 (50%)*	0	3	0.044
	Positive	3 (50%)	9 (100%)	12	
16-34	Negative	4 (40%)	2 (9.1%)	6	0.060
	Positive	6 (60%)	20 (90.9%)	26	
35-49	Negative	<b>1 (33.3%)</b>	<b>2 (18.2%)</b>	3	<b>1.000</b>
	Positive	2 (66.7%)	9 (81.8%)	11	
≥50	Negative	0	1 (12.5%)	1	<b>1.000</b>
	Positive	1(100%)	7 (87.5%)	8	

\*The percentage showed in this table represented the percent of negative and positive cases in each age group.

Regarding the relation to histopathological subtype, all cases with mixed cellularity subtype express LMP-1 (21/50) and the digital labeling index was significantly high compared to other

subtype (p=0.028), followed by nodular sclerosis subtype where 10/11 case were positive for LMP-1 (Table 5).

**Table 5. Relation between LMP-1 and Histological subtypes of Hodgkin’s Lymphoma**

Marker	Parameter	Subtype	No.	Mean±SEM	P value
LMP-1	Intensity	Mixed Cellularity	21	0.24±0.07	0.028
		Nodular Sclerosis	11	0.25±0.06	
		Lymphocyte Rich	6	0.48±0.40	
		Lymphocyte Depleted	12	0.44±0.34	
		<b>Total</b>	50	0.32±0.23	
	Fractional area	Mixed Cellularity	21	12.36±15.34	0.503
		Nodular Sclerosis	11	10.12±10.88	
		Lymphocyte Rich	6	8.67±10.67	
		Lymphocyte Depleted	12	6.20±8.62	
		<b>Total</b>	50	10.10±12.50	
	Digital Labeling Index	Mixed Cellularity	21	52.62±47.58	0.307
		Nodular Sclerosis	11	40.48±39.27	
		Lymphocyte Rich	6	35.69±40.54	
		Lymphocyte Depleted	12	23.27±29.79	
		<b>Total</b>	50	38.32±41.66	

In the present study Bcl-2 showed positive staining in 66% of HL patients versus 80% of control

(p=0.248). Table 6 showed the digital analysis parameters in control and HD.

**Table 6. Comparison of the digital parameters of Bcl-2 in control and Hodgkin's lymphoma cases**

Parameter	Control	Lymphoma	P value
Intensity	0.85±0.13	0.53±0.37	<0.001
Fractional area	4.56±1.02	19.00±6.44	0.330
DLI	5.40±1.27	52.45±10.37	0.047

DLI = digital labeling index

However, by using Digimizer analysis software for grading the intensity of the stained sections, 48% (24/50) of Hodgkin's cases showed strong expression and 16% (8/50) showed moderate expression and only one case showed weak expression, whereas 50% (10/20) of control subjects showed weak expression and 20% (4/20) showed negative expression as shown in table 7 .

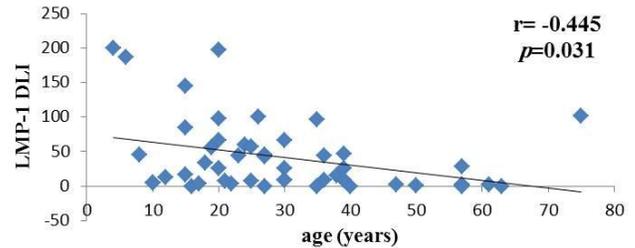
**Table 7. Distribution of lymphoma and control cases into different grades of intensity of Bcl-2 expression**

Intensity grade	Control	Lymphoma	Total
Negative	4(20%)	17(34%)	21
Weak	10(50%)	1(2%)	11
Moderate	6(30%)	8(16%)	14
Strong	0	24(48%)	24
<b>Total</b>	<b>20(100%)</b>	<b>50(100%)</b>	<b>70(100%)</b>

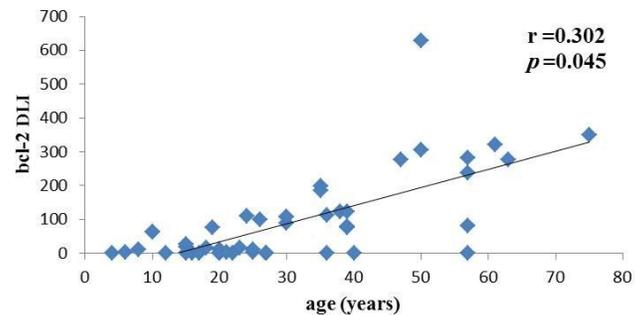
In relation to the histopathological subtypes ,the Intensity, Fractional area and Digital Labeling Index of Bcl-2 immunohistochemical expressions did not significantly differed in relation to the histological subtypes of the tumor when applying ANOVA test. However Lymphocyte Depleted Hodgkin's lymphoma showed the highest Digital Labeling Index of Bcl-2 expression followed by Mixed Cellularity subtype.

By applying spearman rank linear correlation, there was significant positive correlation between the age of the patients and the three digital analysis parameters. Figure 4 showed the negative correlation between the age and Digital labeling

index of Bcl-2 expression Furthermore Bcl-2 expression was high in patients older than 35 years old but this association was not statistically significant.

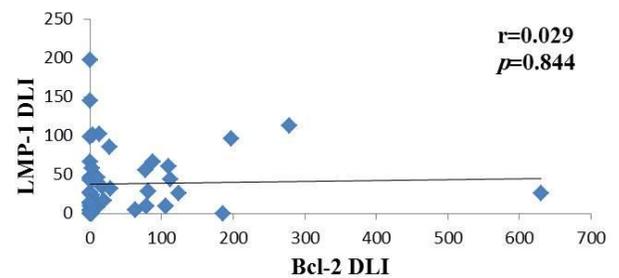


**Figure 3. Correlation between LMP-1 DLI and age**



**Figure 4. Correlation between Bcl-2 DLI and age**

By applying spearman rank linear, there was no significant correlation between LMP-1 and Bcl-2 expression in Hodgkin's lymphoma cases ( $p=0.844$ ).



**Figure 5. Correlation between LMP-1 DLI and Bcl-2 DLI**

## Discussion

In current study the age of most cases was between 10-39 years and more than half cases were below 27 years old. This was concordant with the results of Iraqi Cancer Registry in 2006 which showed that most of the Hodgkin lymphoma (HL) cases were between 15-35 years<sup>(6)</sup> and it was similar to other Iraqi studies done in 2007, 2005, 2004 and 2001<sup>(17-20)</sup> and to studies done in other Arab countries like Kuwait, Jordan and Egypt<sup>(21-23)</sup>. Thus we may conclude that in Iraq the age distribution of HL followed the pattern in developing countries in which the disease occurred earlier than in developed countries.

The slight male predominance observed in this study was in agreement with Iraqi Cancer Registry (ICR) results in 2006<sup>(6)</sup> and in other Iraqi study in 2005<sup>(20)</sup>, but it differed from Al-Safi results in 2007 which stated that incidence of Hodgkin lymphoma was equal in both male and female<sup>(17)</sup>. This could explain by the small number of the samples.

Similar to this study, other studies done in other Arab countries like Kuwait in 2003<sup>(21)</sup> and Egypt in 2010<sup>(23)</sup> and worldwide<sup>(24-26)</sup> showed that there was male predominance with ratio of 1.2-2.4:1.

Similar to the results of ICR in 2006<sup>6</sup> and other local studies in 2005<sup>(18)</sup>, 2003<sup>(27)</sup>, 2001<sup>20</sup> and a Kuwaiti study in 2003<sup>(21)</sup>, the mixed cellularity was the commonest histological subtype.

Cervical lymph nodes were the commonest site involved by tumour which was in agreement with the results of Iraqi study done in 2007<sup>(19)</sup>, Turkish study in 2005<sup>(29)</sup> and a Kuwaiti study done in 2003<sup>(21)</sup>.

In the current study, LMP-1 expression was positive in 90% of HD versus 60% of control group. This high expression was comparable to an Iraqi study that was done by Al-Safi in 2007<sup>(17)</sup> in which LMP -1 was found in 75% of Hodgkin lymphoma cases and also in line with other developing countries reaching 63% in Egypt<sup>(23)</sup>, 60% in Nigeria<sup>(30)</sup>, 82% in India<sup>(31)</sup>, and 93% in Iran<sup>(32)</sup>, whereas it was less common in developed countries, with percentages of 20-50% for North American<sup>(33)</sup> and European cases<sup>(34)</sup>, and 39% in China<sup>(26)</sup>.

Although LMP-1 EBV antigen was detected in HL and control subjects, the expression was significantly higher in HL and since LMP-1 is the major EBV oncogene and is essential for B-cell immortalization; thus we may conclude that the presence of the virus have played an important role in the pathogenesis of the disease.

In the present study all patients below 16 years, were infected with EBV and showed high expression of LMP-1. These results were in agreement with Al-Safi study which revealed that EBV expression was highest in childhood<sup>(17)</sup>. This followed the pattern of EBV expression in developing countries such as Kuwait<sup>(21)</sup>, Jordan<sup>(22)</sup> and Iran<sup>(35)</sup>. And this in contrast to the results found in USA<sup>(33)</sup>, United Arab Emirates<sup>(36)</sup>, and Netherlands<sup>(34)</sup>, in which high frequency of LMP-1 expression was seen in young adult. In developing countries, infection usually occurs in early childhood and usually passed unnoticed and the vast majority will be persistently infected with a reservoir of infection in memory B-cells this may lead to Hodgkin's Lymphoma to develop in childhood group<sup>(21)</sup>. Whereas in industrialized countries primary infection is often delayed until adolescence and frequently results in infectious mononucleosis (IM)<sup>(7)</sup>.

The high expression of LMP-1 in mixed cellularity HL that was seen in the present study was in concordance with several studies done in Jordan<sup>(22)</sup>, China<sup>(26)</sup> and Rio de Janeiro<sup>(37)</sup>.

Bcl-2 is an antiapoptotic protein, it was detected in 66% of HD cases, and this was in line with results of Rassidakis *et al*<sup>(38)</sup>, Wang and Taylor<sup>(39)</sup>, Kim *et al*<sup>(40)</sup> and Adelusola studies<sup>(30)</sup>, which found that Bcl-2 expression was detected in HRS cells in 61%, 56.45%, 43.7%, 56% and 40% of the cases respectively. Moreover the highest expression was detected in the aggressive Lymphocyte depleted subtype followed by mixed cellularity. This result was concordant with results of Flangea *et al*<sup>(41)</sup> and it explain the role in the pathogenesis of the disease, since overexpression of Bcl-2 may result in accumulation of cells in the G0 phase of cell cycle<sup>(14)</sup>, causing resistance to chemotherapeutic drugs and radiation therapy thus Bcl-2 was considered as a

critical cellular factor contributing to the pathogenesis and progression of cancer<sup>(16)</sup>.

Regarding the relation with age, the result of the present study was in agreement with Rassidakis *et al* study which had found that there was a significant association between Bcl-2 expression and patients older than 45 years old<sup>(38)</sup>. Thus we may propose that Bcl-2 expression is closely related to the age of the patients and is often associated with shorter survival time and generally poorer clinical outcomes<sup>(42)</sup>.

Lastly, in current study there was no significant correlation between LMP-1 and Bcl-2 expression in classical Hodgkin's lymphoma, this result was in line with the result of Cickusic *et al*<sup>(43)</sup> study. Although escape from apoptosis represents the major oncogenic event in CHL pathogenesis<sup>(44)</sup>, and LMP-1 upregulates cellular Bcl-2 and its homologues<sup>(45)</sup>, therefore, interaction between LMP-1 and Bcl-2 may be a critical factor contributing to the pathogenesis and progression of cancer. However both markers act independently on each other. So we may conclude that LMP-1 and Bcl-2 are independent biological markers in CHL.

The study conclude that the use of Automated Cellular Image Analysis System to quantitate immunohistochemical staining (Digimizer software) is easy to use, more objective, semiquantitative flexible image analysis software and is simple to perform, The LMP-1 was detected in 90% of Hodgkin's lymphoma compared to 60% of control subjects however the expression of LMP-1 was strong and moderate in lymphoma cases, whereas it was weak in all control subjects. Thus we may suggest a role for the virus in the pathogenesis of Hodgkin's lymphoma. Bcl-2 was detected in control subjects as well as in Hodgkin lymphoma cases but the intensity of immunostaining of Bcl-2 was significantly stronger in lymphoma cases ( $p=0.001$ ) which may suggest that escape from apoptosis represents the major oncogenic event in CHL pathogenesis. The EBV antigen LMP-1 and Bcl-2 were independent markers in HL.

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## D240 as a Potential Marker that Differentiate Verrucous Carcinoma from Squamous Cell Papilloma

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### Abstract

- Background** Verrucous carcinoma is a distinct variant of oral squamous cell carcinoma characterized by slow growth and rare metastases. It may present diagnostic difficulties as it may be inaccurately diagnosed as squamous cell papilloma.
- Objective** The study performed a comparative immunohistochemical staining for both entities to obtain a possible method of differentiation.
- Methods** The study involved 13 samples of oral verrucous carcinomas and 10 samples of oral squamous cell papillomas which were stained immunohistochemically with antibodies to the lymphatic endothelial marker D240.
- Results** In all samples, the entire epithelium of verrucous carcinomas was positively stained with D240 whereas only the basal cell layer of squamous cell papillomas was positive.
- Conclusion** D240 could be used as a differentiating marker between oral verrucous carcinomas and squamous cell papillomas.
- Keywords** Verrucous carcinoma, Squamous cell papillomas, D240.

### Introduction

Verrucous carcinoma (VC) is a rare variant of oral squamous cell carcinoma which was first described by Ackerman in 1948<sup>(1)</sup>. It is distinct in its slow growth and ability to become locally aggressive if not treated properly. However, even with local tumor progression, regional or distant metastasis is rare<sup>(2)</sup>. Its occurrence originally was related to the use of chewing tobacco or snuff, although this was never substantiated by controlled epidemiologic investigations. Moreover, HPV appears to be of etiologic significance<sup>(3)</sup>, although not supported universally. VC predominantly occurs in older people, the majority of cases being observed in individuals in

their sixth decade or later, and has a higher incidence in males<sup>(4)</sup>.

On clinical examination, VC appears as a relatively well-circumscribed, elevated, nodular mass with a surface that may be pebbled, papillary, verrucous, or smooth. Depending on the degree of surface keratinization, it varies in color from white to red to admixtures of both<sup>(5)</sup>.

VC is broadly based and invasive, with plump papillary invaginations of thickened and infolding epithelium that lack the usual cytologic criteria of malignancy<sup>(4)</sup>. The exophytic surface is covered by abundant orthokeratin and/or parakeratin that also fill the crevices of deep surface invaginations. Because the lesions are well differentiated,

superficial biopsies often do not yield a diagnosis. Clinical correlation can be of great value because lesions tend to be more impressive visually than microscopically<sup>(5)</sup>.

The clinical and histologic differential diagnosis of VC includes benign and malignant squamous proliferations, including reactive inflammatory epithelial hyperplasia, squamous papilloma, conventional squamous cell carcinoma (SCC), and papillary squamous cell carcinoma (PSCC)<sup>(6)</sup>. Lack of cellular atypia serves to rule out conventional SCC and PSCC whereas distinguishing VC from squamous papilloma and reactive inflammatory epithelial hyperplasia may be more problematic<sup>(4, 7)</sup>.

D240 is a monoclonal antibody to podoplanin, it was primarily employed as a specific marker for lymphatic vessels, however; it was shown to be of use as a differentiation marker in a number of tumors<sup>(8,9)</sup> and as a marker for malignant cells in other studies<sup>(10)</sup>.

This study compared the immunohistochemical staining pattern between VC and squamous cell papilloma using a lymphatic vessel marker (D240).

**Methods**

The archives of the department of oral pathology at the college of dentistry / Baghdad University were retrospectively reviewed for formalin fixed – paraffin embedded biopsy samples for verrucous carcinomas and squamous cell papillomas. The samples were checked for size adequacy and fitness to the histological diagnostic criteria, three samples were excluded due to these reasons. Five µm thickness tissue sections were cut from each tissue sample and mounted on positively charged slides (Fisher scientific, USA).

Slides were baked in hot air oven at 65 °C overnight. Sections were sequentially dewaxed through a series of xylene, graded alcohol and water immersion steps. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide followed by blocking the nonspecific antibody binding with normal goat serum this was followed

by the application of the primary antibodies for D240 (Dako Cytomation - USA) with a dilution of (1:100). Lymphatic vessels were considered as appositve control to the marker. The slides were incubated for 1 h at 37 °C and then kept at 4°C in a humid chamber overnight. Next day, after washing with PBS, biotinylated antimouse IgG were applied to the sections, incubated and rinsed with a stream of PBS. Conjugated antibodies were visualized with DAB chromogen. Sections were counterstained with Mayer’s hematoxylin for 1-2 min, dehydrated and mounted. Then a microscopical examination of the slides was performed.

**Results**

The archival review resulted in tissue samples from twenty three patients, thirteen of verrucous carcinomas and ten of squamous cell papillomas (Table 1 and figures 1, 2, 7, 8).

Diffuse positive immunohistochemical staining was shown in all verrucous carcinoma tissue samples extending from epithelial top to bottom in addition to strong positivge staining of the small sized lymph vessels in the underlying connective tissue stroma (Figures 3-6). Whereas only the basal cell layer of the squamous cell papilloma tissue samples showed positivity for D240 monoclonal antibody with strong positivity in large lymph vessels at the intervening papillae and underlying connective tissue stroma (Figures 9-12).

**Table 1. Study samples’ age and sex**

Tissue sample	No.	Age (years)	Sex	
			Female	Male
VC	13	56.07±16.32	5	8
SCP	10	26±17.66	4	6

VC = Verrucous carcinoma, SCP = Squamous cell papilloma

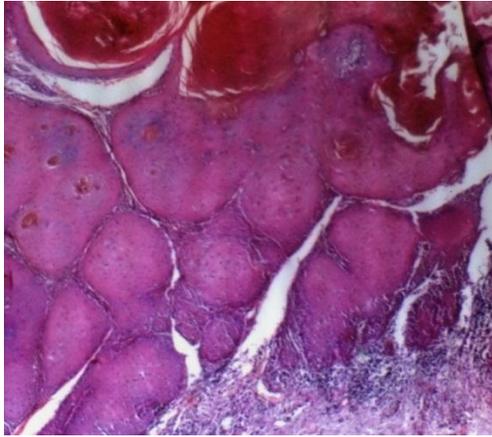


Figure 1. Verrucous carcinoma with surface keratin (H&E) X40

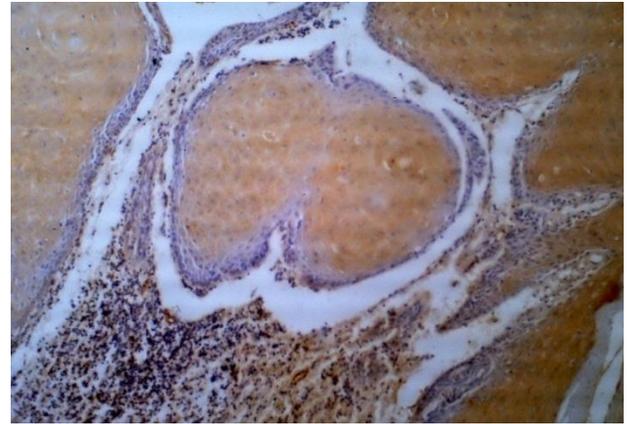


Figure 4: Verrucous carcinoma demonstrating diffuse staining pattern throughout the epithelial layers (Immunohistochemistry with D2-40) X100



Figure 2. Verrucous carcinoma showing bulbous rete ridges and subepithelial infiltration of inflammatory cells (H&E) X40

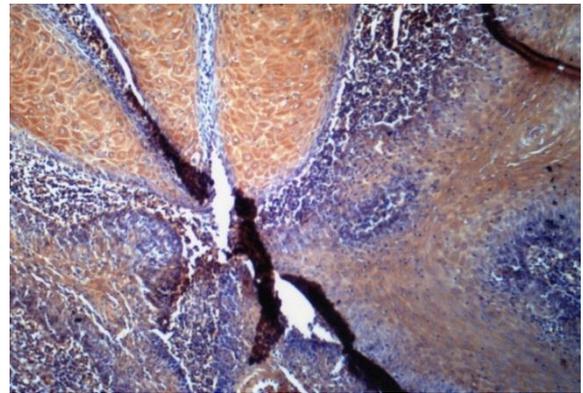


Figure 5. Diffuse tumor cell positivity of verrucous carcinoma and positive small lymphatic vessels peritumorally (Immunohistochemistry with D2-40) X200

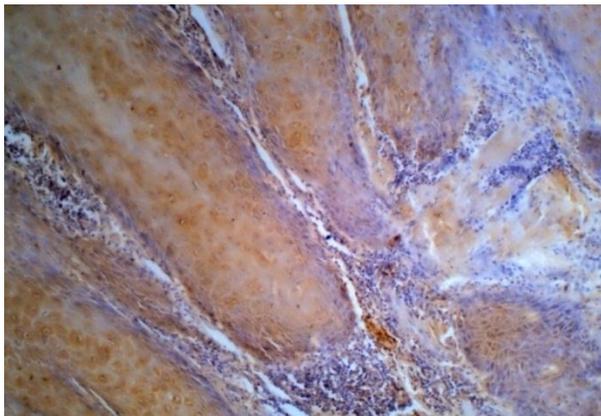


Figure 3. Verrucous carcinoma demonstrating diffuse staining pattern throughout the epithelial layers (Immunohistochemistry with D2-40) X100

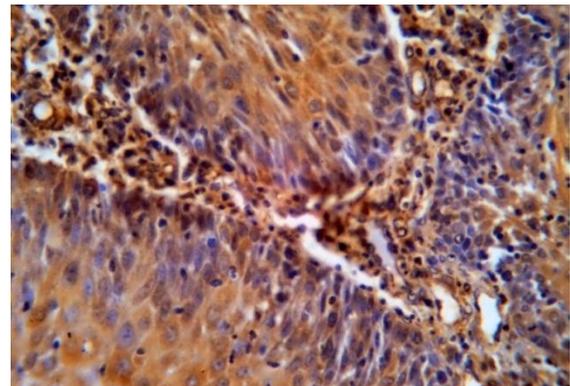


Figure 6. Advanced front of verrucous carcinoma with diffuse positivity for D2-40 antibodies with immunopositive small lymphatic vessels in-between (Immunohistochemistry with D2-40) X400

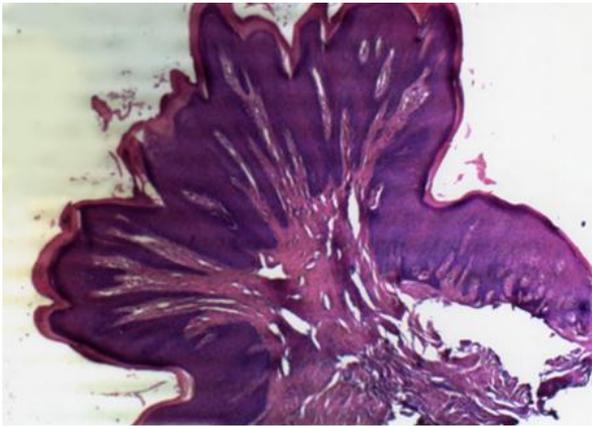


Figure 7. Squamous cell papilloma (H&E) X40

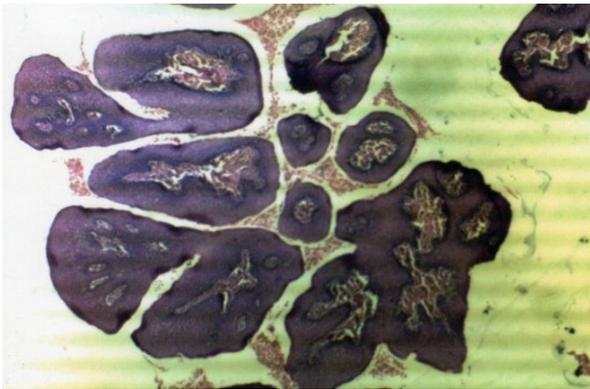


Figure 8. Squamous cell papilloma cut in cross section (H&E) X40

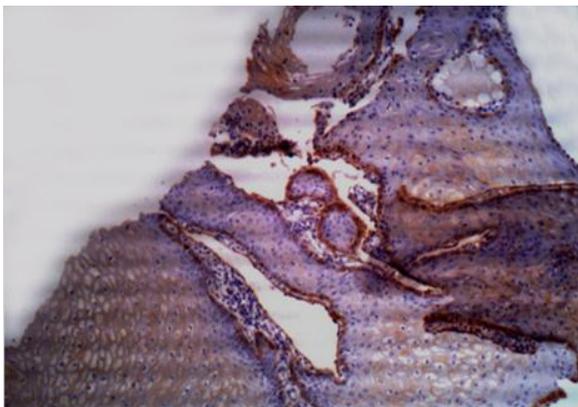


Figure 9. Strong basal cell positivity in squamous cell papilloma tissue sample (Immunohistochemistry with D2-40) X100

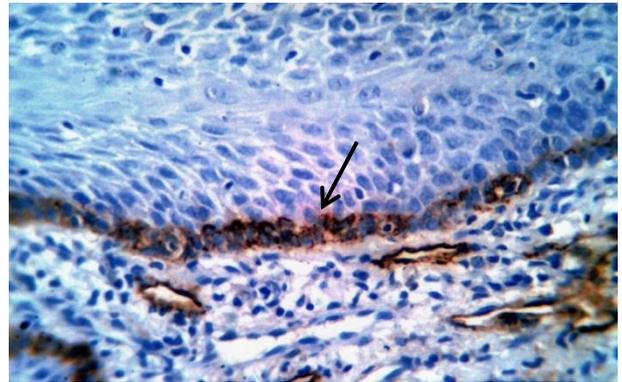


Figure 10. Squamous cell papilloma tissue sample demonstrating strong basal cell positivity with numerous positive subepithelial lymph vessels (Immunohistochemistry with D2-40) X400

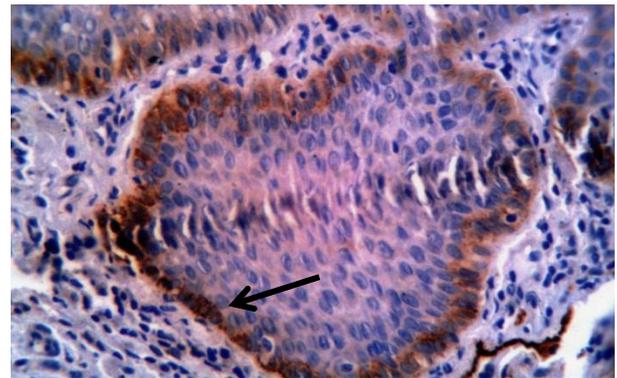


Figure 11. D2-40 immunopositivity confined to the basal cell layer in a squamous cell papilloma tissue sample (Immunohistochemistry with D2-40) X400

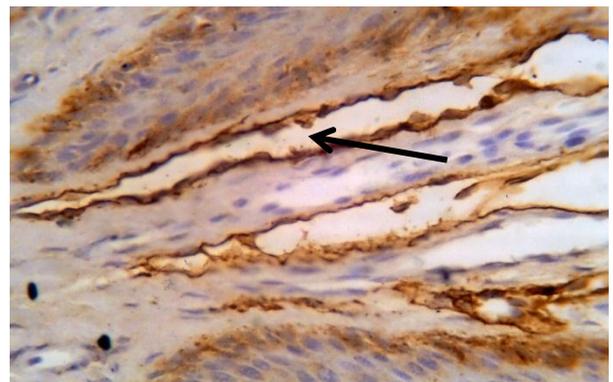


Figure 12. D2-40 positive large lymphatic vessels between positive basal cell layer in a squamous cell papilloma tissue section (Immunohistochemistry with D2-40) X400

## Discussion

Verrucous carcinoma has been the subject of a debate concerning its diagnostic features and mode of treatment since its discovery<sup>(1,4)</sup>. It is a differentiated variant of squamous cell carcinoma and may present diagnostic difficulties as it may be erroneously diagnosed as squamous papilloma<sup>(11)</sup>. Determining the DNA content by nuclear cytometry on Feulgen-stained histologic sections has been reported to be diagnostically useful in detecting cells with abnormal DNA content in VC; this finding may be helpful in differentiating VC from benign lesions<sup>(12)</sup>. Recording nuclear size with image analysis has been suggested to be helpful in differentiating VC from squamous papilloma, as the cells in VC are, in general, larger (>300 µm) than those in papillomas (<250 µm)<sup>(11)</sup>. D240 is a monoclonal antibody directed against podoplanin, which is a 38 kDa type-1 transmembrane glycoprotein<sup>(13)</sup> which has been reported to be expressed occasionally in normal epidermal basal cells<sup>(14)</sup>. It is widely used as a specific marker for lymphatic endothelial cells and lymphangiogenesis in many species, as podoplanin is expressed on lymphatic but not on blood vessel endothelium<sup>(15)</sup> which was demonstrated in the lymphatic and vascular endothelial cells in this study, moreover; it has been reported to be upregulated in human squamous cell carcinomas<sup>(14)</sup>. This study is the first of its kind describing IHC of D240 monoclonal antibodies verrucous carcinoma in comparison to squamous cell papilloma. Previous studies were either based on cytomorphological features or counting mitotic figures<sup>(11,12)</sup>, immunohistochemical staining with D240 showed positivity confined to the basal cell layer in all the samples of squamous cell papillomas whereas the positive staining in verrucous carcinoma samples involved all the layers of epithelium, reflecting the disparity of the epithelia amid the lesions and facilitating their differentiation. The resulting staining pattern could be attributed to normal physiologic regeneration within basal cell layer in normal

epithelium and, in this study; squamous cell papilloma epithelium.

Consequently, and with no statistical analysis required, this method of comparative staining could be used to differentiate verrucous carcinomas from squamous cell papillomas.

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## Histopathological Changes in Adult Male Rat's Liver Induced by Continuous Darkness

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### Abstract

- Background** It is well known that liver is an endocrine as well as exocrine gland; it also synthesizes, accumulates, detoxifies and transports certain substances. Melatonin is the principle hormone of the pineal gland, which is mainly secreted at night and it is definitely documented to regulate the physiology of all tissues and cells keeping their normality.
- Objective** This work is designed to study the effect of continuous darkness on hepatic tissues.
- Methods** Adult Wister albino rats were kept in complete 24 hours darkness for successive 4 periods. Rats were divided into 16 groups. Group II, III, IV and V were left in continuous darkness for 2, 4, 6 and 8 weeks, respectively. Group I<sup>+</sup>, Group I<sup>++</sup>, Group I<sup>+++</sup>, and Group I<sup>++++</sup> were the control groups for group II, III, IV and V correspondingly. After the last day of the dark period for each group, the animals were dissected under effect of anesthesia. The liver was weighed and right lobe of liver was processed for study its histopathology.
- Results** The results showed no important histopathological effect on short and medium periods, while on long periods; there was histopathological changes represented by clear lobulation and inflammatory cell infiltrations.
- Conclusion** Continuous darkness affects the hepatic tissues of rats depending on the length of exposure.
- Key words** Melatonin, Darkness, Liver injury.

### Introduction

Despite decades of investigations on the darkness hormone namely melatonin; researches are still going on, about this hormone, with great deal of interest <sup>(1,2)</sup>. Melatonin is the principle hormone of the pineal gland and its level is well known to undergo an undulating level through daylight and night darkness, and it hits the highest point level at night <sup>(3)</sup>. It has receptors in all bodily tissues and cell, through which it thought to regulate their function <sup>(4)</sup>.

It is well known that liver is an endocrine as well as exocrine gland; it also synthesizes, accumulates, detoxifies and transports certain

substances <sup>(5)</sup> and its integrity is principally vital for whole body tissues and organs, so the effect of any environmental events on hepatic tissues could be critical for the entire body healthiness, such as alcohol or drugs administration <sup>(6)</sup>.

Melatonin has hepatoprotective potential in various models of oxidative stress and reduces liver damage after sepsis, hemorrhagic shock, ischemia/perfusion and in various models of toxic liver injury

These effects of melatonin on liver are mediated by its influence on hepatic antioxidants enzymes, nitric oxide signaling, hepatic cytokine & heat shock protein expression <sup>(7)</sup>.

The aim of the present study is to assess the "histopathological changes" in liver tissue, in response to gradually increasing periods of continuous darkness in adult male rats.

**Methods**

Forty healthy mature ten week old male Wister albino rats were kept individually in wire meshed stainless steel cages room temperature 22±2°C, fed controlled pellet diet and tap water was provided for drinking *ad libitum*. They were divided into 8 groups, each consisting of 5 rats. Group I<sup>+</sup>, Group I,<sup>++</sup> Group I,<sup>+++</sup> and Group I<sup>++++</sup> were the control of group II, III, IV, and V respectively. All of the 4 control groups were put on 12:12 light – dark cycle. Group II, III, IV, and V were put in continuous darkness for a period of 2, 4, 6 and 8 weeks respectively. All the 8 groups were kept in and were put individually in–wire meshed. At the last day of 1<sup>st</sup> couple of weeks, rats of group II with its control group (Group I<sup>+</sup>), were sacrificed under diethyl ether anesthesia effect, the whole liver was weighed and the right lobe of liver was removed, fixed in Boun's

solution immediately and processed through for histopathological study by light microscopy, using serial paraffin sections of 5 µm thickness stained with haematoxyline and eosin <sup>(8,9)</sup>. At the same manner the rats of group III was dissected at the end of the 4<sup>th</sup> weeks also with its control group (Group I<sup>++</sup>). The rats of group IV with its control group (Group I<sup>+++</sup>), were managed at same way at end of the 6<sup>th</sup> week, and rats belong to group V with its control group (Group I<sup>++++</sup>), were operated on at end of the 8<sup>th</sup> week. Histopathological as well as anatomical examinations were performed. Biostatistical analysis was done to evaluate the significance of results by analysis of variance, using student-t-test <sup>(10)</sup>.

**Results**

Descriptive as well as morphologic studies were done, as follows:

Body & liver weight: There was no significant effect of continuous darkness on the over all body weight as shown in (Table 1).

**Table 1. Effect of continuous darkness on the body weight of 10 week old male rats**

Time of keeping rats in continuous darkness	Body wt. of rats at 1 <sup>st</sup> day of experiment	Body wt. of rats at last day of experiment	Difference in body wt.
Control (Group I <sup>+</sup> )	411.17±62.9	437.95±74.9	26.78±11.9
2 week continuous darkness	419.84±77.1	443.81±75.2	23.97±10.8
Control (Group I <sup>++</sup> )	410.97±65.1	454.42±51.1	43.45±17.1
4 week continuous darkness	422.03±82.9	461.11±80.2	39.08±15.5
Control (Group I <sup>+++</sup> )	423.23±44.0	491.96±79.0	68.73±23.2
6 week continuous darkness	416.00±72.8	489.09±90.1	73.09±25.2
Control (Group I <sup>++++</sup> )	418.02±74.6	511.19±75.2	93.17±32.6
8 week continuous darkness	405.25±64.1	503.25±87.7	98.00±34.1

Results were expressed in mean ± SD of 5 rats.

All differences were statistically not significant (P>0.05) when compared with its control.

Liver weight was not affected in group II, whereas it was significantly affected in group III, IV, and V (Table 2). Liver weight to body weight ratio was also estimated (Table 3). Liver weight was increased in group III and still more increased in group IV, and the outer surface of

the organ was clearly irregular. Then after, at group V the liver shrank significantly and the outer surface of the organ was markedly nodular.

**Table 2. Effect of continuous darkness on liver weight of 10 week old male rats**

Time of keeping rats in continuous darkness	Liver weight at autopsy (mg)
Control (Group I <sup>†</sup> )	15080±121.3
2 week continuous darkness	15119±169.3
Control (Group I <sup>††</sup> )	15271±101.2
4 week continuous darkness	15312±115.2*
Control (Group I <sup>†††</sup> )	15382±123.4
6 week continuous darkness	14981±118.1*
Control (Group I <sup>††††</sup> )	15506±122.5
8 week continuous darkness	13294±116.1*

Results were expressed in mean ±SD of 5 rats.  
\* = P<0.001)

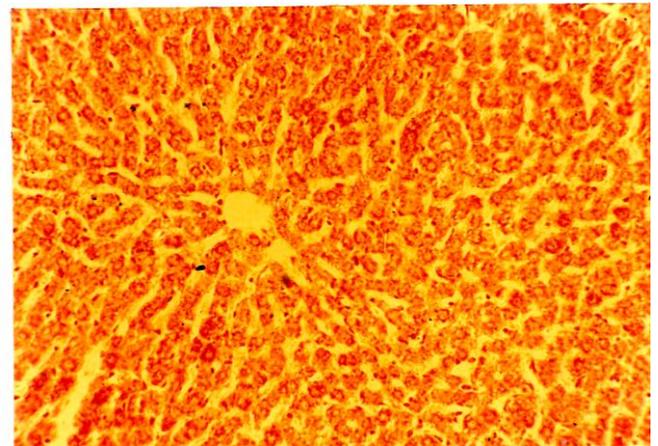
**Table 3. Effect of continuous darkness on liver weight to body weight ratio in 10 week old male rats**

Time of keeping rats in continuous darkness	Liver weight/100g BW
Control (Group I <sup>†</sup> )	3.443±0.18
2 week continuous darkness	3.406±0.20
Control (Group I <sup>††</sup> )	3.360±0.18
4 week continuous darkness	3.320±0.19
Control (Group I <sup>†††</sup> )	3.126±0.17
6 week continuous darkness	3.063±0.16*
Control (Group I <sup>††††</sup> )	3.033±0.21
8 week continuous darkness	2.641±0.19**

Results were expressed in mean ± SD of 5 rats.  
\* = P<0.01, \*\* = P<0.001

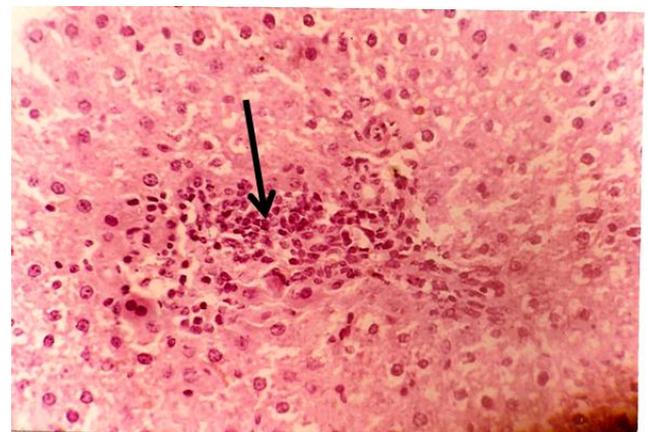
**Histological observations:**

The liver of the control groups showed normal histological structure; consists of epithelial, liver cells (hepatocytes); arranged into interconnected plates forming hepatic cords separated by vascular sinusoids, the hepatocytes extend radially from the central vein toward the periphery forming the hepatic cords (Figure 1).



**Figure 1. Liver tissues of 10 week old male control rat (H&E stain X125)**

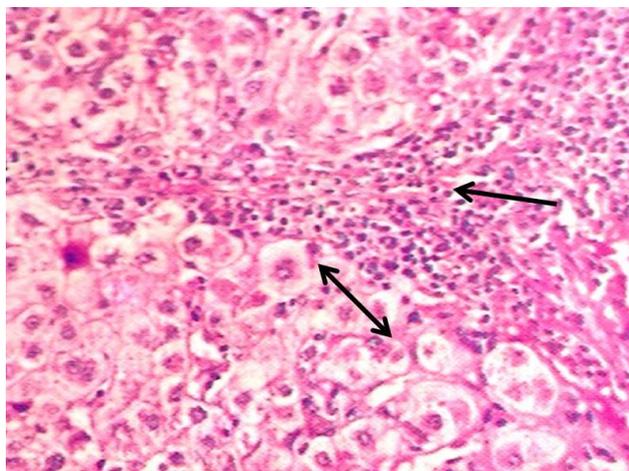
In **group II**: no any clear abnormalities were noticed in the liver architecture, apart from a little infiltration of mononuclear inflammatory cells seen on the portal tract. Nuclear vacuolation was noticed and some cytoplasmic fat vacuoles (Figure 2).



**Figure 2. Liver tissues in male rat exposed to 2 week continuous darkness. Little infiltration of mononuclear inflammatory cells (arrow) seen on the portal tract. Nuclear vacuolation was noticed and some cytoplasmic fat vacuoles. (H&E×125)**

In **group III**: The liver tissue showed swelling of a few hepatocytes, blurring of the septal-parenchymal junction with heavy infiltration of connective tissues with lymphocytes, macrophages, granulocytes. Apoptotic

hepatocytes noticed concomitantly with large vacuolated cells. Acidophil bodies also noticed (Figure 3).



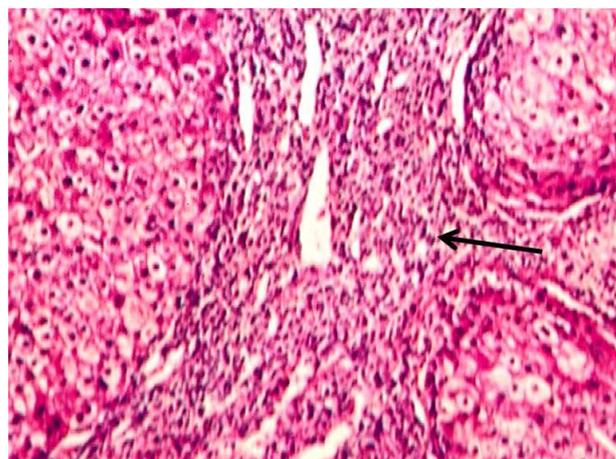
**Figure 3.** Liver tissues in male rat exposed to 4 week continuous darkness. Necrosis was seen blurring the septal-parenchymal junction, and obvious swelling of adjacent liver cell cytoplasm (double head arrow). Severe infiltrated with different types of inflammatory cells was illustrated in the connective tissue (one head arrow), and unusual ductular proliferation is seen. (H&E×250)

In **group IV**: There was nodular formation; consisting of aggregated hepatocytes, and the nodules were surrounded by thick bands of fibrous connective tissue, infiltrated with large number of mononuclear cells and macrophages. There was a variation in the size of hepatocytes and their nuclei, with moderate amount of fatty vacuoles within those hepatocytes. A large number of apoptotic cells in parenchyma and mononuclear cells infiltration both in septal and parenchymal constituents (Figure 4).

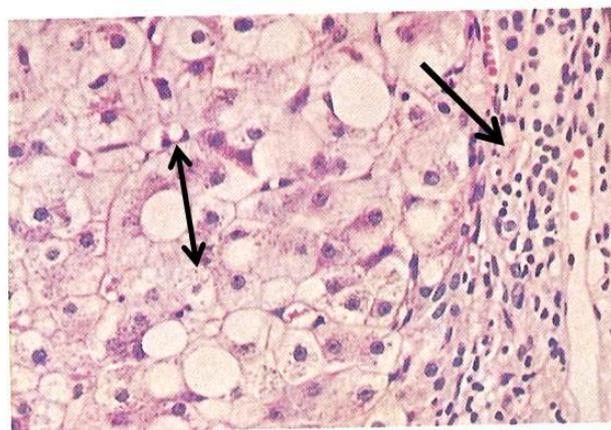
In **group V**: Abnormal liver architecture was clearly seen, with wide fibrous septal bands, radiating from the portal tract to surround the lobules. Those lobules consisted mainly from hepatocytes with a large number of mononuclear cells.

Prominent fatty vacuolation, was seen within the hepatocytes. Apoptotic hepatocytes seemed

to be much more abundant in this group (Figure 5).



**Figure 4.** Liver tissues in male rat exposed to 6 week continuous darkness. Bands of fibrous connective tissue surrounding a population of hepatocytes forming nodular compartments of liver tissues. Connective tissue infiltration with mononuclear cells (arrow). There was discrepancy in the size of hepatic cells and their nuclei. Fatty change was present. (H&E. ×250)



**Figure 5.** Liver tissues in male rat exposed to 8 weeks continuous darkness. A more apparent nodular organization of the hepatic tissue was viewed. The lobules were surrounded by short connective tissue bands (one head arrow). A clear fatty change was seen (double head arrow). (H&E ×250)

### Discussion

The continuous darkness was well documented to increase the endogenous melatonin, the principle pineal neuro-hormone <sup>(3,4,11)</sup>, and the

melatonin is known to causes no effect on body weight in rats <sup>(12,13)</sup>; this might be because melatonin administration does not affect food intake in rats, as it was noticed in our work. The increase of liver weight induced by continuous darkness which was proportionate with length time exposure to darkness, might be explained by the fact that melatonin can reach all body tissues and cells, acting through specific receptors in all body tissues <sup>(14,15)</sup>. Once melatonin reaches any bodily tissue it exerts its action immediately, and melatonin has a dose-dependent physiologic action <sup>(16,17)</sup>. In the present study no significant hepatomegaly occurred, on those animals kept for 2 weeks of continuous darkness, however there was hepatomegaly accompanied with longer period of darkness. These results could be discussed by the fact that melatonin has damaging effect only when excreted on high level <sup>(15- 17)</sup>. The hepatomegaly is essentially the consequence of hepatocyte death which could be of 2 types; the ballooning degeneration leading to massive increase in cell size or, apoptosis; this is also leads to hepatomegaly since these apoptotic cells are entrapped within a large gathering of inflammatory mononuclear cells leading to increasing volume of cellular parenchyma <sup>(6)</sup>. The rise in built and thickening of connective tissue bands also could contribute to that hepatomegaly. This large increment in septal thickness is due to the increase in production of fibro-collagenous tissue whenever there are any injurious events to the liver <sup>(6,18)</sup>. Also the melatonin has specific effect on fibroblast cells which are the active collagen - secreting cells and the basic forming cells of the connective tissues <sup>(6,19)</sup>. The other provider to hepatomegaly could be the dilated blood vessels, because melatonin has a well-known vasodilator action <sup>(20,21)</sup>.

The shrink and regression in size of liver was so clear at toxic effect of long dark period , that might be discussed by the fact that liver gets regression and shrink after any toxic damaging effect leading to fibrosis and scaring preventing

the regenerating hepatocytes from expanding the parenchymal mass <sup>(6,18)</sup>.

The blurring of the septal-parenchymal junction might be caused by the beginning of increased in connective tissue bulk as discussed previously. The swelling of hepatocytes is always seen in any destructive effect that results from the buildup of fat and water as well as proteins which are normally is exported <sup>(6)</sup>.

The heavy infiltration of connective tissues and parenchyma, with lymphocytes, macrophages granulocytes and other mononuclear cells, is due to the inflammatory process within the liver tissues, since these are the principal cells in any inflammatory process <sup>(6,18)</sup>. Apoptotic cells are seen in any programmed cell death, to replace the damaged cells by new healthy ones <sup>(6,21)</sup>.

The large vacuolated hepatocyte is the form of cell that represents the intermediate type of liver injure, their cytoplasm seen filled with large and small vacuoles of fat, predominantly triglycerides, what is called steatosis, accordingly, these hepatocytes are named steatotic hepatocytes <sup>(6)</sup>. Acidophil bodies noticed at this work in fact, were the dead hepatocytes demonstrating current liver damage <sup>(6,18)</sup>.

In the last group the hepatic lobules that entrapped within thick bands of fibrous connective tissues were formed by almost normal hepatocytes. The enlightenment for that could be the fact that a new generation of hepatocytes, is formed in an attempt of the liver to substitute the dead hepatocytes <sup>(6,17,18)</sup>.

The present study about the histopathological effect of continuous darkness on the liver both macroscopic and microscopic reveals no injurious effect on short period of continuous darkness, whilst it induced ultimately destructive effect with long period of continuous darkness.

The severity of destructive and damaging effects seemed to be correlated with the length of continuous darkness, which could be due to graduated amount of melatonin secretion, because the effect of melatonin is proportional to its level <sup>(12,23-25)</sup>. The time-course (30 days), for rats, was regarded as a long period according to

Peltier *et al* <sup>(25)</sup>. We conclude that hepatic tissues are affected by periods of darkness depending on the length of exposure.

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## Observing the Outcome of using NeuroAid [MLC 601] on a sample of Iraqi Stroke Patients

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### Abstract

**Background** Stroke is one of the major causes of morbidity and mortality throughout the world, and carries greater economic costs. [MLC 601] originates from Traditional Chinese Medicine approved in 7 countries as drug that can aid post-stroke recovery.

**Objective** To assess [MLC 601] efficacy in improving outcomes of Iraqi patients' stroke.

**Methods** Two hundred ischemic stroke patients and 17 intracerebral hemorrhage patients were participated in this study; they took [MLC601] at the onset of their disease for 3 months and were assessed monthly for the motor power using modified Rankin scale mRs scale, speech, and visual field assessments.

**Results** mRs grade [4-5] were changed from (zero out of 159) at onset to (89 out of 55) at first month and to 98 out of 134 ischemic stroke patients after 3 months; also mRs grade [4-5] were changed from (1 out of 17) at onset to (12 out of 17) at first month and to 12 out of 17 intracerebral hemorrhage patients after 3 months. In 44% of the enrolled patients with aphasia were improved. Visual field assessments showed improvement in 43% of the patients with homonymous hemianopia after 3 months [MLC 601] treatment.

**Conclusion** [MLC 601] is associated with improvement in all post stroke disabilities and placebo controlled trial is crucial to assess the benefit of it.

**Key words** Stroke, intracerebral hemorrhage, MLC 601, NeuroAid

### Introduction

Stroke is the sudden onset of focal neurologic symptoms due to ischemia or hemorrhage in the brain <sup>(1)</sup>. It is the commonest neurological disorder admitted in the general medical and neurological wards (accounting for 50 % of the neurological wards admission) <sup>(2)</sup>. Although stroke death rates decreasing substantially in the United States from 1996 to 2005 <sup>(3)</sup> stroke still the second leading cause of death worldwide <sup>(4)</sup>, and is the

commonest cause of morbidity worldwide <sup>(2,4)</sup>. The high mortality and morbidity of stroke poses a high economic and social burden on the society <sup>(5)</sup>. To date, no effective treatment has been found that reduces stroke-induced disabilities. [MLC 601] has recently been approved in 7 countries as a treatment that can support post-stroke recovery <sup>(6)</sup>. [MLC 601] originates from traditional Chinese medicine and is a combination of nine herbal

components and five animals components<sup>(7)</sup>, trials of [MLC 601] in China found that patients receiving [MLC 601] were 2.4 times more likely to be independent at 1 month after stroke than the control group<sup>(6)</sup>. The neuroproliferative and neuroprotective effects of [MLC 601] (and hence its potential role in neuroplasticity after stroke) have been recently established in animal models of stroke and ischemia<sup>(8)</sup>. MLC601 (NeuroAid) were provided by Moleac (Singapore). The composition of MLC601 (0.4 g per capsule) was the following: 0.57 g Radix astragali, 0.114 g Radix salvia miltiorrhizae, 0.114 g Radix paeoniae rubra, 0.114 g Rhizoma chuanxiong, 0.114 g Radix angelicae sinensis, 0.114 g Carthamus tinctorius, 0.114 g Prunus persica, 0.114 g Radix polygalae, 0.114 g Rhizoma acori tatarinowii, 0.095 g Buthus martensii, 0.0665 Hirudo, 0.0665 g Eupolyphaga seu steleophaga, 0.0285 g Calculus bovisartifectus, 0.0285 g Cornu saigae tataricae<sup>(8)</sup>.

In our study we summarize reported neurological improvements in Iraqi stroke patients who used Neuraid as part of their treatment.

## Methods

Two hundred and seventeen patients with stroke were admitted into Al-Kadhimiya Teaching Hospital and Hospital of Neurosciences from January 2007 to January 2011 was included in the study. The patients and their companions' written consent were taken before participating in this study and the study was approved by ethical committees of Alkindy College of Medicine.

We excluded unconscious patients and those with minor stroke at the onset of the disease.

The [MLC 601] dose received was 4 tablets, 3 times per day<sup>(8)</sup> for 3 months [MLC 601] was given in addition to the patients other treatment like antiplatelet, anticoagulant, lipid-lowering, antihypertensive, hypoglycemic drugs and other medications.

The patients were assessed medically and neurologically at time of admission and thereafter

monthly for 3 months after discharge from the hospital. All patients were sent for brain CT scan, the residual disability was assessed according to modified Rankin scale [mRs]<sup>(9)</sup> at onset and monthly thereafter.

Six speech domains were assessed: fluency, comprehension, naming, repetition, writing and readings (if the patient could read and write prior to the stroke). Visual field was assessed only for the patients with field defects using perimetry at onset and at first and third months post stroke. For the homonymous heminopia, we considered any enlargement in the visual field using perimetry as an improvement.

We lost contact with 25 patients for unknown reasons mainly because of the unstable security state in Iraq which prevent them to maintaining follow up.

## Results

Two hundred patients had ischemic stroke, and 17 had intracerebral hemorrhage. We had 133 male participants out of the 200 ischemic stroke patients, and 11 males out of the 17 intracerebral hemorrhage patients. Their age ranged between 24-83 years (Table 1). Seventy three patients were females, while 144 were males, majority are between 40-69 years of age. Age and gender distribution of patients is present in tables 1 and 2.

Table 3 showed that only one out of 17 intracerebral hemorrhage patient in the group with mRs below grade 2; and 12 out of 17 in the first and third month.

We considered G2-5 mRs score to reflect patients' dependence, while G0-1 was equivalent to the patient being independent. Table 4 shows that zero out of 159 ischemic stroke patients group with mRs score of G2-5; 89 out of 155 in the first month and 98 out of 134 in the third month were had such mRs score 2 of treated group.

Out of the 21 aphasic patients, 9 improved and 12 did not after 3 months. Also, 18 out of the 37

patients with visual field defects improved (Tables 5 and 6).  
 Twenty patients stopped taking the drug. Causes of treatment discontinuation were: no benefit in

11 patients, large dose in 5 patient, side effects in 3, and patient will in only 1 patient.

**Table 1. Gender distribution of patients among stroke types**

Sex	Ischemic stroke	Intracerebral hemorrhage	Total (%)
Male	133	11	144 (66.3%)
Female	67	6	73 (33.6%)
<b>Total (%)</b>	<b>200 (92.2%)</b>	<b>17 (7.8%)</b>	<b>217</b>

**Table 2. Age and sex distribution of ischemic stroke patients**

Sex	Age Groups (Years)							Total
	20-29	30-39	40-49	50-59	60-69	70-79	80-	
Male	10	13	13	27	49	13	8	133 (66.5%)
Female	2	3	9	20	21	8	4	67 (33.5%)
<b>Total (%)</b>	<b>12 (6%)</b>	<b>16 (8%)</b>	<b>22 (11%)</b>	<b>47 (23%)</b>	<b>70 (35%)</b>	<b>21 (10.5%)</b>	<b>12 (6%)</b>	<b>200</b>

**Table 3. Grade of disability according to mRs Score in patients with ICH at the onset, 1 month and after 3 months afterwards**

	G5	G4	G3	G2	Total G2-5 (%)	G1	G0	Total G1-0 (%)	Total
onset	83	1	3	4	16 (94.1%)	1	0	1 (5.9%)	17
1 <sup>st</sup> month	28	2	2	1	5 (29.4%)	3	9	12 (70.6%)	17
3 <sup>rd</sup> month	11	2	2	1	5 (29.4%)	3	9	12 (70.6%)	17

(G2-5 being dependent and G0-1 being independent)

**Table 4. Grade of weakness according to mRs Score of muscle power in patients with ischemic stroke at onset, after 1 month and after 3 months afterwards**

	G5	G4	G3	G2	Total G2-5 (%)	G1	G0	Total G1-0 (%)	Total
onset	83	30	29	17	159 (79.5%)			41 (20.5%)	200
1 <sup>st</sup> month	28	17	11	10	66 (33%)	59	30	134 (67%)	200
3 <sup>rd</sup> month	11	6	6	13	36 (18%)	43	55	164 (82%)	200

**Table 5. Aphasic patients' results and distribution across types of defects**

Type of defect	Improved (%)	Not (%)	Total
<b>Global</b>	4 (30.8%)	9 (69.2%)	13
<b>Motor</b>	2 (50%)	2 (50%)	4
<b>Sensory</b>	1 (50%)	1 (50%)	2
<b>Others*</b>	2 (100%)	0 (0%)	2
<b>Total</b>	9 (42.8%)	12 (57.1%)	21

\*Trascortical and subcortical aphasia

**Table 6. Visual field problems patients**

Visual Field defect	Improved (%)	Not (%)	Total
<b>Cortical blindness</b>	0 (0%)	5 (100%)	5
<b>Homonymous heminopia</b>	18 (56.25%)	14 (43.75%)	32

### Discussion

In the present study we use mRs scaling system to assess the motor function and we divided the patients into those with MRC score G2-5 (and hence who were independent), and those with mRs score of G0-1 (who were dependent). Other studies used Fugl-Meyer Assessment score (FMA), National Institutes of Health Stroke Scale (NIHSS) and Functional Independence measure scale (FIM) which are so detailed and takes longer time than (mRs) scale and difficult to apply routinely<sup>(6,7,8)</sup>. We only excluded comatose patients and those with minor stroke; this is another difference from other studies which also excluded intracerebral hemorrhage, atrial fibrillation, infarction of the basilar artery system systemic diseases<sup>(6-8,10)</sup> to have an idea about the recovery whatever the type of stroke and whatever the risk factors. This observational study depend on the improvement from depend to independ; although the sample size is small, the result is clearly more than what expected in the measurement of motor recovery with [MLC 601]<sup>(11)</sup>.

We used [MLC 601] in 17 patients with intracranial hemorrhage. Although the number is low, this is the first study involving intracranial hemorrhage

patients as the previous trials excluded intracranial hemorrhage<sup>(6-8,10)</sup>.

Our results again, as seen in the tables, demonstrate the improvement in functional ability and reduction in the morbidity of stroke that we observed in our patients in comparison with the natural history<sup>(11)</sup>; the result is comparable to Venketasubramanian et al<sup>(7)</sup>, and Kong et al study<sup>(10)</sup>. Venketasubramanian et al<sup>(7)</sup> suggested that [MLC 601]'s effectiveness in improving stroke recovery may be related to its role in neuronal protection reconnection and plasticity.

Regarding patients with aphasia, our study showed full improvement in 44% of the enrolled patients, all of whom kept some degree of dysarthric speech. The result was definitely more than what we expected as a natural history of aphasia after stroke<sup>(12)</sup>. To our knowledge, no other trial assessed the effect of [MLC 601] on the defects in the speech component after stroke.

Assessment of the field defects after stroke is another unique feature that we included in our study; the results of cortical blindness was shown in table 6, which revealed no beneficial effect of [MLC 601] on cortical blindness. The results showed improvement in 43% of the patients.

Regarding side effects only one patient developed repeated epileptic fits (causing the patient to consequently stop the drug). One patient developed renal failure (which may not be related to the treatment); others developed gastric upsets; no fatal reaction was reported in patients with and without side effects. Most patients complained about the dosage (being 4 capsules per dose); 5 patients stopped [MLC 601] because of this large dose.

In conclusion, although a larger cross match trial is needed for better evaluation, this study observed that [MLC 601] use was associated with high proportion of functional recovery in our patients. This study can be a starting point for future research. As we do not have highly effective medications that aid in the post stroke recovery, it is beneficial to investigate the available new alternatives like [MLC 601].

We conducted this study (which may not be the most robust method to investigate [MLC 601] benefits) as it is currently the most feasible given the situation in Iraq. However, our results can justify future research using case-control or cohort study designs. In Iraq, randomized clinical trials are almost impossible to conduct, but colleagues in other countries may be invested in that as a potential future direction. As we acknowledge our limitations and the potential sources of bias in our results; we also recommend better studies with larger samples and with double blinding techniques to reflect the true effects of [MLC 601] on stroke patients.

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## Effect of Blood Flow Rate on Dialysis Adequacy in Al-Kadhimiya Teaching Hospital

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### Abstract

**Background** Adequacy of dialysis is one of determinants of morbidity and mortality. The Blood flow rate is one of important factors of adequacy of dialysis in patients with Hemodialysis.

**Objectives** To assess the effect of blood flow rate on adequacy of dialysis.

**Methods** Seventy patients maintained on regular Hemodialysis in the dialysis unit, Al-Kadhimiya Teaching Hospital. Their body weight and blood urea level before and after hemodialysis sessions were recorded, volume of ultra-filtration, duration of dialysis and blood flow rate were documented in a checklist. Both (kt/v) and (URR) were determined at three different pump speed (150-200), (201-250) and (251-300) ml/min. During hemodialysis, the hemodynamic status and vital signs of patients were monitored and controlled.

**Results** Efficiency of dialysis was calculated using the standard formulas. Paired t-test showed no significant difference in dialysis efficiency between the three groups.

**Conclusion** Higher rate of inadequacy of hemodialysis, and no significant correlation was observed between BFR groups.

**Key words** Hemodialysis, Blood flow rate, Adequacy of dialysis

### Introduction

Patients with end stage renal disease are unable to sustain life without dialysis support<sup>(1)</sup>. Hemodialysis, refer to the transport process by which a solute passively diffuse down its concentration gradient from one fluid compartment (either blood or dialysate) into the other<sup>(2)</sup>. During (HD), the waste products and electrolytes move between the dialysate and blood<sup>(3)</sup>. The dialysate flow is countercurrent to blood flow through the dialyzer to maximize the concentration gradient between the compartments and therefore to maximize the rate of solute removal<sup>(4)</sup>.

Dialysis delivery should be adequate to not only improve quality of life and also to prolong survival<sup>(5)</sup>. The aims of dialysis are thus, to

decrease morbidity, increase quality of life and prolong life span<sup>(6)</sup>. To achieve these aims, dialysis must be performed effectively, effective (HD) is one of important factors that plays a role in decreasing morbidity and mortality of patients<sup>(7)</sup>, and in effective dialysis is one of factors causing mortality of these patients<sup>(8)</sup>. There are many surveys that indicate the relationship between dose of dialysis and morality of patients; they concluded that inadequate dose of dialysis increase duration of hospitalization and over all cost of care and complications<sup>(9)</sup>.

One method of assessing dialysis dose is calculation of (kt/v). This index reflects the efficiency of dialysis and correlated with mortality and morbidity rate of patients<sup>(10)</sup>.

Dialysis dose can also be assessed measuring the urea reduction ratio (URR) <sup>(11)</sup>. The (URR) can be assessed by measuring the blood urea level before and after dialysis <sup>(12)</sup>.

The results of many surveys show that achieving a (kt/v) of (1.2-1.3) or more and (URR) of (65%) or more is effective in improving prognosis of patients on (HD) <sup>(13)</sup>. Therefore achieving this goal remain are the aims of dialysis. Many factors can increase (kt/v) and (URR) including use of high quality filter, increase blood flow rate (BFR), increase flow of dialysate and dialysis time <sup>(14,15)</sup>.

Increasing duration of dialysis is a useful method for increasing (kt/v), but it is not always possible because of economic factors and intolerance of patient <sup>(16)</sup>. Also, increasing the flow rate of dialysis leads to increase diffusion of urea from blood to the dialysate, but the affect cannot be prolonged <sup>(17)</sup>.

According to united state Renal Data system (USRDS), increasing (kt/v) by 0.1 can result in reducing partial risk of cardiovascular system and infections <sup>(18)</sup>, and each (0.1) reduction of (Kt/v) can increase mortality by (5-7) years in dialysis patients <sup>(19)</sup>, available literature suggest that usage of more effective dialyzer and increase BFR and Increase dialysis duration can all increase efficiency of Hemodialysis <sup>(20)</sup>.

It should be remembered that increase of BFR not always lead to highest clearance of blood urea, thus increasing BFR by (100%) from 200 ml/min to 400 ml/min can increase blood urea clearance by 33% <sup>(21)</sup>.

The study intended to assess the effect of blood flow rate on adequacy of hemodialysis in patients with (ESRD) under going regular (HD).

## Methods

The study was carried out on samples of patients (cross sectional study) in dialysis unit at Al-Kadhimiya Teaching Hospital, assessment of adequacy of HD in patients with ESRD underwent different range of BFR during HD, which are (150-200), (201-250) and (251-300) ml/min.

## Patients' selection

70 patients were selected randomly, 46 males and 24 females, with age range between (28-70) years (48±13 mean and SD) on regular on hemodialysis sessions a bout (2-3) sessions per week (2.6±0.4, mean and SD), and each session lasting (2-4) hours (3.3±0.4 mean and SD) the vascular access used was an arterio-venous fistula in (55) patients, and dual lumen catheter in subclavian vein in (15) patients, ethically there were acceptances from the patients

## Methods

BFR was grouped in three readings which are (150-200), (201-250) and (251-300) ml/min and the patients were classified patient according to these groups.

Dialysis machine used is GAMBRO AK95S and all patients used hollow fiber dialyzer (GAMBRO) with synthetic membrane; surface area (1.5-1.7) m<sup>2</sup>. The dialysate fluid consisted of flowing constituents sodium 140 mmol/l, potassium 2.0 mmol/l, calcium 1.5 mmol/l, magnesium 0.5 mmol/l, chloride 111.0 mmol/l, bicarbonate 32.0 mmol/l, acetate 3 mmol/l, osmolality 290 mmol/l and dialysate flow rate 500 ml.

In dialysis sessions patient body weight (pre and post dialysis) were recorded and ultrafiltration pressure, trans- membrane pressure and BFR recorded from machines.

During HD, the Clinical vital signs (pulse rate, blood pressure and temperature of patients) were recorded and controlled appropriately.

Dialysis efficacy was measured by using two types of formula which are (URR) and (kt/v) and this formula as follow <sup>(14)</sup>

$$\text{URR} = (\text{urea pre} - \text{urea post} / \text{urea pre}) * 100\%$$

Where URR is ratio of the relationship between two different numbers or quantities, Urea that uses is BUN, BUN = blood urea / 2.141

Another formula is  $kt/v = -\ln(1 - \text{URR})$

Where K refer to dialyzer clearance, T = time of dialysis, V refer to pt. body water volume.

*Statistical analysis*

Statistical analysis was performed using SPSS14.0 the Chi-square (X) test was used, p-value < 0.05 were considered as statistically significant.

The main possible causes of renal failure in this study group were hypertension they were 50% of patients and the least was glomerulonephritis.

## Results

**Table 1. Prevalence of the main possible causes of CKD on HD**

Etiology of CKD	No. of patient	Percentage
Hypertension	35	50%
DM	25	36%
Unknown	5	7%
Pylonephritis	3	4%
GN (on clinical and histopathological base)	2	3%

GN = glomerulonephritis

The URR 65% or more were six patients at BFR (150-200), (10) patients at BFR (201-250) and Five patients at BFR (251-300) ml/min. The (Kt/v)

1.3 or more in two patients at BFR 150-200, two patients at BFR (201-250) ml/min and one patients at BFR (251-300) ml/min.

**Table 2. Distribution of patients according to URR in three different BFR**

URR		BFR (ml/min)			P. value
		150-200	201-250	251-300	
< 65%	Mean±SD	57.3 ± 5.19	56.40 ± 5.97	56.50 ± 5.06	0.258
	%	(13%)	(43%)	(14%)	
	No.	9	30	10	
> 65%	Mean±SD	76.6 ± 1.63	87.8 ± 2.30	67.6 ± 2.40	0.01
	%	(9%)	(14%)	(7%)	
	No.	6	10	5	
<b>Patient No.</b>		15(22%)	40(57%)	15 (21%)	70

**Table 3. Distribution of patients according to Kt/v in three different BFR**

(Kt/v)		BFR(ml/min)			P. value
		150-200	201-250	250-300	
0.5-0.8	Mean±SD	0.65 ± 0.105	0.64 ± 0.093	0.59 ± 0.068	0.821
	%	(11.5%)	(28.5%)	(7%)	
	No.	8	20	5	
0.9-1.2	Mean±SD	1.03 ± 0.12	1.01 ± 0.107	0.97 ± 0.075	0.765
	%	(7%)	(30%)	(8.5%)	
	No.	5	21	6	
>1.3	Mean±SD	1.45 ± 0.071	1.4 ± 0.14	0.1 ± 0.01	0.45
	%	(3%)	(3%)	(1.5%)	
	No.	2	2	1	
<b>Patient No.</b>		15(21.5%)	43(61.5%)	12(17%)	

## Discussion

The current study found that higher rate of inadequacy of hemodialysis mainly in the group of (201-250) BFR and there were no significant difference between BFR groups.

The current result of the study run in contrast with the findings of Kim and his colleagues showed that by increasing the BFR by 15-20 % in patients with low efficiency dialysis (kt/v less than 1.2), efficiency of dialysis would increase<sup>(22)</sup> According to study of S.R Borzou and his colleagues that increase BFR will increase efficiency of HD which in turn will reduce the morbidity and mortality of patients on HD<sup>(23)</sup>.

Taziki, Lesan, Chaara and bloombergen and their colleagues assessed the effectiveness of increase BFR on clearance of potassium and phosphate with dialysis and showed that increase clearance the BFR was effective in increase clearance of potassium but was not effective in phosphate clearance<sup>(24-27)</sup>.

The explanation of higher rate of inadequacy of HD in current study despite using high rate of BFR are different factors not only the difference in blood flow rate:

Malnutrition, anemia, short time of dialysis session, premature cessation of sessions of HD , infection, inadequate blood flow from vascular access, hypotension episodes, technical reasons, the design of the study and the sample size might play a role .

In conclusion there were high rate of inadequacy of hemodialysis, and no significant effect of increasing blood flow rate on hemodialysis adequacy

The low dose of dialysis / week plays an important role in this result.

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## Stroke in Iraqi children: Experience of Children Welfare Teaching Hospital

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### Abstract

**Background** Stroke in childhood is serious disorder about which little is published.  
**Objective** To determine demographic and presenting features of children with stroke.  
**Methods** A cross sectional study was conducted on cases of pediatric stroke admitted to Children Welfare Teaching Hospital during May 2008-August 2011. Cases classified radiologically into ischemic and hemorrhagic types. Basic data and clinical presentation were gathered.  
**Results** Sixty nine children (49 boys, 20 girls), aged 1 month-14 years were enrolled. Annual hospital admission rate was 54.2 /100000; boys:girls ratio was 2.5:1, girls were younger than boys. Ischemic stroke was found in 58% of cases. Patients with hemorrhagic stroke were younger than those with ischemic type. Those aged <1year account for 55.1% (82.8% of patients with hemorrhagic stroke) while half of those with ischemic type aged 1-5 years. The commonest presentation in both types was seizure, mostly among infants. About 2/3 of patients were from Baghdad.  
**Conclusion** Stroke in children is more common among boys. Ischemic type is commoner than hemorrhagic. Those with hemorrhagic stroke tend to be younger than ischemic. The majority of children with hemorrhagic type present at age younger than one year. Seizure was the most common presentation in both types.  
**Key words** Stroke; Iraqi children; Ischemic; Hemorrhagic

### Introduction

Stroke is increasingly recognized as a cause of childhood disability and lifelong morbidity: population-based estimates of the annual incidence of childhood stroke (ischemic and haemorrhagic) range from 2.3 to 13.0 per 100,000 children and incidence rates in neonates are closer to 1 per 5000 live births<sup>(1)</sup>. Stroke is among the top ten causes of death in children in United States<sup>(2)</sup>. Although considered rare by adult standards, stroke is more common in children than brain tumours. Subtypes include<sup>(3)</sup>:

- Arterial ischemic stroke (AIS)
- Cerebral sinovenous thrombosis (SVT).
- Haemorrhagic stroke (HS).

Childhood ischemic stroke can include both arterial ischemic stroke (AIS) and sinovenous thrombosis (SVT)<sup>(4)</sup>.

Stroke in childhood can have many causes. Diagnosis is often delayed owing to low clinical suspicion and the need to exclude the frequent mimics of stroke in childhood<sup>(1)</sup>. Stroke in childhood has long been thought of as a rare and benign occurrence. However, advances in non-invasive neuroimaging have led to increased recognition of this disorder in children who might otherwise have received a diagnosis of hemiplegic cerebral palsy. Furthermore, the idea that children recover well from stroke has been contradicted by the results of outcome studies that show a high rate of lifelong morbidity: 10% of children who

have a stroke die; 20% have further stroke; and 70% have seizures or other neurological deficits. About a half of incident childhood strokes are ischemic, and the incidence is higher in boys than it is in girls<sup>(1)</sup>.

The clinical presentation of stroke in children varies according to age, underlying cause, and stroke location. The most common presentations include hemiplegia and seizure in ischemic stroke, headache and vomiting in hemorrhagic stroke, and headache and decreased level of consciousness in children with cerebral venous thrombosis<sup>(5)</sup>.

The current study aimed to calculate the prevalence of stroke among patients admitted to Children Welfare Teaching Hospital / Baghdad-Iraq, to study some demographic characteristics of patients with stroke and to throw light on the most common presenting symptoms of patients with stroke.

## Methods

### Study Design

A cross sectional study was carried out to review all cases with stroke admitted to Children Welfare Teaching Hospital (CWTH)/4<sup>th</sup> ward during the period from May, 2008 to August, 2011. Ethical approval was obtained from the Research Ethical Committee - Human Resources Development and Training Center - Ministry of Health, Iraq.

### Setting

Children Welfare Teaching Hospital is the tertiary pediatric referral center for Baghdad City. Annual admission is about 12,000 child/year. Revision of the ward registry and medical records was performed during the period from 1<sup>st</sup> of May to 1<sup>st</sup> of October, 2011.

### Sampling Technique

Selection of Participants; Case identification for ischemic and hemorrhagic stroke was based on the 4<sup>th</sup> ward (neurology department) registry which commenced by seniors responsible for patients' diagnoses and management. All cases of stroke aged from 1 month to younger than 14 years who were admitted to the 4<sup>th</sup> ward

[neurology department] during the study period were recorded in that registry.

Cases were classified into ischemic and hemorrhagic stroke as follow<sup>(6)</sup>:

1. Ischemic stroke; Acute ischemic stroke was defined as acute neurologic deficits lasting more than 24 hours and caused by cerebral ischemia, with neuroimaging showing parenchymal infarction.
2. Hemorrhagic stroke; Hemorrhagic stroke was defined as an acute neurologic deficit lasting more than 24 hours, with neuroimaging showing intracranial hemorrhage.

The initial and most available neuroimaging used to confirm the diagnosis of both types was Computerized Tomography Scan (CT-brain, to be followed (if available) by Magnetic Resonance Imaging (MRI) sequences, and according to availability, Magnetic Resonance Angiography (MRA) was used in cases of arterial ischemic stroke or hemorrhagic type while Magnetic Resonance Venography (MRV) was used in cases of cerebral venous thrombosis. Patients included were those registered as to have stroke, ischemic stroke, hemorrhagic stroke, intracranial hemorrhage, intracerebral hemorrhage and those diagnosed with cerebral venous thrombosis. Ninety cases were found in the ward registry; only 69 were included, of which the medical records of only 25 cases were accessible at the time of the study because the hospital was under refurbishment, Twenty one cases were excluded according to the following criteria:

1. Patients with suspected (no definite) diagnosis of stroke.
2. Patients diagnosed later to have ADEM (Acute Disseminated Encephalomyelitis) or other stroke mimics.
3. Cases with traumatic intracranial hemorrhage.
4. Cases with hemiplegia but normal neuroimaging [as the patients' eligibility required a radiological diagnosis of ischemic stroke or lesion consistent with it].

Thus information contained in the ward registry was adopted, focusing on demographic information like age of first diagnosis, residence, types and sex. The presenting features of 25 patients were obtained from their medical records.

**Statistical analysis**

SPSS, version 16, was used for data input and analysis. Discrete variables presented as numbers and percentages and continuous variables presented as Median and mean ± SD (standard deviation). Chi square test for independence was used to test the significance of association between discrete variables. Mann-Whitney test for two independent samples was used to test the significance in observed difference in mean of continuous variables; this test was used as the age was shown to have extreme values with significant departure from normality according to Semivnov-Kolmogorov test for the assumption of normality. Findings with P value less than 0.05 were considered significant.

**Results**

Out of the ninety patients only 69 were included in the study, as the rest were excluded according to the exclusion criteria mentioned in the methodology. Among each 1000 patients admitted to the Children Welfare Teaching Hospital during the period of study, 1.8 patients presented with stroke, and the Annual hospital frequency rate of stroke /100,000 was 54.2 (Table 1).

Among the 69 patients; 49 were boys (71%) and 20 were girls (29%). Their ages ranged from 1-168 months with a mean of 25.8 months ± 37.2 SD and a median age of 10 months. Girls tend to be younger than boys yet the differences were statistically not significant (Table 2).

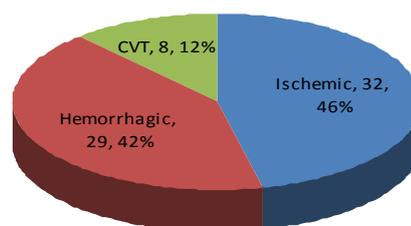
**Table 1. Patients admitted to General Pediatric Wards during the study period**

Variables	No.
Total admissions	37457
Total cases of stroke	69
Stroke / 1000 admissions	1.8
Annual hospital frequency rate of stroke /100000	54.2

**Table 2. Distribution of the study group by age at first diagnosis (in months) and gender\***

Age in months	Males (49)	Females (20)	Total (69)
Range	1-168	1-120	1-168
Mean±SD	28.3±40.5	19.8±27.4	25.8±37.2
Median	10	4	10

Patients with stroke were classified into; ischemic stroke (32 patients (46%), hemorrhagic Stroke (29 patients (42%)) and Cerebral Venous Thrombosis (CVT) (8 patients (12%) as shown in Figure 1. Cerebral venous thrombosis and arterial ischemic stroke can be included under the topic of ischemic stroke.



**Figure 1. Distribution of the study group by type of stroke**

Table 3 showed the distribution of the study group by type of stroke and gender. Although boys dominated girls in both ischemic stroke (72.5%) and hemorrhagic type (69%), yet the association was statistically not significant.

**Table 3. Distribution of the study group by type of stroke and gender\***

Type of stroke	Males		Females		Total	
	No.	%	No.	%	No.	%
Ischemic Stroke	29	72.5	11	27.5	40	100
Hemorrhagic Stroke	20	69	9	31	29	100
<b>Total</b>	<b>49</b>	<b>71</b>	<b>20</b>	<b>29</b>	<b>69</b>	<b>100</b>

\* The association between sex and type of stroke was statistically not significant ( $\chi^2=0.1$ ,  $df=1$ ,  $p>0.05$ )

When age (in months) was considered, table 4 showed that patients with hemorrhagic stroke were significantly younger than those with ischemic type (Mann Whitney,  $P < 0.05$ ), and on stratifying the two groups according to gender, girls with hemorrhagic type were significantly

younger than those with ischemic type (Mann Whitney,  $P < 0.05$ ). The same results were reached for boys. Girls were younger than boys in both subtypes, yet the differences were statistically not significant (Mann Whitney,  $P > 0.05$ ).

**Table 4. Distribution of the study group by age (in months), gender and type of stroke**

Gender	Type	P (Mann-Whitney)		
		Hemorrhagic	Ischemic	
Female	Range	1-96	2-60	0.012
	Median	2	18	
	Inter-quartile range	1-4	3-60	
	No.	9	11	
Male	Range	1-60	1-168	0.002
	Median	4	24	
	Inter-quartile range	2-10	6-60	
	No.	20	29	
Group Total	Range	1-96	1-168	<0.001
	Median	3	21	
	Inter-quartile range	2-9	6-60	
	No.	29	40	

On distributing the patients on three major age groups, (less than one year, 1-5 years and more than 5 years), table 5 showed that generally more than half of them were aged less than one year (55.1%), (34.8%) aged between 1-5 years and only (10.1%) were older than five years, the

majority of patients (82.8%) with hemorrhagic type were younger than one year and half (50%) of those with ischemic type were within 1-5 years of age and the association was statistically highly significant ( $\chi^2=14.8$ ,  $df=2$ ,  $p<0.05$ ).

**Table 5. Distribution of the study group by type of stroke and age groups\***

Age groups (years)	Ischemic Stroke		Hemorrhagic Stroke		Total	
	No.	%	No.	%	No.	%
< 1 year	14	35	24	82.8	38	55.1
1 – 5 years	20	50	4	13.8	24	34.8
> 5 years	6	15	1	3.4	7	10.1
<b>Total</b>	<b>40</b>	<b>100</b>	<b>29</b>	<b>100</b>	<b>69</b>	<b>100</b>

\* The association between types of stroke and age was statistically highly significant ( $\chi^2=15.5$ ,  $df=2$ ,  $p<0.005$ )

On reviewing patient's records; although the number of reviewed records was small, yet table 6 showed that the most common presentation among patients with both types was seizure (87.5% in hemorrhagic type and 70.6% in ischemic one), the second common presentation among patients with ischemic type was disturbed consciousness (47.1%),

hemiplegia and/or paresis (35.4%) and only (17.7%) of them presented with vomiting, whereas vomiting came in the second place among patients with hemorrhagic type (62.5%). 25% of those with hemorrhagic type presented with either bulging fontanel or pallor.

**Table 6. Distribution of the study group by type of stroke and presenting complaint\***

Presenting Complaint	Ischemic Stroke (17)		Hemorrhagic Stroke (8)		Total (25)	
	No.	%	No.	%	No.	%
Seizure *	12	70.6	7	87.5	19	76
Disturbed consciousness	8	47.1	1	12.5	9	36
Vomiting	3	17.7	5	62.5	8	32
Hemiplegia and /or paresis	6	35.3	0	0	6	24
Bulging fontanel	0	0	2	25	2	8
Pallor	1	5.9	2	25	3	12

\*95%CI for differences between proportion of seizure as a presenting symptom in both types of stroke (-0.19 – 0.41); the difference was statistically not significant P> 0.05

Regarding seizure as presenting symptom, table 7 showed that most of those presented with seizure were below 1 year of age (79%).

**Table 7. Distribution of patients with seizure as presenting symptom by age groups**

Age groups	Seizure	
	No.	%
Less than one year	15	79
≥ 1year	4	21
<b>Total</b>	<b>19</b>	<b>100</b>

Table 8 showed the distribution of patients by residency. It was found that most of the patients (63.8%) were from Baghdad city, Kut was the second governorates in number of patients (11.6%) followed by Diyala governorate (7.3%).

**Discussion**

Up to our knowledge this is the 2<sup>nd</sup> study that described the experience of this hospital with

children who were admitted with features of stroke. The previous study<sup>(7)</sup> included patients with stroke as those who were presenting with hemiplegia only without pointing to the inclusion and /or exclusion criteria or the neuroimaging as a definitive diagnostic criteria. The current study showed that the calculated annual hospital frequency rate of stroke was 54.2/100,000 children. This result is much higher than what was reported in two studies performed in Saudi Arabia<sup>(8,9)</sup>. The Children Welfare Teaching Hospital is a tertiary care center which drains a very wide area and this may partially explain this difference.

The mean age at first diagnosis was found to be 25.8 ± 37.2 months with a median of 10 months. A work done on Saudi children by Salih et al<sup>(9)</sup> demonstrated similar age trend, whereas a study in Melbourne<sup>(6)</sup> showed an older mean age than the current study, this could be attributed to including children up to 18 years of age in that study rather than 14 years, and it may be attributed to differences in the etiology and sociodemographic

characteristics between developing countries like Saudi Arabia and Iraq and developed countries like Australia. A non-significant trend of earlier age of presentation among females was shown in the current study. By reviewing the literature, no obvious explanation was found which prompted the need for further study to shed the light on this topic.

**Table 8. Distribution of the study group by type of stroke and residency**

Place of Residence	Stroke	
	No.	%
Baghdad	44	63.8
Kut	8	11.6
Diyala	5	7.3
Anbar	3	4.3
Missan	2	2.9
Other	7	10.1
<b>Governorates</b>		
<b>Total</b>	<b>69</b>	<b>100</b>

The current study showed that ischemic stroke is more common than hemorrhagic type which is consistent with other studies<sup>(5, 9-11)</sup>.

Stroke incidence as estimated from a state-wide hospital discharge database emphasize that boys carry higher risk for all stroke types than do girls<sup>(11)</sup>. A Canadian study<sup>(13)</sup> found a 3.6:1 male: female ratio for neonatal Cerebral SVT. Saudi Arabian<sup>(14)</sup> study found a male: female ratio of 1.6:1 for neonatal arterial ischemic stroke. A population-based Californian study relying on administrative data found that boys had a higher incidence of childhood (non-neonatal) stroke than girls for both ischemic and hemorrhagic stroke types with a relative risk of 1.25 (95% CI, 1.11 to 1.40) for ischemic stroke<sup>(12)</sup>.

Normann et al provide provocative evidence that the male child's risk for arterial ischemic stroke or cerebral sinovenous thrombosis is linked to androgen availability<sup>(15)</sup>.

Specifically, elevated plasma levels (>90<sup>th</sup> percentile for age and gender) of the principal

circulating androgen, testosterone, were found to be associated with a 4-5-fold increased risk of cerebrovascular disease after adjustment for pubertal status, cholesterol, and hematocrit. Furthermore, among the boys, there was a dose-response relationship such that for each 1nmol/l increase in testosterone level, the odds of stroke were increased. This novel finding is the first to address androgens in pediatric stroke and is consistent with the rather sparse literature that androgens impact ischemic outcomes and mechanisms of brain damage<sup>(16)</sup>.

A significant trend that patients with hemorrhagic stroke tend to present at earlier ages than ischemic stroke and this was the same trend when stratified for sex, yet on reviewing the literature no explanation for this significant trend was found.

The risk of stroke in children is greatest in the first year of life and peaks during the perinatal period<sup>(17)</sup>. The current study showed that more than half of stroke events in children present at the infancy period (the hemorrhagic type is over-represented in the first year (82.3%) in comparison to the ischemic type (35%)). These results were confirmed in a previous study<sup>(18)</sup> which showed large proportion (36%) of ischemic stroke events to occur in patients less than 12 months of age.

Symptoms like vomiting, bulging fontanel were common features in patients with hemorrhagic stroke and that's consistent with other studies<sup>(6,19,20)</sup>. Weakness was the main presenting feature in 6 (35.3%) patients with ischemic stroke and in none of those with hemorrhagic stroke and that's similar to what have been reported in other pediatric studies<sup>(1,6)</sup>, but this proportion was well below what other studies reported because this table view constellation of presenting symptoms that prompted the family to seek medical advice rather than the signs and symptoms that were recorded after examination by the physicians.

This study found that seizure was the most common presenting feature in the setting of acute childhood stroke (76%) with non-

significant trend to be more common in hemorrhagic than in Ischemic types. The study done by Zimmer et al<sup>(21)</sup> demonstrated the age trend with seizures being more frequent in younger children when looking at childhood arterial ischemic stroke. Wide ranges exist in the literature regarding the incidence of early seizures in children following stroke, ranging from 34 to 53.8%<sup>(22)</sup>.

Youngest children tend to have higher rates of early seizure in the stroke setting. The occurrence of higher seizure rates in children than in adults is likely due to immaturity of the neural networks leading to imbalances in excitatory and inhibitory amino acids. This, in turn, can cause increased excitation or decreased inhibition which, can lead to increased susceptibility to develop seizures<sup>(22,23)</sup>. Other studies found that this presentation can be more common than hemiparesis especially in the neonatal period<sup>(24,25)</sup>.

Nearly two thirds (63.8%) of the patients in the current study were from Baghdad city, where the hospital is, and as a tertiary center many cases are referred to this center from nearby governorates.

We conclude that pediatric stroke is not uncommon in Iraqi children; arterial ischemic stroke is over-represented in children less than 12 months of age, furthermore hemorrhagic strokes present in children younger than ischemic stroke. Rapid assessment and diagnosis can be achieved by increasing awareness of pediatricians that vomiting, headache, and altered conscious states are indicators of serious intracranial pathology, warranting urgent neuroimaging. Childhood ischemic stroke appears to be more common in boys regardless of age and stroke subtype. Further exploration of this gender difference could shed light on stroke mechanisms in both children and adults. Complete stroke registries are necessary to provide information for future studies. Further studies evaluating a larger population in different clinical settings are

required to provide a more comprehensive picture of stroke in children in this area.

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## Diagnostic Accuracy of (FNAC) Biopsy in Palpable Mammary Lesions

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### Abstract

- Background** Fine Needle Aspiration Cytology (FNAC) has advantage of providing a diagnosis before the time of surgery; this situation enable the patient and surgeon to discuss and decide the type of surgery to be done and may obviate the need for a 2 stage procedure in surgical management of breast cancer.
- Objectives** To study the accuracy, sensitivity and specificity of FNAC of solid breast mass compared to histopathological examination.
- Methods** A retrospective study includes 126 female patients palpable solid breast mass aged 17 to 67 years with masses in the breast. FNAC and histopathological studies was done to all of them.
- Results** Fifty six patients had mass in the Rt. Breast and 70 (57.4%) had mass in the Lt. breast. 58 (47.5%) masses diagnosed as malignant (54 true-positive and 4 false negative) with diagnostic accuracy of 93% (54 of 58). Sixty four patients (52.5%) were diagnosed as benign (62 true-negative and 2 false positive) with diagnostic accuracy of 96.8% (62 of 64).
- Conclusions** FNAC is simple, quick and relatively low cost procedure, with minimal patient discomfort; it is helpful in reducing the number of breast biopsies done for benign breast disease. It can provide a diagnosis before the time that operation is performed and this may help to obviate the need for two stage procedure in surgical management of breast cancer.
- Keywords** Solid breast mass, FNAC, Histopathological examination.

### Introduction

Breast lump is the most common symptom associated with breast cancer; between 9% and 11% of breast lumps result in a diagnosis of breast cancer<sup>(1-3)</sup>.

The prevalence of breast cancer among women who present with a breast lump increases with age from 1% for women 40 years of age and younger to 9% for women between 41 and 55 years of age to 37% for women aged 55 years and older<sup>(2)</sup>. In Iraq breast cancer had remained the commonest malignancy of female accounting for 16% of all cancers in Iraqi patients with cancers and with a general trend toward an increase in younger age group<sup>(4,5)</sup>.

If a circumscribed non-calcified solid mass is palpable, the recommended management is usually to obtain a tissue diagnosis, even when, according to morphologic criteria, the mass is probably benign<sup>(6-9)</sup>. The rationale behind this recommendation is the absence of published data on the safety and efficacy of periodic imaging surveillance for palpable circumscribed non-calcified solid breast masses<sup>(10)</sup>. Although previous studies have shown the safety and efficacy of periodic imaging surveillance for non-palpable or palpable circumscribed non-calcified solid breast masses on sonography, but pathologic diagnoses were not obtained in all cases<sup>(11,12)</sup>. Moreover, follow-up of a palpable mass with benign morphology may be more risky

than follow-up of a non-palpable lesion. On the chance that the lesion is malignant, the risk for metastasis is higher, since palpable masses are usually larger than non-palpable lesions<sup>(13)</sup>.

The three main areas where Fine Needle Aspiration Cytology still plays a major role are the following: (a) diagnosis of benign disease in symptomatic palpable lumps as part of triple assessment; (b) staging of breast carcinoma, in particular preoperative axillary lymph node FNAC and intraoperative sentinel node imprints; and (c) diagnosis of metastatic disease at distant sites following treatment for carcinoma<sup>(14)</sup>.

Fine-needle aspiration biopsy uses a small-gauge needle (21- to 25-gauge) to obtain fluid and cellular material from a breast lump or suspicious area of breast texture. Samples are obtained from the entire lump or suspicious area by multiple passes with one puncture<sup>(15)</sup>.

In fact, Fine Needle Aspiration Cytology has advantage of provide a diagnosis before the time that operation is performed, this situation enable the patient and surgeon to discuss and decide the type of surgery to be done and may obviate the need for a 2 stage procedure in surgical management of breast cancer<sup>(16)</sup>.

Fine-needle aspiration cytology is a very useful test, relatively rapid and inexpensive, less invasive owing to finer needle size and is easier/safer in certain lesions, such as very small lesions, lesions just under the skin or very close to the chest wall<sup>(17,18)</sup>.

The aim of this study was to study the accuracy, sensitivity and specificity of Fine Needle Aspiration Cytology of solid breast mass compared to histopathological examination, and to study the distribution of breast masses according to the patient's age and site of involvement.

## Methods

This retrospective study was done between April 2007 and March 2010, the study included 126 patients with palpable solid breast mass.

All the patients were examined by a pathologist and a surgeon; they had palpable evidence of breast lump on clinical examination.

The patients were examined by U/S using a high-resolution sonography system (Sonoline Versa pro, Siemens Medical System) using a 7-10 MHz linear array transducer, US characteristics that were evaluated included: size of lump, margin (well defined or ill defined), shape (regular or irregular), consistency (whether solid or cystic), presence of calcification. Only those patients with solid breast masses were referred for Fine Needle Aspiration Cytology, patients with cystic masses were excluded from the study.

The Fine Needle Aspiration Cytology was done using fine needle (G 20 or 21). The aspirated material was spread on 2 slides and fixed in 90% alcohol, stained by Hematoxylin and Eosin (H and E) and examined under light microscope. Those aspirates that had yielded insufficient or inadequate materials for diagnosis were excluded from the study of surgical specimens mastectomy.

The final diagnosis of breast masses was confirmed by subsequent histopathological examination of the excised specimen. The type of surgery ranged between excisional biopsy for probable benign lesions and simple mastectomy with axillary sampling for probable malignant lesions.

All the aspirated and biopsy materials were examined in the laboratories of Al-Kadhimiya teaching hospital and some Private laboratories, Baghdad, Iraq.

Statistical analyses were done by using the program SPSS (version 14 for Microsoft Windows). Statistical significance was indicated by a value of less than 0.05.

## Results

The study population included 126 females with palpable solid breast mass. The mean age was 56 years (range, 17-67 years). 56 patients had mass in the Rt. Breast and 70 (57.4%) had mass in the Lt. breast.

Of 126 breast masses 58 (47.5%) diagnosed as malignant (54 true-positive and 4 false negative) with diagnostic accuracy of 93% (54 of 58). Sixty four patients (52.5%) were diagnosed as benign

(61 true-negative and 2 false positive) with diagnostic accuracy of 96.8% (62 of 64). The overall diagnostic accuracy of the procedure was 95% (116 of 126), sensitivity 93%, and

specificity 97.5%. Table (1) shows the final pathological diagnoses and results of FNAC.

**Table 1. Final pathological diagnoses and results of Fine Needle Aspiration Cytology**

	Final pathological Diagnosis	No.	Accurate diagnosis by FNAC	
			No.	Diagnostic accuracy
<b>Malignant lesions</b>	Invasive ductal carcinoma	50	48	93%
	Invasive lobular carcinoma	6	4	
	Ductal carcinoma in situ	2	2	
<b>Benign lesions</b>	Fibroadenoma	34	34	96.8%
	Fibrocystic disease	20	18	
	Duct ectasia	6	6	
	Fat necrosis	4	4	
<b>Total</b>		126	116	91%

The breast masses (both benign and malignant) were more common in the upper outer quadrant of the breast, followed by upper inner quadrant,

lower outer quadrant, central peri-areolar region lastly lower inner quadrant as shown in table 2.

**Table 2. The distribution of lump in relation to quadrant of the breast**

Site	Benign		Malignant		Total	
	No.	%	No.	%	No.	%
Upper outer	34	53.4%	32	55.2%	66	54%
Upper inner	16	25%	10	17.3%	26	21.3%
Lower outer	8	2.5%	8	13.8%	16	13.1%
Central	2	3.2%	6	10.3%	8	6.6%
Lower inner	4	6.2%	2	3.4%	6	5%

The mean age of patients with malignant masses was 45.5±0.8 years (mean ± SD) compared with 36.4±0.2 years in those with benign masses (P<0.01)

The peak incidence of malignant conditions was in the 6<sup>th</sup> decade of life, while the peak incidence of benign conditions was in the 3<sup>rd</sup> decade as shown in table 3.

**Table 3. The distribution of benign and malignant lesions of the breast in relation to age groups**

Age (years)	Benign		Malignant	
	No.	%	No.	%
10-19	4	6.3	-	-
20-29	34	53.1	-	-
30-39	14	21.8	6	10.3
40-49	8	12.5	16	27.6
50-59	4	6.3	34	58.6
60-69	-	-	2	3.4

## Discussion

Breast Cancer is the most frequent cancer in women worldwide with 1.05 million new cases every year and represents over 20% of all malignancies among females<sup>(19)</sup>. It is important to understand the relationship of histological type to etiology, and to allow separation of entities with distinct etiologies. Histology as a prognostic factor has been well documented. Patients with histology of Infiltrating duct carcinoma (IDC) have a poor survival compared to other types<sup>(3)</sup>.

There is controversy in the literature about the role of combining fine needle aspiration cytology (FNAC) and needle core biopsy (NCB) in the assessment of breast lesions. Some studies favor FNAC over NCB as a less expensive, faster, and more sensitive test<sup>(6,20)</sup>. Others criticize the use of FNAC as the only pathological diagnostic test, particularly the assessment of non homogenous micro calcification containing breast lesions<sup>(21)</sup>, as well as the inability of FNAC to distinguish invasive from in situ malignancy<sup>(17,22,23)</sup>. Some authors recommend combining the two techniques in selected cases<sup>(24)</sup>.

FNAC is an effective method of diagnosing carcinoma of the breast and can prevent unnecessary surgery for benign disease in women of any age without increasing the risk of missing cancer<sup>(25)</sup>. FNAC had many advantages over surgical biopsy, because it is simple, rapid, relatively painless (that is why it is accepted and tolerated by patient), it does not need local or general anesthesia, inexpensive and reliable method for diagnosis of breast mass<sup>(24)</sup>.

In order to achieve a zero false positive rate (100% specificity) in FNAC, however the pathologist must be conservative and refuse to diagnosis malignancy if there is the least bit of uncertainty<sup>(25)</sup>. Avoiding false positive must lead necessarily to an increase in uncertain diagnosis in women with breast cancer and this explains the decrease sensitivity.

In order to reduce false negative and suspicious diagnosis as much as possible and in order to get high accuracy of FNAC is limited by the skill of the surgeon in obtaining the specimen (ensure

that appropriate area is biopsied) and by the experience of cytopathologist in its interpretation<sup>(23)</sup>.

In compare result of FNAC to histopathological examination only four (3.2%) cases of 126 was found to be false negative on subsequent histopathological examination, its similar to result of other studies whereas the false negative rate between 0-4%<sup>(20,18)</sup>. Condition associated with false negative results, include small tumor size, fibrotic tumors, well differentiated tumors (because individual cell some time lack obvious malignant changes)<sup>(19-21)</sup>.

The false positive result was 2 patients (1.6%) which is comparable to a previously reported study (of surgical specimens mastectomy)<sup>(18)</sup>.

The distribution of the masses between left and right breast were unequal with increased incidence in the left side and this result match a previous study in which increase incidence of left sided cancer has been noted<sup>(17)</sup>.

Regarding the site of predilection of breast lump to the quadrant of the breast its similar to both benign and malignant lesions, most common quadrant affected was upper outer quadrant accounted 54%, which is similar to other studies<sup>(15)</sup>.

The mean age for benign lesions was 36.4 years while that of malignant was 45.5 years, which is lower than mean age of malignant tumors of the breast as reported<sup>(16)</sup>.

Our study showed an overall diagnostic accuracy of 95%, sensitivity 94%, and specificity 96% breast, which is similar to other studies<sup>(10,18)</sup>.

We conclude that FNAC is simple, quick, inexpensive and reliable method of establishing tissue diagnosis and the positive clinical and FNAC for malignancy may obviate the need for a two stage procedure in surgical management of breast cancer.

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## Lower Gastrointestinal Bleeding: An Etiological Study

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### Abstract

**Background** Lower gastrointestinal bleeding (LGIB) is defined as bleeding from a source below the ligament of Treitz. Most series which studied the etiology of the acute LGIB showed that Colonic diverticulae and angiodysplasia were the commonest etiology identified, ranging from (17- 40%, 2-30% respectively). Colitis including the inflammatory bowel disease, account for 6-30% of cases. Uncommon causes of LGIB include colonic neoplasia in 11-14% and anorectal lesions in 4-10% (mostly due to hemorrhoids,). Other less common cause is small bowel source in 2-9%; rare causes include Dieulafoy lesion and colonic ulcerations.

**Objective Methods** To verify the etiology of LGIB in Iraqi patients. The study group included 100 patients who were suffering from acute lower gastrointestinal bleeding and referred to the endoscopy unit in the Gastroenterology and Hepatology Teaching Hospital. After the initial history, physical examination and laboratory studies were obtained; all patients with hemodynamic instability were resuscitated with intravenous fluid and blood transfusion. Colonoscopy performed within 12 hours of hospitalization (with or without an upper endoscopy), with the aim of reaching up to the cecum in all cases.

**Results** Of 100 patients (55 female, 45 male) 78% presented with haematochesia, 11% with red maroon stool with malaena, and 11% with malaena only. Bleeding due to colitis was the most frequent diagnosis, which is reported in 38 patients. The diagnosis of anorectal lesions and colonic neoplasia were the second and third most common diagnosis (21%, 12% respectively). Colonic diverticulae in 12 patients, colonic angiodysplasia in 11 patients and small intestinal source is 5 patients and 1 patient with colonic Dieulafoy lesion.

**Conclusions** This study showed that inflammatory bowel disease; colorectal polyps including post polypectomy bleeding, diverticulosis, angiodysplasia, and hemorrhoids were the most common causes of lower gastrointestinal bleeding.

**Key words** Lower gastrointestinal bleeding

### Introduction

Lower gastrointestinal bleeding (LGIB) is defined as bleeding from a source below the ligament of Treitz, it occurs at a rate of 20 per 100,000 population, which is about one fifth as frequently as upper gastrointestinal bleeding <sup>(1)</sup>.

The incidence of LGIB is higher in men and increases with age, presumably because of the high frequency of diverticulosis and vascular disease in older men <sup>(2)</sup>. Hematochezia, which is the most common presenting symptom for lower gastrointestinal hemorrhage, can be variously

described as bloody diarrhea, blood and clots per rectum, maroon-colored stool, or blood mixed with the stool, hematochezia can occur from bleeding anywhere in the gastrointestinal tract, and about 10% of patients who present with hematochezia have an upper gastrointestinal source of bleeding<sup>(3,4)</sup>.

Few data on prognostic variables for lower gastrointestinal bleeding exist; the following clinical data were associated with severe bleeding: heart rate  $\geq 100$ /minute; systolic blood pressure  $\leq 115$  mm Hg; syncope; a non-tender abdominal examination; bleeding per rectum during the first 4 hours of evaluation; aspirin use; and more than two active comorbid conditions<sup>(5-7)</sup>.

It is important to determine the color of the stool. Such information appears to be the most informative<sup>(8)</sup>. Bright red blood commonly indicates a distal colonic source or a rapidly bleeding proximal (small intestinal or gastric) source, whereas black stool indicates a slowly bleeding right colonic or more proximal source. Accordingly, in patients with apparent massive LGIB, it is important to exclude upper gastrointestinal hemorrhage by examining an aspirate from a nasogastric tube.

A thorough history and physical examination often point to the correct diagnosis. For example, LGIB in elderly patients is commonly caused by colonic diverticula or vascular ectasias, whereas in young patients, infectious or inflammatory conditions are more likely<sup>(9,10)</sup>. A prior diagnosis of hemorrhoids or inflammatory bowel disease is important.

A history of NSAIDs intake, radiation therapy, previous surgery (particularly vascular surgery), and constipation, a change in bowel habits, and anorectal disease or trauma are important to consider in making a correct diagnosis<sup>(11)</sup>. Symptoms that are associated with bleeding, such as abdominal pain or diarrhea, suggest specific diagnoses. A recent history of anorexia or weight loss or an abdominal mass found during physical examination may indicate an underlying malignancy.

Historically, the two major causes of acute LGIB were thought to be diverticulosis and angiodysplasia<sup>(12-14)</sup>.

Most series which studied the etiology of the acute LGIB showed that diverticular bleeding was the commonest etiology identified ranging from 17 to 40% of LGIB episodes<sup>(15,16)</sup>. Despite the fact that most diverticulae are located in the left colon in western individual, a number of series suggest that bleeding diverticulae occur more often in the proximal colon<sup>(17-20)</sup>.

Angiodysplasia are ectatic blood vessels seen in the mucosa and the submucosa of the GI tract, with 1 to 2% incidence in the colon at autopsy and colonoscopy<sup>(21,22)</sup>. Angiodysplasia are identified most commonly in elderly patients; more than two thirds of affected patients are older than 70 years of age<sup>(23-25)</sup>. Approximately three quarters of bleeding angioectasias are identified in the right colon<sup>(26-28)</sup>.

Colonic neoplasia (including postpolypectomy bleeding) is uncommon cause of LGIB, if not rare, bleeding can be the presenting symptom of colonic neoplasia and the cause of LGIB in 11 to 14 % and are thought to bleed mainly from erosions on the luminal surface<sup>(29)</sup>.

Postpolypectomy bleeding occur in 2-3% of patient undergoing polypectomy and is thought to occur from separation of the polyp before there is adequate coagulation of the blood vessel in the stalk, or from sloughing of the coagulum in case of delayed bleeding<sup>(30)</sup>.

Colitis, including the inflammatory bowel disease, infectious colitis, radiation colitis and ischemic injury, can account for 6% to 30% of acute lower intestinal bleeding<sup>(31)</sup>.

Severe GI bleeding is said to account for 6% to 10% of emergency surgical resections for ulcerative colitis. Ulcerative colitis and crohns' disease is responsible for 2% - 8% and 1% of cases of lower GI bleeding respectively<sup>(32,33)</sup>.

Radiation therapy induces inflammatory changes in the bowel wall and can result in mucosal telangiectases that bleed<sup>(34)</sup>.

Colonic ischemia can also present with acute lower GI bleeding. Few series reported it in 3% of cases<sup>(35)</sup>. Reports of vasculitis as a cause of lower GI bleeding include polyarteritis nodosa, Wegener's granulomatosis, and rheumatoid vasculitis, this is caused by ulcerating necrotizing process resulting in hemorrhage<sup>(31)</sup>.

Less common causes of acute LGIB include anorectal lesions in 4-10% (including hemorrhoids, solitary rectal ulcer syndrome, and rectal varices). Hemorrhoids prevalence rate of 76% have been reported in patients with acute LGIB, but an etiological relationship is established only in 2-9% of cases<sup>(36)</sup>.

Other less common cause are small bowel source in 2-9%, rare causes include Dieulafoy lesion and colonic ulcerations<sup>(37)</sup>. Cases of aortocolonic fistula formation with bleeding have rarely been reported months or years after aortic graft surgery<sup>(38)</sup>.

The source of bleeding cannot be identified definitively in a substantial number of patients<sup>(2, 39)</sup>. The diagnosis should be sought in all patients with lower GI bleeding unless their overall prognosis is too poor to warrant further tests.

Urgent colonoscopy has been defined variably as one performed within 12-48 hr of admission. Urgent colonoscopy enables a final diagnosis of colonic lesions in 74-90% of patients, this diagnostic accuracy is better than that of angiography<sup>(40,41)</sup>.

The majority of episodes of acute LGIB cease spontaneously, regardless of source, but patients with continuing or recurrent bleeding require intervention to stop the bleeding<sup>(42)</sup>.

## Methods

The study group included 100 patients who were suffering from acute lower gastrointestinal bleeding and referred to the endoscopy unit in the Gastroenterology and Hepatology Teaching Hospital between February 2009 and October 2010. After the initial history and physical examination were performed, laboratory studies

were obtained, including hematocrit, platelet count, prothrombin time, activated partial thromboplastin time and type, and cross match.

All patients with hemodynamic instability (defined as either a resting heart rate over 100 beats per minute; resting systolic blood pressure below 90 mm Hg; or evidence of end-organ compromise, specifically lightheadedness, syncope, chest pain, or dyspnea at the time of hospitalization) were resuscitated with intravenous fluid and blood transfusion.

All patients were prepared with a polyethylene-glycol-based purgative administered either orally (1 cup every 15 min) or by nasogastric tube (250 mL every 15 min) for patients unable to comply with oral intake. The goal was 4 to 6 L of purge requiring 3-4 hr. to clean the colon.

Patients then received conscious sedation with IV pethidine 50mg and diazepam 10mg while monitoring heart rate, blood pressure, and oxygen saturation.

Colonoscopy performed within 12 hr of hospitalization with or without an upper endoscopy. A standard video colonoscope (Olympus, Tokyo, Japan) was used for all patients with the aim of reaching up to the cecum in all cases.

When evidence of more than 1 potential site of bleeding was identified, the probable cause of bleeding was judged by the presence of ongoing hemorrhage or adjacent evidence of recent bleeding.

Push enteroscopy was performed if the source of bleeding remained undiagnosed after both colonoscopy and upper endoscopy. Other investigations were done in some patients in whom the source of the bleeding was not identified on initial endoscopic screen, these tests were included small intestinal barium study, mesenteric angiography (done by seldinger technique using JR catheter and iohexol contrast media) and intraoperative enteroscopy; this is judged according to the clinical situation of the patients and the haemodynamic state.

Statistical analysis was performed using the SPSS software package, Version 7.5. The chi square test was used to compare categorical data.

**Results**

Seventy eight patients (78%) were presented with haematochesia, eleven patients (11%) with red maroon stool with malaena and eleven patients (11%) with malaena only. Twelve patients (12%) had haeomodynamic instability at the time of the presentation. Multiple comorbidities were

reported in 27% of the patients, which include (DM, cardiovascular disease, cerebrovascular disease and renal failure). Fifteen patients only had history of aspirin and NSAIDs use before the development of the bleeding.

The mean hemoglobin level of our patients at the time of presentation was 9.7±1.2 g/dl and the mean blood transfusion units received was 2.6±1.2. The demographic characteristics of the study group are shown in table 1.

**Table 1. The demographic characteristics of the study group.**

Feature	Number, %, Mean±SD	
<b>Total No. of patients</b>	100	
<b>Age (Years)</b>	36.1±20.1	
<b>Gender</b>	Males	45
	Females	55
<b>Aspirin &amp; NSAID Use (%)</b>	15%	
<b>Hemodynamic Instability (%)</b>	12%	
<b>Comorbidities (%)</b>	27%	
<b>Units of blood Transfused</b>	2.6±1.2	
<b>Hb level at presentation</b>	9.7±1.2	
<b>Length of the hospital stay(days)</b>	6.7±1.3	

All patients underwent total colonoscopy other diagnostic studies which had been done include OGD (esophagogastroscopy) in 22 patients, barium follow through in 10 patients, enteroscopy for 12 patients, angiography for 2 patients and 1 patient underwent lapratomy with intraoperative enteroscopy.

Bleeding due to colitis was the most frequent diagnosis, which is reported in 38 patients; ulcerative colitis in 28 patients, crohns colitis in 4 patients (assuming that there are no other causes of the bleeding), infective colitis in 4 patients,

drug-induced colitis in 1 patient and ischemic colitis in another 1 patient).

The diagnosis of anorectal lesions and colonic neoplasia as the cause of the bleeding were the second and third most common diagnosis (21%, 12% respectively).

Other etiology included, in the order of frequency, colonic diverticulae in 12 patients, colonic angiodysplasia in 11 patients and small intestinal source is 5 patients and 1 patient with colonic Dieulafoy lesion. Table 2 showed the etiology of the bleeding.

**Table 2. The Etiology of Bleeding**

Cause	Frequency	Percent	
<b>Diverticulae</b>	12	12%	
<b>Colonic Angiodysplasia</b>	11	11%	
<b>Colitis (total = 38)</b>	ulcerative Colitis	28	
	Crohn's Colitis	4	
	Infectious	4	38%
	Ischemic	1	
	Drug induced	1	
<b>Colonic neoplasia (total = 12)</b>	Colonic polyp	6	
	Colonic carcinoma	4	12%
	Post-Polypectomy bleeding	2	
<b>Anorectal Lesions (total = 21)</b>	Piles	8	
	Solitary Rectal Ulcer	4	21%
	Rectal polyp(non-neoplastic)	9	
<b>Small Intestinal Source (total = 5)</b>	Typhoid ulcer	2	
	T.B. ilietis	1	5%
	Intestinal Telengectasia	1	
	Meckls diverticulum	1	
<b>Colonic Dieulafoy lesion</b>	1	1%	

Statistical analysis of the etiological data in different age group showed that the diverticular bleeding is the most frequent cause in patient's age more than 50 years (33.3%) followed by angiodysplasia (27.7%). Colitis was the most common cause of bleeding in age group below 50 years old (55.3%).

The children (age < 12 years), non- neoplastic rectal polyp is the most frequent cause of lower G.I.T bleeding (52.9%). The etiology in different age groups is shown in table 3.

During the hospitalization of the patients, spontaneous cessation of the bleeding occurred in 79 patients. Endoscopic control of the bleeding was attempted in the remaining 21 patients, and it was successful in 17 patients (6 patients with diverticular bleeding; 8 patients with angiodysplasia; 2 with postpolypectomy bleeding and one patient with colonic dieulafoy lesion).

The remaining 4 patients in whom endoscopic therapy failed or cannot be done were referred for emergency surgery (1 with Meckles disease, 1 with intestinal telengectasia and 2 patients with diverticular bleeding).

The mortality was reported in 3 patients (1 with bleeding dieulafoy lesion, 1 patient with ischemic colitis and 1 patient with diverticular bleeding).

**Discussion**

Lower GI bleeding encompasses a wide clinical spectrum ranging from trivial bleeding to massive hemorrhage with shock. Lower GI bleeding is more common in men than in women, which is different from our study group which showed slight female predominance (55 versus 45 patients), and the incidence rate increases with age, with a greater than 200-fold increase from the 3rd to the 9th decades of life <sup>(2)</sup>.

Table 3. The etiology in different age groups

Etiology	Age <12 (17 patients)	Age <50 (47 patients)	Age >50 (36 patients)	Total (100)
Diverticular disease	-	-	12(33.3%)	12
Colonic angiodysplasia	-	1(2.1%)	10(27.7%)	11
Colitis	7 (41.1%)	26 (55.3%)	5 (13.8 %)	
IBD	2	26	3	
Drug induced	1	-	-	38
Infective	4	-	1	
Ischemic	-	-	1	
Colonic Neoplasia	-	3 (6.3%)	9 (25%)	
Colonic cancer	-	1	3	12
Colonic polyp	-	1	5	
Post polypectomy	-	1	1	
Anorectal lesion	9(52.9%)	12 (25.5%)	-	
Solitary rectal ulcer	-	4	-	21
Pile	-	8	-	
Rectal polyp	9	-	-	
Small intestinal source	1(5.8%)	4(8.5%)	-	
Typhoid ulcer	-	2	-	
T.B ileitis	-	1	-	5
Telengectasia	-	1	-	
Meckls diverticulum	1	-	-	
Colonic Dieulafoy lesion	-	1(2.1%)	-	1

In a study done by peura DA, etal on patients with lower gastrointestinal bleeding showed that less hemodynamic instability than those with upper gastrointestinal bleeding and show less frequent orthostasis (19% versus 35%), need less frequent blood transfusions (36% versus 64%), and present with higher hemoglobin levels<sup>(6,7)</sup>. This is also seen in our patients were only 12% of them showed hemodynamic instability and 38% had received blood.

Another study done by Strate et al on 252 patients with acute lower gastrointestinal bleeding found predictive factors which increase the likelihood of a severe course or recurrence of bleeding: heart rate >100/min; systolic blood pressure <115 mmHg; history of acetylsalicylic acid use and more than two active comorbid

conditions<sup>(5)</sup>. This is also seen in some of our patients, 3 of them died because of aspirin use and associated comorbidities.

The frequency of the source of colonic bleeding reported varies from one publication to the next. Epidemiologic and historical features should be considered. For example, in patients with lower GI bleeding over the age of 65, colonic angiodysplasia, diverticular hemorrhage, or ischemic colitis, are most common, while in young patients, infectious or inflammatory conditions are more likely<sup>(9, 10)</sup>. This is comparable to our findings which showed that diverticular and angiodysplasia bleeding was more common in patients over the age of 50 year (33.3%, 27.7% respectively). While colitis was the most common cause in those below 50 years (55.3%).

We have found that the most common cause of lower GI bleeding in our patients was inflammatory bowel disease (32%), this is higher than what reported in the literature (16%), which can be attributed to young age of most of our patients<sup>(9)</sup>. However, the frequency of the colonic diverticulae and angiodysplasia as a cause of lower GI bleeding, in our study, was (12%) and (11%) respectively, this finding is less than those reported in the literature (17-40%) for diverticular bleeding and up to (30%) for angiodysplasia bleeding<sup>(42)</sup>, which can be attributed to relatively small number of our patients were above the age of 50 years.

A striking finding in our study was that anorectal lesions were responsible for (21%) of causes of lower GI bleeding which is much more than reported in other series (4-11%), this can be explained by that our hospital is a tertiary referral center which receive large number of pediatric patients in whom rectal polyps are the most common cause of lower GI bleeding.

Other less frequent causes of lower GI bleeding were reported with lower frequencies in our study for example colonic neoplasia (including postpolypectomy bleeding) account for 12% of cases of haematochezia, hemorrhoids are reported to account for 8% of acute lower GI bleeding episodes, small intestinal source of bleeding is encountered in 5% of cases. These findings are comparable to those found in other series which reported incidence of 11-14%, 4-10% and 2-9% for bleeding from colon neoplasia, hemorrhoids and small intestinal source of bleeding respectively<sup>(9,25,29,36,37)</sup>.

Prognosis in lower GI bleeding varies; however, since most acute lower GI bleeding is self-limited, outcomes are typically favorable. Spontaneous cessation of acute lower gastrointestinal bleeding is seen in about 79% of our patients, and it is similar to other studies which reported spontaneous cessation rate of 80 %<sup>(43)</sup>.

Indeed, the mortality rate associated with lower GI bleeding is generally considered to be less than

5% and when it occurs, is often a result of comorbid conditions and the need for emergency surgery is in the range of 5%<sup>(2)</sup>. which is also reported in our study 3% and 4% for mortality and emergency surgery respectively.

In conclusion, there are numerous lesions that may be responsible for lower GI bleeding. Our study showed that inflammatory bowel disease; colorectal polyps including post polypectomy bleeding, diverticulosis, angiodysplasia, and hemorrhoids were the most common causes of lower gastrointestinal bleeding. Colonoscopy allows for diagnosis in most patients with lower gastrointestinal bleeding. Lower intestinal bleeding generally has a less severe clinical presentation and course and typically favorable outcomes.

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## The Value of Ultrasound and Color-Doppler Features in the Assessment of Single Solid Thyroid Nodule

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### Abstract

**Background** The use of ultrasound (US) in the assessment of thyroid disease has greatly increased the detection of small thyroid nodules unrecognized at clinical examination.

**Objective** To determine the accuracy of the diagnosis of the nature of solitary thyroid nodule by ultrasound in comparison with histopathological findings and to correlate the different sonographic and color-Doppler (CFD) findings with the results of histopathology of resected nodules.

**Methods** The nodule size, echogenicity, presence/absence of calcification, lesion margins and vascular pattern of 63 patients with solitary thyroid nodule referred for US assessment).

**Results** Twenty four patients (38.1%) had malignant thyroid nodules and 39 patients (61.9%) had benign nodules as confirmed by histopathology. The large nodules show benign histopathological finding more than the small nodules (no significant difference; 56.5% vs. 25.4%, respectively). Histologically-confirmed malignant lesions show hypoechoic appearance and calcification more than benign nodules. The Malignant lesions presented more frequently than did benign nodules as solid hypoechoic appearance and irregular or blurred margins (52.2% vs. 47.8%;), and intranodular vascular pattern with calcification (63.3% vs. 36.4%) and the sensitivity and specificity by ultrasound in the evaluation of these nodules will be more and have highly diagnostic accuracy (58.3%, 79.49%, 71.5% respectively) in comparison to the former feature (50%, 71.79%, 63.5% respectively).

**Conclusion** We conclude that the typical appearance of nodules in thyroid carcinoma is irregular hypoechoic mass with internal vascularity and calcifications. Uncommon appearances of carcinoma include hyperechoic texture, intrinsic hypovascularity, and sharp regular contours. Uncommon sonographic features were found to occur more often than expected.

**Keywords** Thyroid nodule, ultrasound, color Doppler

### Introduction

The thyroid gland is an endocrine gland; this means that it manufactures hormones that are released into the bloodstream, which then act as messengers to affect cells and tissues in other parts of the body <sup>(1)</sup> The use of US in the assessment of thyroid disease has greatly increased the detection of small thyroid nodules unrecognized at clinical examination <sup>(2)</sup>.

Thyroid nodules are common, but thyroid cancer is rare. Palpable nodules (usually >1.5 cm) are found in approximately 5% of the population. The prevalence of non-palpable nodules is even higher, occurring in an estimated 40% to 50% of the population. In contrast, the American Cancer Society estimated that there were only 19,500 new cases of thyroid cancer in 2001, representing 1.5% of all new cancers <sup>(3)</sup>. During

the past decade, the use of high-resolution sonography has resulted in high rates of detection of thyroid nodules, but characterization of nodules as either benign or malignant remains problematic because of considerable overlap in sonographic features<sup>(4)</sup>.

Ultrasonography is often the first imaging modality employed to evaluate a thyroid nodule since it is readily accessible, inexpensive, and noninvasive, and it requires no radiation exposure. Ultrasonography is effective at delineating intrathyroidal architecture, distinguishing cystic from solid lesions, determining if a nodule is solitary or part of a multinodular gland, and accurately locating and measuring a nodule<sup>(5)</sup>. It has the added advantage of demonstrating any associated lymphadenopathy in the para-tracheal region, the most commonly involved lymph node region for metastasis<sup>(6)</sup>.

The first use of thyroid ultrasonography was more than 30 years ago to differentiate solid and cystic thyroid lesions<sup>(7)</sup>. Ultrasonography relies on the emission of high-frequency sound waves that are reflected as they pass through tissue of various impedances. The current technology of high-resolution ultra-sonography uses sound frequencies between 7.5 and 14 MHz, allowing visualization of solid or cystic nodules as small as 2 mm. With improvements in technology, high-resolution ultrasonographic equipment has become more affordable and available so that many endocrinologists are now well trained in its applications and use office-based equipment for evaluation of thyroid nodules<sup>(7)</sup>.

Our aim is determine the accuracy of the diagnosis of solitary thyroid nodule with ultrasonographic findings in comparison with histological finding and to correlate the sonographic ultrasound (US) and color-Doppler (CFD) findings with the results of histopathology of resected nodules to establish:

- 1) The relative importance of US features as risk factors of malignancy; and
- 2) A cost-effective management of thyroid nodules.

## Methods

From January 2011 to May 2011, sixty three patients (from 20 to 70 yr old, mean age: 38.2±14.7 year; (males 17 and females 46), with

solitary thyroid nodule were referred from US department in Baghdad Teaching Hospital to Baghdad hospital surgical center for further assessment.

Ultrasound investigations used an ultrasonographic scanner (Philips HD11) equipped with a 7.5-10 MHz linear transducer for morphological studies 4.5-7 MHz for color flow Doppler evaluation. The CFD examinations were performed with biplanar scanning. Examinations were conducted and recorded by two skilled sonographers according to a standard procedure; the amplifier gain was adjusted in each case at a level to block the appearance of random color noise.

The following ultrasound parameters were assessed in all nodules:

- Nodule diameter (maximum diameter as evaluated by sagittal and transverse scans)
- echogenicity (iso-, hyper- or hypoechoic)
- presence/absence of calcification
- lesion margins: well-defined or blurred
- vascular pattern (along the maximum diameter of the nodule:

Type 0, absence of flow signals

Type 1, vascular flow in peripheral position

Type 2, intranodular flow with multiple vascular images.

All cases were confirmed pathologically by FNA, thyroidectomy, or both.

Adequate cytological material was classified as benign (colloid nodules, lymphocytic thyroiditis, cystic goiters), malignant (papillary carcinoma, medullary carcinoma, anaplastic carcinoma) or suspicious (including follicular or Hurthle cell neoplasms). Cases with benign cytology (or repeated inadequate smears) underwent clinical and biochemical control; to rule out overlooked malignancies. All patients with suspicious or malignant cytology underwent surgery

**Statistical Analysis:** Clinical, ultrasound, cytological and histological findings were separately recorded and blind-processed for statistical evaluation. Comparison of frequency distributions used the  $\chi^2$  test. Univariate and multivariate (logistic regression analysis) with 95% confidence interval were calculated to assess the

relationships between ultrasound criteria and histological outcomes.

The diagnostic value of ultrasound criteria was also assessed in terms of sensitivity, specificity, likelihood ratio, positive/negative predictive value and efficiency. The relative risk of malignancy was evaluated by logistic regression analysis. The significance level was set at *P* less than 0.05.

Overall, 63 nodules were examined by US, of which 25 (39.7%) were <1 cm in diameter, and 38 (60.3%) were >1 cm in diameter. After the assessment of sonographic features, patients were referred thus,

there were a total of 24 thyroid nodules representing carcinoma. Patients with treated thyroid disease without surgery were excluded from this study.

Those patients complaining from hyper-thyroidism have increase in thyroid function test (increase in T4 and/or increase T3) with decrease TSH, Each patient underwent a physical examination have a characteristic feature which elicited in table 1.

**Table 1. Characteristic feature of patient with thyroid nodules and laboratory finding**

Patients		Benign N = 39	Malignant N = 24
Laboratory investigation	T3	1.6662±0.482	1.284±0.292
	T4	87.85±21.81	78.9±17.12
	TSH	2.88±0.11	4.17±1.9
Age		39.48±15.05	36.33±14.20

**Results**

Sixty three patients (from 20 to 70 year old, mean age: 38.2±14.7 year; (46 females and 17 males) with solitary solid thyroid nodule underwent US / CDI examination for evaluation

**Ultrasound investigations**

The sonographic features of the nodules are given in Table 2. The solitary thyroid nodule was identified and sonographically characterized in each patients, which show that there is no significant difference between groups of small nodules and large nodules in the sonographic features (p<0.05).

**Table 2. Sonographic features of the nodules**

Sonographic Finding		Small nodule <1cm N= 25	Large nodule ≥1cm N = 38	Total
Echoic	Hyper	4 (6.3%)	7 (11.1%)	11 (17.5%)
	Iso	7 (11.1%)	11 (17.5%)	18 (28.6%)
	Hypo	14 (22.2%)	20 (31.7%)	34 (54%)
Calcification	No	12 (19%)	19 (30.2%)	25 (49.2%)
	Yes	13 (20.6%)	19 (30.2%)	38 (50.2%)
Margin	Define	14 (22.2%)	17 (27%)	31 (49.2%)
	Blurred	11 (17.5%)	21 (33.3%)	32 (50.8%)
Vascularity	type 0	5 (7.9%)	9 (14.3%)	14 (22.2%)
	type1	5 (7.9%)	9 (14.3%)	14 (22.2%)
	type2	15 (23.8%)	20 (31.7%)	35 (55.6%)

### Histological features

Twenty four patients (38.1%) were histologically confirmed to have malignant nodule. The remaining 39 had benign nodules (61.9%). The prevalence of malignancy was lower in small vs. large nodules (14.0% vs. 23.8%,  $p < 0.05$ ). However, the benign histopathological finding were more common in large than in small nodules also but there is no significant difference (56.5% vs. 25.4% respectively,  $p > 0.05$ ).

### Ultrasound finding and histological features:

The relationships between ultrasound findings and histological features show that hypoechoic appearance was more common in histologically-confirmed malignant lesions than in benign nodules but without significant difference (54.2% vs. 53.8%)

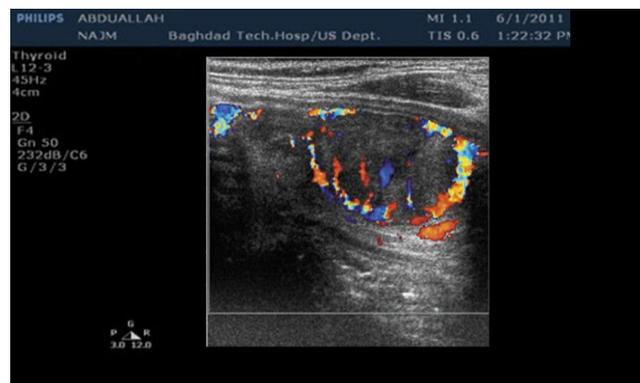
calcifications were more common in histologically-confirmed malignant lesions than in benign nodules (70.8% vs. 38.7%; 0.13:  $p < 0.05$ ; OR 2.1, 95% CI 1.8-2.3), as were blurred margins (66.7% vs. 41%; 0.42:  $p < 0.05$ ; OR 7.1, 95%CI 6.6-7.6), and highly significant difference in central vascularity type 2 (62.5% vs. 25.6%; 0.007:  $p < 0.01$ ; OR 3.2, 95% CI 3.1–3.4) in malignant lesion versus benign one (Figures 1-4).



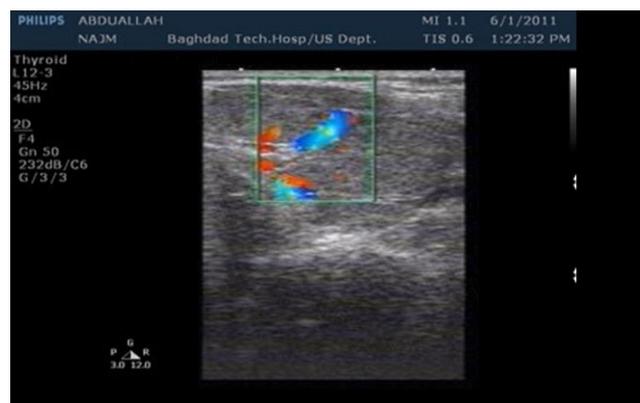
**Figure 1. Longitudinal sonogram of a typical hypoechoic, well-defined, round lesion with a thyroid adenoma**



**Figure 2. Longitudinal sonogram of a papillary carcinoma with coarse calcifications.**



**Figure 3. Showing peripheral vascularity in a thyroid nodule on Color Doppler**



**Figure 4. Showing central vascularity in a thyroid nodule on Color Doppler**

**Ultrasound finding and nodule malignancy:**  
 Table 3 shows the sensitivity and specificity of ultrasound in the evaluation of malignant thyroid nodules and this show that central

vascularity will be more specific and highly diagnostic accuracy (48.7%, 53.97%, respectively).

**Table 3 the predictive value of ultrasound for detection of malignant thyroid nodules**

Finding	Sensitivity	Specificity	PPV	NPV	LR	Diagnostic accuracy
<b>Size ≥ 10mm</b>	66.7%	41.1%	41.1%	66.6%	1.13	50.79%
<b>Hypoechoic</b>	54.2%	46.2%	38.2%	62.1%	1.01	49.2%
<b>Calcification</b>	66.7%	48.46%	40%	65.2%	1.08	49.21%
<b>Blurred margin</b>	67.1%	42.2%	39.5%	65.7%	1.21	50.8%
<b>Vascularity type 2</b>	62.5%	48.72%	42.9%	67.8%	1.22	53.97%

PPV: positive predictive value, NPV: negative predictive value, LR: likelihood ratio

In Table 4 the malignant nodules presented more frequently than did benign nodules as a solid hypoechoic appearance and irregular or blurred margins (52.2% vs. 47.8%), and intranodular vascular pattern with calcification (63.3% vs. 36.4%) and there sensitivity and

specificity by ultrasound in the evaluation of these nodules will be more, and have highly diagnostic accuracy (58.3%, 79.49%, 71.5% respectively) in comparison to the former features (50%, 71.79%, 63.5%, respectively).

**Table 4 the predictive value of ultrasound for detection of malignant thyroid nodules with combination of feature.**

Finding	Sensitivity	Specificity	PPV	NPV	LR	Diagnostic accuracy
<b>Calcification + Vascularity type 2</b>	58.33%	79.4%	63.6%	75.6%	2.84	71.43%
<b>Hypoechoic + Blurred margin</b>	50%	71.79%	52.2%	70%	1.77	63.5%

PPV: positive predictive value, NPV: negative predictive value, LR: likelihood ratio

**Discussion**

The use of US in the assessment of thyroid disease has greatly increased the detection of small thyroid nodules unrecognized at clinical examination<sup>(2)</sup>. Thyroid nodules are shown by US to be present in 30-50% of the population<sup>(8)</sup>. Although most thyroid “incidentalomas” are benign, approximately 5% to 6.5% may be malignant<sup>(9)</sup>. In our study we concentrate on the finding of Grey scale, color and power Doppler of thyroid nodule and exclude other associated findings like cervical lymph-adenopathy.

**Value of US and CFD findings as predictors of malignancy**

US findings are important in predicting malignancy in non-palpable lesions. Although previous reports

have denied that US findings have a predictive role, in our series sensitivity and specificity analysis confirmed that irregular or blurred nodular margins, an intranodular vascular pattern and microcalcifications were closely linked to neoplastic lesions<sup>(10)</sup>. On the other hand, a hypoechoic appearance or the presences of small lesions were not independent risk factors for malignancy in nonpalpable thyroid nodules.

The presence of calcifications and internal vascularity presented a higher specificity for malignancy (79%) than the findings of hypoechoic and irregular margins (71%) but the predictive value of calcifications and internodal vascularity was blunted by their low sensitivity (58%). Our results also confirm that thyroid cancer tends to be

hypervascular. Most commonly, we found a pattern of intrinsic hypervascularity (62.5%) within the malignant nodule rather than perinodular flow (16.7%). Although the perinodular flow pattern or “color halo sign” is a less common pattern of vascularity, our findings suggest that it occurs twice as often as previously thought<sup>(11)</sup>. Although the hypovascular intrinsic flow pattern was uncommon (20.8%), no lesions were found to be completely avascular. This result differs somewhat from the recent findings of Frates et al<sup>(12)</sup> because their series included 2 malignant nodules with no detectible intrinsic flow. However, their methods involved only color Doppler sonography, whereas in our series, both color Doppler sonography and power Doppler sonography were used. It is possible that the greater sensitivity to low flow of power Doppler sonography enabled us to detect even very weak signals in hypovascular nodules that might have appeared avascular on color Doppler sonography. Apart from its value in isolation, vascularity assessment was particularly useful in combination with other gray scale features. There is almost unanimous agreement that the presence of calcifications within a nodule is associated with thyroid cancer. Recently, two retrospective studies with 799 and 1475 nodules, respectively, have suggested that this is the only ultrasonographic finding predicting histological malignancy<sup>(13,14)</sup>. Our data also indicate that intermodal vascularity with intrinsic calcification is the strongest criterion for cancer (2.84, 71.5%). Blurred margins, hypoechoic pattern and size  $\geq 10$ mm have also been associated with malignant lesions in some (but not all) investigations<sup>(14)</sup> our results confirm the predictive value of these features, with a stronger association for hypoechoic and blurred margins.

In conclusion, the present study showed that the typical appearance of thyroid carcinoma is irregular hypoechoic mass with internal vascularity and calcifications. Uncommon appearances of carcinoma include hyperechoic texture, intrinsic hypovascularity, and sharp regular contours. Uncommon sonographic features were found to occur more often than expected. Finally, a cost-effective approach to the use of sonographic examination with specific ultrasound patterns (internal vascularity, calcifications, blurred margins

and hypoechoic appearance) appear to be useful indicators.

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## Surgical Treatment of Suppurative Chondritis, Limited *versus* Radical Chondrectomy

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### Abstract

- Background** Suppurative chondritis of auricle due to burn injury is a devastating complication which usually results in deformed shrunken ugly ear in spite of many modalities for treatment.
- Objectives** To document clinical nature of the injury, the results of various methods of treatment, and to recommend the management protocol of chondritis of burned ear.
- Methods** From Nov. 1998 to Nov. 2010 a prospective study performed on 100 patients 110 ears in Hilla Teaching General Hospital, Al-Kindy Teaching General Hospital on surgical treatment of suppurative chondritis. All cases were due to flame burn, and all were given prophylactic systemic antibiotics.
- Results** Forty six of patients were males (46%), 54 females (54%), ages ranged from 1 year 35 years with mean 24years, partial thickness burn 80 patients (80%), full thickness burn 20 patients (20%) . Patients treated in three groups; the first group 20 ear (18%), treated by only stab wound drainage which resulted in 100% recurrence, the second group 20 ears (18%) treated by limited wound excision which resulted in 85% recurrence, the third group 70 ears (64%) treated by radical wound excision which resulted in 10% recurrence, total loss of auricle occurred in 10%, moderate deformity occurred in 80% of ears, mild deformity resulted in 10% of ears.
- Conclusion** Surgical treatment of Suppurative chondritis gives superior results by radical excision.
- Key words** Chondritis, Suppurative chondritis, Burned ears, Radical chondrectomy, Deformed ear.

### Introduction

The blood supply of the cartilage of the external ear is poor, should it becomes infected, and it quickly liquefies<sup>(1)</sup>. Some authors mentioned that the cartilage has no intrinsic blood supply and thus has the potential to develop chondritis<sup>(2)</sup>.

Burns of the ears could be partial thickness which usually heal with little or no deformity, or full thickness which lead to exposure of the underlying cartilage leading to desiccation and focal necrosis, but the majority of those patients don't develop chondritis.

Early treatment of burn is essential to avoid disablement<sup>(3)</sup>.

The key and most important factor in treating ear burns is to prevent the development of suppurative chondritis because it's exceedingly painful and difficult to eradicate and require surgical treatment and the result is shrunken, misshapen ear<sup>(2)</sup>.

Prevention is the key, as the treatment of an established infection frequently leads to disastrous consequences<sup>(4)</sup>.

1. Avoidance of pressure on the injured ear by avoiding usage of pillows and dressing and the only dressing applied to the pinna should be antibiotic cream<sup>(5)</sup>. If necessary foam can be placed around the pinna to further prevent pressure. Specific head gear can be fashioned to

perform the same role. Local edema may predispose to thrombosis of central vessels, so adjustment of fluid resuscitation and elevation of the head of the patient may be of some value in prevention of suppurative chondritis<sup>(2)</sup>.

2. Topical antibacterial to control bacterial proliferation as Mefanide burn cream which is agent of choice which suppressed chondritis significantly<sup>(6)</sup>. Systemic antibiotics prophylactically have no influence as shown in many studies<sup>(4)</sup>.

Suppurative chondritis is a devastating complication of auricle burn in which secondary bacterial infection is superadded to thermal damage of the ear cartilage<sup>(7)</sup>. Chondritis usually seen 3-5 weeks post burn but has occurred as early as 11 days and as late as 9 weeks post burn<sup>(8)</sup>.

The most common bacteria that causes perichondritis is *Pseudomonas aeruginosa* and it has been found that once chondritis occur the auricle never returns to normal<sup>(9)</sup>. The majority of the burned auricles heal on conservative treatment. The incidence varies in different studies from less than 3% to 20% or more.

Partial or full thickness burns, and sometimes develop after reepithelialization, has occurred. No one can predict which ear will develop chondritis, which may occur in partial or full thickness burns and sometimes develop after reepithelialization<sup>(9)</sup>.

Suppurative chondritis may present as dull ear pain increasing in intensity within hours, (springing sign), recent onset of pain, redness, warmth, and swelling suggest the presence of chondritis<sup>(9)</sup>.

Early diagnosis and treatment are essential to limit the progression of infection and necrosis and to minimize deformity, by complete removal of all non-viable tissues<sup>(10)</sup>.

Different modalities for treatment tried:

1. Anterior and posterior poly ethylene drains for Antibiotic irrigation (Wanamaker 1972; Basiouny et al<sup>(11)</sup>).
2. Iontophoresis: By using Antibiotic solutions. (LaForest and Cofrancesco)<sup>(12)</sup>. apparently successful management of Suppurative ear

chondritis does suggest a clinical potential for the use of the procedure<sup>(12)</sup>.

3. Excision and drainage leaving the posterior skin intact subsequent wound granulation and epithelialization may occur<sup>(13)</sup>.
4. Extensive incision filleting the ear open with drainage and moist packing immediately up on diagnosis<sup>(14)</sup>.
5. Grant described the use of dermabrasion of the skin and necrotic cartilage followed by skin grafting within 48 hours<sup>(15)</sup>.
6. Treatment at the institute of surgical research (Dowling, Foley, and Moncrief, 1968, Mills, 1988) consisted of either; Formal debridement with incision and bivalving of the ear and excision of all non-viable cartilage, or; as now more common.

Prompt local debridement of infected tissues after early recognition of the process with a single layer of fine mesh gauze soaked in antibacterial solution between the skin flaps and the dressing changed daily until secondary closure<sup>(16)</sup>.

## Methods

For the period of November 1998 to November 2010, 100 patients (100 ears with suppurative chondritis) were studied prospectively at Hilla Teaching General Hospital and Al-Kindy Teaching General Hospital. The patient age ranged from 1 to 35 years with a mean of 24 years.

The age, sex, time from burn to detection of chondritis (Table 1), thickness of burn (Figure 1) prophylactic antibiotics, type of bacteria, number and type of operations to treat chondritis (Table 2) and recurrence were studied.

The treated patients were grouped into 3 categories:

- a. First group treated by stab wound drainage comprised 20 ears.
- b. Second group treated by limited excision comprised 20 ears.
- c. Third group treated by radical excision included 70 ears.

**Table 1. Duration of clinical detection of chondritis and its percentage**

The duration of clinical detection	Percentage
2-3 weeks	70%
3-4 weeks	25%
5-8 weeks	5%



**Figure 1. (A) Partial thickness ear burn  
(B) Full thickness ear burn**

**Table 2. Type of surgery and percentage of recurrence of chondritis**

Type of surgery	Percentage
Stab wound drainage	100%
Limited excision	85%
Radical excision	10%

The patients were evaluated including the burned ear. In case of limited swelling, we incise

on it directly with radical cartilage and necrotic tissue excision while if the swelling is wide, bivalving incision (incision along the helical rim) with drainage of pus and radical excision of the infected necrotic cartilage (which is soft while normal cartilage feels granular) and other tissues (Figure 2), with irrigation of the cavity with normal saline and sometimes adding Gentamicin solution, and povidone iodine solution followed by inserting fine mesh gauze soaked with povidone iodine solution 10% then soft dressing with mild pressure and sending the pus for bacteriological study to differentiate the type of microorganism and its sensitivity to antimicrobial drugs.



**Figure 2. (A) Bivalving helical incision. (B) Bivalving /Radical excision**

Twenty four to forty eight hours later, the dressing and the mesh gauze removed after soaking with normal saline, irrigation with normal saline and mild squeezing and milking of the drained cavity, then insertion of another smaller piece of gauze and redressing for an

additional 24 hours, after that if no pus collection found then cleaning and irrigation, then dressing. This procedure repeated daily until good healing insures which take about 7-10 days; and the ear leaves exposed later on.

After that the patient followed once weekly until complete healing which take 6-8 weeks. Pre and postoperative systemic antibiotic used such as Amikacin (15 mg/Kg/day), Gentamicin (5 mg/Kg/day), Carbenicillin (300 mg/Kg/day), or others according to culture and sensitivity tests. In all procedures swab from the drained pus sent for bacteriological study and sensitivity test.

### Results

The study comprised 46 males (46%) and 54 females (54%) with mean age of 24 years. The anterior surface of the ear involved in 85% and both surfaces in 15%, partial thickness burn in 90%, and full thickness burn in 10%. Unilateral chondritis; 90 patients (90%), bilateral chondritis 10 patients (10%). All cases are due to flame burn.

The results of bacteriological culture showed growth of pseudomonas aeruginosa in 62%, klebsiella in 22%, and E.colli, staphylococcus aureus, and proteus in 16% of the cultures.

Total loss of auricle occurred in 10% of ears, moderate deformity of auricle occurred in 80% of ears and mild deformity of auricle occurred in 10% of ears (Figure 3).

### Discussion

The skin of the ear is attached to the perichondrium without any subcutaneous tissue for protection so it is highly vulnerable for injury during facial burns in which ear burn is a common finding<sup>(17)</sup>.

In our study all cases are due to flame burn that is because the majority of cases of facial burns were due to flame burn because of wide usage of direct flame in the vast majority of domestic and industrial activities with carelessness and absence of strict adherence to safety measures and precautions at home or at work place, for that reason we used to receive sever ear burns, some of them came late to us and were badly

managed by different types of people like nursing staffs pharmacist other family members, people who use herbals for treatment of burned patients or selling these products and others.



**Figure 3. (A) Mild deformity (B) Severe deformity**

Prophylactic systemic antibiotics given to all patients from admission and changed according to the results of culture and sensitivity tests.

Daily follow up and close observation of patients for signs of chondritis this was very important in early detection and early treatment.

In spite of systemic antibiotics and local antimicrobial application no one can guarantee its prevention and nobody can predict which ear will develop chondritis, that can follows superficial or deep burns, and which may occur as early as 11 days or after complete reepithelialisation, as late as 9 weeks post burn ; these findings corresponds with the studies accomplished by many authors<sup>(8,9)</sup>.

Three ways of surgical treatment performed and compared with each other. The first group; treated by just stab wound drainage (20 ears), this performed just for pus drainage to relief pain until preparations for excision under general anesthesia were completed the recurrence rate in this group was 100% of the stabbed cases

The second group; treated by limited wound excision (20 ears) under general anesthesia in which the suspicious tissues were left unexcised, the recurrence rate was 85% of the excised cases.

The third group; treated by radical wound excision (70 ears), in which no suspicious tissues left behind the recurrence rate was 10% of the excised cases.

From this study we have found that the recurrence of chondritis is high in limited excision and low in radical excision; this because of inadequate excision of the abscess cavity leaving behind some infected and necrotic tissues which will result in further multiplication of infecting bacteria, and reaccumulation of pus and further damage to the cartilage and soft tissues, and reappearance of other clinical pictures; this finding corresponds with other studies performed in different countries<sup>(8,9,16)</sup>.

*Pseudomonas aeruginosa* found to be the most common bacteria that is because in our burn units the most common bacteria is *pseudomonas* and as a consequence of hospital acquired infection Suppurative chondritis found to be due mostly to this microorganism; this finding corresponds with other studies in different localities in the world<sup>(18,11)</sup>.

Deformity of the auricle is found to be in the majority of cases of moderate to severe deformities that's because of the nature of the disease process which is well known that once it start it is very difficult to stop and usually result in catastrophes'; these findings corresponds to other studies accomplished in different sites worldwide<sup>(8,9,18)</sup>.

In addition to these factors, the compliance and cooperation of our patients and their companions concerning positioning to avoid

pressure on the ears and taking medications and timing of surgeries were poor which will be reflected on the severity of the infection and deformity.

### Conclusion

Suppurative Chondritis of burned ear is a devastating complication which once occurred is difficult to treat. Prevention of suppurative chondritis is far better than treatment of established infection.

In spite of all precautions and ways of treatment systemically and locally, and in spite of all what has been written and we believe that it is a preventable complication, nobody can predict which ear will develop chondritis and which will not. No evidence for the role of prophylactic antibiotics in prevention of chondritis.

Surgical treatment of chondritis should be of radical excision without leaving any suspiciously non-viable cartilage and other tissues otherwise recurrence rate will be very high. Once chondritis occurred the auricle will never return to normal and result in deformed misshapen shrunken auricles.

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## Early Presentation of Bilateral Morgagni Hernia in an Infant Case Report

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### Abstract

In this report, a rare case of bilateral Morgagni hernia is enlightened in a 7-month old infant weighing 6.5 kg, presented with shortness of breath and fever for 5 days duration. There was a history of the same attack at 2 and 5 months of age and treated as a chest infection. He was admitted to pediatrics emergency unit for investigations and treatment. Plain chest radiography revealed retrosternal bowel herniation and Barrium-enema revealed bilateral big Morgagni hernia. Reduction of herniated contents (transverse colon and omentum) done with repairing of bilateral big Morgagni hernia through transabdominal approach. This case is a very rare and interesting one, because of an early bilateral presentation, and the fact that it occurred in a male infant.

### Introduction

The majority of congenital diaphragmatic hernias occur through the left posterlateral foramen of Bochdaleck and commonly these patients are symptomatic. Hernia through the foramen of Morgagni, on the other hand, is rare in children, representing 1-6 % of all types of congenital diaphragmatic hernia. It is usually asymptomatic and discovered accidentally, or if symptomatic it produces variable nonspecific symptoms which include respiratory or vague gastrointestinal symptoms, and because of this the diagnosis is usually delayed<sup>(1-3)</sup>.

Morgagni hernia is the rarest type of congenital diaphragmatic hernia. Most of the patients are females and 92% of the hernias have a hernial sac. The majority of Morgagni hernias are right-sided with rare only left sided and bilateral occurrence because of the protection provided by the pericardial sac<sup>(4,5)</sup>. The rarity of Congenital Morgagni hernia as

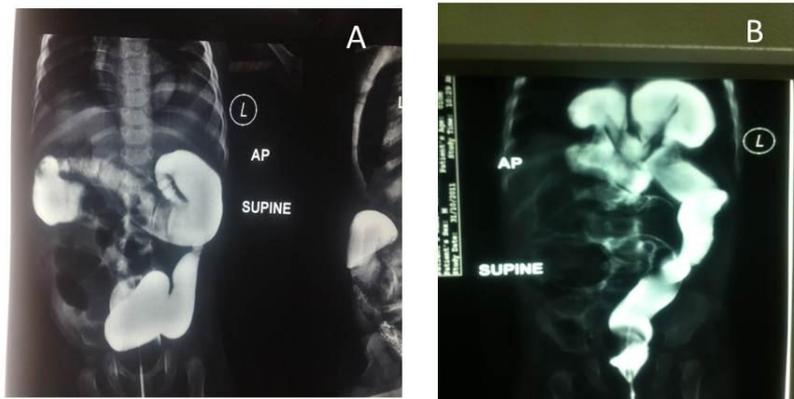
well as the vagueness, variability and non-specificity of symptoms lead to the delayed diagnosis. In the majority of those with bilateral Congenital Morgagni hernia, the diagnosis of bilaterality is made intraoperatively<sup>(6,7)</sup>. This report describes the diagnosis and repair of a big, bilateral Morgagni hernia.

### Case Report

A seven-month-old male infant weighing 6.5 kg was presented to the emergency room with severe dyspnea, cough and fever for five days duration. There was no history of trauma but previous twice hospital admissions due to chest infection. Physical examination revealed stable vital signs. Auscultation of the chest revealed no audible breath sounds in the bilateral lower sites of the chest. The laboratory results were in normal limits. Electrocardiogram was normal and echocardiogram measured normal cardiac chamber, volumes and ejection fraction. He had no gastrointestinal symptoms.

Posteroanterior chest radiography showed presence of air-filled loops of bowel retrosternally. Barrium (Ba)-enema showed presence of loops of colon on both retrosternal regions (Figure 1-A). The patient was diagnosed as bilateral Morgagni hernia. He was admitted to the pediatric ward for medical treatment of his chest infection and stayed for one week until he became stable and then transferred to the pediatric surgical ward for surgical interference which was done as an emergency through trans-abdominal approach. Two

hernial openings were identified on both sides of sternum and reduction of transverse colon and omentum was done with excision of two hernial sacs (Figure 2). After reduction of the herniated contents into the peritoneal cavity, primary repair of the diaphragmatic defects was performed with nonabsorbable silk mattress sutures. The patient made an uneventful recovery. He was discharged on the 7<sup>th</sup> day postoperatively, and was well after six month follow-up.



**Figure 1. A** Ba-enema showing bilateral Morgagni hernia at time of admission. **B** Ba-enema two months postoperatively



**Figure 2. A & B** Intraoperative bilateral Morgagni hernia, **C** Repair of Morgagni hernia

### Discussion

Anatomically, the foramen of Morgagni is a small anterior retro-sternal defect extending from the sternum medially to the eight costal cartilages laterally. Berman *et al*<sup>(3)</sup> in 1989 had reported only 18 cases of Morgagni hernia over a 40-year period from a large tertiary hospital, but no bilateral Morgagni hernia was reported in this study, and all of

those cases were diagnosed after the age of ten years. However, this reported case was diagnosed in infancy and was bilateral which is very rare. Pokornay *et al*<sup>(8)</sup>, in a series of 74 patients with congenital diaphragmatic hernia had found only 4 (5.4%) with Morgagni hernia with only one bilateral Morgagni hernia was diagnosed at 15 years of age.

Most of the Morgagni hernia has a hernial sac. Hernial sac frequently contains the omentum, transverse colon and rarely stomach or liver<sup>(5)</sup>. The present case had hernial sac that contained omentum and transverse colon. Patients with Morgagni hernia are usually asymptomatic. Among symptomatic patients; the complaints included shortness of breath, thoracic pain, nausea, vomiting, abdominal distension, and abdominal pain. The present case presented with shortness of breath, cough and fever (chest infection). The use of computed thoracoabdominal tomography as a diagnostic tool for Morgagni hernia has increased the reliability of preoperative diagnosis. Bilateral, big Morgagni hernia of the present case was diagnosed with chest radiography and Ba-enema without the need for CT scan. The treatment of Morgagni hernia is surgical and is indicated always after diagnosis because of the risk of visceral complications such as obstruction or strangulation.

Both transabdominal and transthoracic approaches are recommended in surgical repair of Morgagni hernia. Transthoracic repair has been used by Kilic *et al*<sup>(9)</sup> with favorable results. They recommended transthoracic approach because it provides sufficient exposure, easy repair of the hernial defect and facilitates the release of pericardial adhesions. But, they also reported that transabdominal approach should be favored, particularly in cases with bilateral hernial sac as in our patient. Transabdominal approach via laparotomy is superior in recognition and management of malrotation and for dealing with visceral complications than transthoracic approach. The present case was treated with an elective laparotomy and there were no postoperative complications. In conclusion, Morgagni hernia is a rare surgical problem. Bilateral Morgagni hernia is extremely rare and patient usually asymptomatic and discovered incidentally. Preoperative diagnosis may be aided by chest radiography and Ba-enema as in our current case and in questionable cases by

CT scans. The current treatment of a Morgagni hernia is surgical repair (open or laproscopically) because of the risk of visceral herniation and strangulation. Transabdominal approach is the preferred technique for reduction and dealing with visceral complications. The laparoscopic approach has the advantage that tissue trauma is kept to a minimum compared with the traditional open approach. The laparoscopic techniques have included a direct suture technique where the diaphragm is sutured to the retrosternal tissues using a Keith needle or inclusion of the whole of the upper abdominal wall in the repair with extracorporeal knots<sup>(10)</sup>.

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