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Editorial:

Serum lipids: A new look for an old subject.

Ghassan A.A. Al-Shamma PhD.

During the last four decades a large number of clinical reports focused on the role of dyslipidemia in the development of atherosclerosis and its consequences (1). All protocols of such studies or reports recommended 12-14 hour fasting blood specimens for the measurements of serum lipids (2). That was reported to be essential to stabilize these lipids especially the triglycerides (TG).

However recent reports have stressed on the effects of postprandial hypertriglyceridemia and hyperglycemia on the endothelial function and inflammation (3), and considered the postprandial surge of serum triglycerides a potent atherogenic factor and an important cause of cardiovascular disease. That was attributed to the increased levels of remnant lipoproteins in the blood in the postprandial state which promote arterial wall damage and cholesterol deposits (i.e. atherosclerosis) (4), and to lipolysis of triglyceride rich lipoprotein which release preformed mediators of oxidative stress (e.g., 9 or 13-hydroxy octadecadienoic acid) that may influence endothelial cell function in vivo by stimulating intracellular reactive oxygen species production (5).

In Iraq, a recent report focused on a limited number of coronary artery disease cases and healthy control subjects, had pointed out the absence of a significant difference between the fasting and postprandial distribution of serum lipids among the three groups of cases and controls: desirable, borderline and undesirable (6).

However as long as people are usually 16-18 hours/day in the fed state, prediction of the disease risk in the postprandial state would be better than in the fasting state, with better assessment for the prognosis of atherosclerotic lesions which occur in the postprandial state. It may also improve the assessment of TG-lowering therapeutics.

Finally it could be said that even with the above mentioned remarks it may take a time to shift from the fasting to the non-fasting serum lipid tests, but however the medical community now has a good reason to look carefully at the use of postprandial serum lipid tests since they would have the potential to identify people whose risk of heart disease is not reflected by traditional fasting tests; and when this becomes valid focusing on the type of food will be of great importance in this respect specially when we are dealing with the highly processed, calorie-dense, nutrient-depleted diet favored in many cultures over the world and which would frequently lead to exaggerated supraphysiological postprandial spikes in blood glucose and lipids (7).

References
The Value of Local Application of Hydrogen Peroxide Solution at the Site of Wound after Mastectomy for Breast Carcinoma in Reducing Local Recurrence of the Tumor.

Taqi Saadoon Atiyah FICMS.

Abstract

Background: Hydrogen peroxide is produced in normal cells of the body by peroxisomes. Cancer cells have lower respiration rates than normal cells therefore they grow better under low oxygen concentration, and introducing high oxygen levels could retard their growth or kill them. On these bases hydrogen peroxide solution had been applied locally at the site of wound after mastectomy for breast carcinoma to decrease the risk of local recurrence of the tumor.

Objective: To evaluate the significance of local application of hydrogen peroxide solution at the site of wound after mastectomy for patients with breast carcinoma in reducing local recurrence of the tumor, and to observe whether it is safe or not.

Patients and Methods: One hundred female patients with breast carcinoma and underwent mastectomy in Baghdad hospitals were involved in this study. Patients were divided into two identical groups each with fifty patients. Group 1, patient's wounds were treated locally by hydrogen peroxide solution after mastectomy; whereas the other group (control) did not treat with it. Patients had been followed for survival, recurrence, and complications for 15 years.

Results: In group 1; there was no local recurrence, while in the control group, the local recurrence of the tumor was 14% . In group 1, the five, ten, and fifteen years survival were 60%, 14%, and 6% respectively without detectable complications; while in the control group, the 5 year survival was 24%, and no patient survive more than 7 years.

Conclusion: Local application of hydrogen peroxide solution at the site of wound after mastectomy for breast carcinoma is safe and may be effective in improving survival rate and reducing local recurrence of the tumor.

Key words: Breast carcinoma, local application of hydrogen peroxide solution, local recurrence.

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Introduction

Cancer cells have lower respiration rates than normal cells, therefore they grow better under low oxygen concentrations, and that introducing higher oxygen levels could retard their growth or even kill them. These facts are the major theoretical foundation for oxygen therapy and were the results of the work of Warburg, the winner of Nobel Prize for medicine in 1931 and his observations in this field (1).

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Hydrogen peroxide (H₂O₂) is produced normally inside certain cells of the body directly by cytoplasmic peroxisomes. Hydrogen peroxide acts as a cytotoxic, digestive, opsonic, and growth inhibitory factor. H₂O₂ induce cellular injury by lipid peroxidation, protein interaction and DNA damage; so it is a natural substance that formed inside normal cells of the body as a part of its defense mechanism (2).

Breast tumors are frequently infiltrated by large number of macrophages. These may contribute to carcinoma cell oxidative stress, as tumor-associated macrophages have been shown to deliver a sublethal oxidative stress to murine mammary tumor cells. This may be due to oxygen radical production by the macrophage (3).
The killing of cells by ionizing radiation is most likely the result of direct formation of hydroxyl radicals from the radiolysis of water (2). Radiotherapy and photodynamic therapy generate oxygen radicals within the carcinoma cells (4). In the same context, some anticancer therapies may add to the oxidative stress within breast carcinomas. The chemotherapeutic agents doxorubicin, mitomycin C, and cisplatin are superoxide generating agents, and part of the mechanism of the action of chemotherapy is a superoxide generating agents (5).

Considering these principles, hydrogen peroxide solution applied locally in the wound after mastectomy for patients with breast carcinoma using its cytotoxic activity to kill any implanted malignant cells that may fail in the wound due to manipulation of the tumor during surgery; considering that hydrogen peroxide is a natural substance that formed inside normal cells of the body causing cell injury and death, and that the cancer cells are relatively anaerobic cells and supplying higher oxygen concentrations surrounding their field cause retardation of their growth and even their death.

There are many studies which shows the effective and safe use of hydrogen peroxide (H₂O₂) in different ways for example: H₂O₂ is administered intravenously, orally, in ear drops, as a nasal spray, as a tooth gel, and via enemas as mentioned in "Clinical literature by Kurt Donsbach runs Hospital Santa Monica." Although some patients develop air embolism after intravenous infusion of hydrogen peroxide which is considered one of its complications; Donsbach claims that injected peroxide will boost oxygen levels which in turns kill cancer cells. Killing cancer cells by supplying more oxygen than they can tolerate (6).

Also soaking an affected body part in hydrogen peroxide solution can cause tumors to separate from the body so that they can be 'wiped away', and that drinking H₂O₂ solution can reduce the size of throat tumors (7).

Local recurrence of cancer following surgery may be due to spillage of cancer cells into the operative field, these cancer cells may be seeded in the wound by direct contact with the primary tumor, with lymph nodes containing metastatic tumor, or with contaminated gloves and instruments. Cancer cells may also enter the wound via cut lymphatics and divided blood vessels (8).

Hence the aim of this study is to assess the efficacy of using local application of hydrogen peroxide (H₂O₂) solution peroperatively following mastectomy for breast carcinoma in reducing local recurrence of the tumor and improve long term survival of the patients "by decrease the risk of distant metastasis through the wound" and to show if there is any complications due to its use.

Patients and methods
A randomized controlled trial was conducted on 100 female patients with breast carcinoma proved by histopathological examination who had mastectomy and axillary clearance "Patey operation" in the period from 1995-2010 with age range from 28-65 years (mean age= 45.52 ± 8.71 years). The patients were divided into 2 identical groups each with 50 patients. All the patients were allocated randomly to be in group 1 (study group) in whom H₂O₂ solution applied locally at the site of the wound after mastectomy, and group 2 (control) in whom no such treatment of the wound done.

Both groups are identical, regarding age group of the patient, histopathological type of the tumor, stage for stage of the breast carcinoma,
and it was as follows in each group: stage I, included 7 (14%) patients, stage II, included 16 (32%) patients, 18 (36%) patients presented with stage III, and 9 (18%) patients were with stage IV. Staging of the tumor done by means of TNM (Tumor – Node – Metastasis) (8), as shown in table 1.

All the patients were followed for 15 years for survival, recurrence and complications.

Informed written consent was taken from the patients before the use of the solution after full explanation of the mechanism of its action and its possible complications (as air embolism or any other unexpected complications).

In group 2(control) patients were submitted to mastectomy and axillary clearance "Patey mastectomy" with absolute hemostasis of the wound using ligatures and electrocautery, and the wound then closed without application of local hydrogen peroxide solution.

In group 1 (study group); after removal of the breast and clearance of the axilla; absolute hemostasis of the wound was achieved to avoid any risk of embolism by H$_2$O$_2$ solution through the capillaries as shown in figure 1.

The exposed axillary vessels were covered by dry gauze packs to prevent local damage of the wall of these vessels by the local application of H$_2$O$_2$ solution as shown in figure 2.

Thenafter, fifty ml of 7%H$_2$O$_2$ solution was applied locally to the surgical wound and allowed to cover the surgical field "the pectoralis facia, axilla, subcutaneous tissue, and the skin edges" and left at the site for two minutes to kill all the malignant cells that may be implanted in the wound due to manipulation of the tumor during surgery as shown in figure 3.

After two minutes of the local application of 7%H$_2$O$_2$ solution and its direct contact with the surgical field; the wound then washed with one pint of normal saline 'to clean it and to remove all the debris and killed malignant cells from the wound as shown in figure 4.

Then the wound was dried by dry gauze packs and closed with drains as in the control group.

Follow up of all the patients were done regularly for 15 years to detect any local, regional, or distant metastasis, and any possible complications that may occurred due to the local use of H$_2$O$_2$ solution at the site of the wound of the patients.

Regular follow up of the patients had been done by clinical examination,labrotery tests' blood cell count and serological tests',X-ray of chest and bones when needed, ultrasound of the wound and of the other breast, ultrasound of the abdomen "to detect any distant metastasis", and fine needle aspiration FNA of any suspicious mass were done.

Results

In the study group no signs of local recurrence were detected in the period of follow up of the patients for 15 years. While in the control group seven (14%) patients had local recurrence in the first postoperative year "five (10%) of them were with stage IV, and two (4%) were with stage III; and unfortunately all of them died in the first postoperative year due to distant metastasis.

The five years survival in the study group was noticed with 30(60%) patients; ten years survival was 7(14%); and the fifteen years survival was 3(6%) of patients "two of the patients were in stage I, and the other patient was in stage II; and those three patients are living for fifteen years without signs of recurrence or detectable complications due to the local application of H$_2$O$_2$ solution to the wound.
The five years survival in the control group was noticed with 12 (24%) and only two patients survived for seven years; both in stage two. No patient survived more than seven years in this group. All the mortality was due to distant metastasis.

The number of patients survival for 5, 10, and 15 years postoperatively in both groups are shown in table (2).

The five years survival in the study group comparing with the control is significant \(X^2=13.3\) and \(P=0.0003\).

The ten years survival in the study group was seven (14%) of patients while the maximum survival rate in the control group was 7 years, which is also significant 'Fisher exact \(P=0.01\).

There are three (6%) patients in the study group survive for 15 years and they are living without obvious site of metastasis or local recurrence, 2 of them with stage I of the disease and the other patient with stage II.

The mortality in the control group in the first postoperative year was seven (14%) patients; all of them were with stage IV. While no mortality in the study group in the first postoperative year which is significant Fisher exact \(P=0.01\).

Table (3) shows the mortality per postoperative year in the study group in comparison with the control and its significance.

All the patients in the study group tolerated the local application of \(H_2O_2\) solution at the site of the wound after mastectomy in the way described above very well without any complain, and there were no detectable side effects or complications observed in the years of regular follow up of the patients till their mortality, and all the mortality were due to distant metastasis which were much less than in the control group.

**Discussion**

Breast cancer is the most common cause of death in middle-aged women in western countries, accounting for 3-5% of all deaths in women. Local spread of the tumor tends to involve the skin and to penetrate the pectoralis muscle and even the chest wall.

"Cancer-en-cuirasse' when the skin of the chest is infiltrated with carcinoma and has been likened to a coat, may be associated with a grossly swollen arm, this usually occurs in case with local recurrence after mastectomy, the condition may respond to palliative systemic treatment but prognosis in terms of survival is poor.

The two basic principles of treatment of breast carcinoma are to reduce the chance of local recurrence and the risk of metastatic spread. \(^{(9)}\)

Hydrogen peroxide is synthesized in normal cells of the body by peroxisomes to kill microorganisms because these intracellular molecules are cytotoxic and cause cell injury and death \(^{(10)}\).

Cancer cells are relatively anaerobic and will die with higher oxygen levels. \(^{(1)}\)

On these principles, the application of 7%\(H_2O_2\) solution at the site of wound after mastectomy for breast carcinoma to kill any malignant cell in the wound after absolute hemostasis by ligatures and electrocautery to decrease the incidence of embolism by hydrogen peroxide which is very important step in the study. The idea is to kill any possible implanted malignant cells in the surgical wound by the cytotoxic effect of hydrogen peroxide and its direct contact with these malignant cells in the hope that this will decrease the incidence of local recurrence of the tumor and even the regional and distant metastasis will be decreased, and so it will improve the survival rate.
and the final results of the management of patients with breast carcinoma. This study showed that it is safe to apply 7% H₂O₂ solution locally at the site of the wound after mastectomy by the same technique mentioned above; it was effective in reducing local recurrence of the tumor and improve long term survival of the patients; and its use carries no detectable local or systemic side effects. There is improvement in survival rate of the patients and low incidence of metastasis in the study group without detectable complications in the period of follow up for fifteen years.

Figure (5) shows one of the patients with advanced stage IV breast carcinoma preoperatively and two years after local application of hydrogen peroxide solution at the site of the wound after surgery without signs of local recurrence or detectable complications.

There were no available studies and researches about the local application of H₂O₂ solution locally in the wound after mastectomy in the literatures, so it is recommended that further studies and researches to be done using larger numbers of patients of identical groups of the same stage of the tumor, same histopathological disease and same age group; to show the significance and value of local application of hydrogen peroxide solution at the site of the wound to decrease the incidence of local recurrence of the tumor and improve long term survival of the patients.

![Figure 1: Patient with mastectomy and axillary clearness for breast carcinoma. Absolute hemostasis was achieved using ligatures and electrocautery to avoid any risk of embolism by H₂O₂ solution. The axillary vessels are marked by arrow.](image-url)
Figure 2: The same patient in figure 1, after secure haemostasis, the axillary vessels is protected by dry gauze pack before the local application of $\text{H}_2\text{O}_2$ solution in the wound "to avoid damage to the wall of these vessels by the solution."
Figure 3: the same patient, using fifty ml of 7% hydrogen peroxide solution to the wound in direct contact with the surgical field and skin edges and left for 2 minutes. "The axillary vessels are well protected by gauze pack"
Figure 4: The same patient, after two minutes the wound washed by one pint of normal saline to remove all the debris and "the killed malignant cells" from the wound. The axillary vessels are still protected by gauze pack.
Figure 5: One of the patients with advanced stage IV carcinoma of the breast in the study group "above" and two years postoperatively "below" without local recurrence.
Table 1: Distribution of patients in the group 1(study group) and group 2 (control) according to the stage of the tumor.

<table>
<thead>
<tr>
<th>Stage of the tumor</th>
<th>Group 1 (study group)</th>
<th>Group 2 (control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>7 (14%)</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>16 (32%)</td>
<td>16 (32%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>18 (36%)</td>
<td>18 (36%)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>9 (18%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>50 (100%)</td>
<td>50 (100%)</td>
</tr>
</tbody>
</table>

$X^2 = 0.0,\quad P = 1.0$

Table 2: The five, ten, and fifteen year's survival of patients in both groups and its significance.

<table>
<thead>
<tr>
<th>Survival</th>
<th>Group 1 (study group)</th>
<th>Group 2 (control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 year survival</td>
<td>30 (60%)</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>5 year dead</td>
<td>20 (40%)</td>
<td>38 (76%)</td>
</tr>
<tr>
<td>5 year TOTAL</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><strong>Significance</strong>:</td>
<td>$X^2 = 13.3,\quad P = 0.0003$</td>
<td></td>
</tr>
<tr>
<td>10 year survival</td>
<td>7 (14%)</td>
<td>0</td>
</tr>
<tr>
<td>10 year dead</td>
<td>43 (86%)</td>
<td>50 (100%)</td>
</tr>
<tr>
<td>10 year TOTAL</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><strong>Significance</strong>:</td>
<td>Fischer exact</td>
<td>$P = 0.01$</td>
</tr>
<tr>
<td>15 year survival</td>
<td>3 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>15 year dead</td>
<td>43 (86%)</td>
<td>50 (100%)</td>
</tr>
<tr>
<td>15 year TOTAL</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><strong>Non significant</strong>:</td>
<td>Fischer exact</td>
<td>$P = 0.1$</td>
</tr>
</tbody>
</table>

Table 3: Mortality of patients in both groups per postoperative year and its significance.

<table>
<thead>
<tr>
<th>Years postoperatively</th>
<th>Mortality in group 1</th>
<th>Mortality in the control</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>nil</td>
<td>7 (14%)</td>
<td>Fisher exact $P = 0.01$</td>
</tr>
<tr>
<td>Two</td>
<td>7 (14%)</td>
<td>7 (14%)</td>
<td>$X^2 = 4.79,\quad df = 2,\quad P = 0.09$</td>
</tr>
<tr>
<td>Three</td>
<td>8 (16%)</td>
<td>14 (28%)</td>
<td>$X^2 = 5.39,\quad df = 2,\quad P = 0.07$</td>
</tr>
<tr>
<td>Four</td>
<td>5 (10%)</td>
<td>10 (20%)</td>
<td>$X^2 = 11.46,\quad df = 2,\quad P = 0.003$</td>
</tr>
<tr>
<td>Five</td>
<td>7 (14%)</td>
<td>6 (12%)</td>
<td>$X^2 = 4.399,\quad df = 2,\quad P = 0.11$</td>
</tr>
<tr>
<td>Six</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
<td>Fisher exact $P = 0.008$</td>
</tr>
<tr>
<td>Seven</td>
<td>5 (10%)</td>
<td>2 (4%)</td>
<td>Fisher exact $P = 0.08$</td>
</tr>
<tr>
<td>Eight</td>
<td>5 (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nine</td>
<td>4 (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ten</td>
<td>4 (8%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
References

Developmental Changes in the Genital Organs of Young Male Mice Associated with Licorice Extract Consumption by Mothers before and During Gestation.

Basima Mohammed Al –Jiboori MSc, Nisreen Khazal Faliah MSc.

Abstract

**Background:** Licorice (*Glycyrrhiza glabra*) is one of the most popular plants and widely consumed as a medicinal herb, which is used mainly in treatment of inflammations and improvement of reproductive performance in both females and males.

**Objective:** To detect the effect of licorice extract consumed by pregnant females on gonadal development of their male offspring.

**Materials & Methods:** Mature female mice (No: 60), were divided into: experimental group (G, 30 mice), which subdivided into 3 equal subgroups: G1, G2 and G3 (10 mice for each), given extract of licorice root (1gm/kg body weight), immersed in distilled water for 3 weeks before mating (G1) or for 6 weeks before and during pregnancy (G2) or for 3 weeks during pregnancy time only (G3). The other main group (C) was considered as control group subdivided as that with the experimental group but they were given distilled water only. Number and weights of male’s newborn were recorded. After 40 days, weights of their bodies and testes were recorded; histological sections were prepared for the testes to detect the presence of mature sperms and to measure the diameters of semineferous tubules and thickness of their germinal walls.

**Results:** Highly significant increase (P<0.01) in litter size of all experimental groups were recorded. Young males born from treated mothers showed significant increase (P<0.05) in weights of both body and testes, and a highly significant increase (P<0.01) in the thickness and diameters of their semineferous tubules in comparison with that born from control mothers. No significant differences were recorded between the three experimental groups.

**Conclusions:** consuming low dose of licorice root extract by pregnant females causes a significant increase in the numbers and weights of the offspring with clear maturation features in the gonads of young males.

Key words: fetal development, male gonads, pregnancy, licorice, mice.

**IRAQI J MED SCI, 2010; VOL.8 (3): 14-19**

Introduction

Plants have been used as source for medical treatment and a major resource for health care since ancient times (1). Licorice (*Glycyrrhiza glabra*) is one of the most popular and widely consumed herbs in the world (2). The two major chemical constituents of licorice are glycyrrhizin and flavonoid which have anti-inflammatory action and may inhibit the breakdown of cortisol produced by the body (3). Licorice root have mild estrogen-like effect, making the herb potentially useful in certain symptoms of premenapasl tension syndrome, polycystic ovary syndrome and menopause by compensating for the decline in estrogen level (4, 5). In general, licorice root improve reproductive performance and improves sperm count as well as semen viscosity (6). Additionally it could minimize the inflammation and irritation of the urethra which can be consequence of coitus (7). Also it was noticed that the consumption of licorice, lead to a significant increase in sperm concentration and motility of oligoasthenozoospermic infertile men (8).
**Materials and Methods**

Sixty mature female Swiss Webster mice (45-49 day age) were divided into two main groups: Experimental (G) and control (C) groups. Experimental G (30 animals) were treated with 1 gm/kg of body weight of licorice root extract (Food Industry Company, Iraq) immersed in 1 ml of distilled water while the control C (30 animals) were given distilled water only. Then the two main groups subdivided into three equal subgroups (G1, G2, G3) where G1 were treated for 3 weeks before mating, G2 were treated for 3 weeks before mating and 3 weeks during pregnancy, and G3 were treated for 3 weeks during pregnancy, while (C1, C2, C3) were given distilled water only by the same volume and periods that used with experimental groups. The licorice extract was prepared by using 1000 gm of licorice in granular powder moistened with boiling water and percolated until the licorice exhausted. Then ammonia solution were added the percolate, filterate and evaporate until black pillar mass having a characteristic sweet taste powder were prepared. Chief component are glycyrrhetinic acid, glycone, salt, flavonoids, volatile oil, acetic acid. Litter size for all experimental and control mothers were recorded. The newborn animals (male only) feed on normal diet, without licorice from both treated and control groups (180 mice) were also divided by the same way of division used with the mothers. After 40 days (around the puberty age), the animals were weighted again using an electrical balance (Sartorius, Germany), then sacrificed to get their testes. Each testis was weighted, then fixed in 10% normal saline and histological sections with thickness of 5 micrometer were prepared using the routine histological technique and stained with Haematoxylin-eosin. Diameters of semineferous tubules together with the thickness of their germinal wall were calculated by a calibrated eye piece (x400) ocular micrometer in order to draw their mean value for individual mice. Data was analyzed using student t-test.

**Results**

1-Effect of licorice extract on the litter size: highly significant increase (P<0.01) in the numbers of pups born from treated mothers compared to that of control groups were recorded with slight non significant increase in their weights. No differences were revealed between the three experimental groups (Table 1).

2-Effect of licorice extracts on young males offspring:

A-Weights: males born from treated mothers (G1, G2, &G3) showed a significant increase in Body weights (P< 0.05) and highly significant increase (P< 0.01) in their testes weights (Table 2).

Comparison with that born from control groups mothers (C1, C2 &C3).

B-Histological study: A highly significant increase (P< 0.01) in the diameters of semineferous tubules in the testes of all experimental males (G1, G2 &G3), was recorded with a well developed germinal epithelial lining. The measurement of the lining thickness was also showed a significant increase (P< 0.05), compared to that of control males C1, C2&C3 (Table 2). Mature sperms with full formed head and tail appeared clearly free in the lumen of semineferous tubules of the experimental
animals, while those belong to the control groups showed immature stages of spermiogenesis with pyramidal shape and attached to the apex of Sertoli cells (Figure 1 and 2).

Table 1: Effect of licorice extracts administration to pregnant females on the number and weights of their offspring.

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>G1</th>
<th>C2</th>
<th>G2</th>
<th>C3</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weights of Pups (g)</td>
<td>1.21 ± 0.010</td>
<td>1.23 ± 0.097</td>
<td>1.22 ± 0.0127</td>
<td>1.33 ± 0.016</td>
<td>1.03 ± 0.015</td>
<td>1.24 ± 0.012</td>
</tr>
<tr>
<td>Number of pups/pregnant</td>
<td>4.06 ± 0.1</td>
<td>8.13** ± 0.13</td>
<td>3.86 ± 0.12</td>
<td>8.76** ± 0.13</td>
<td>3.96 ± 0.13</td>
<td>8.96** ± 0.15</td>
</tr>
</tbody>
</table>

**: highly significant increase (P < 0.01).

Table 2: Effect of licorice extract administration to pregnant mothers on the body weight, testis weight, thickness and diameters of semineferous tubules of young males.

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>G1</th>
<th>C2</th>
<th>G2</th>
<th>C3</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight of young male mice (g)</td>
<td>16.21 ± 0.04</td>
<td>19.58** ± 0.08</td>
<td>16.42 ± 0.07</td>
<td>19.75** ± 0.10</td>
<td>16.5 ± 0.09</td>
<td>20.1** ± 0.12</td>
</tr>
<tr>
<td>Weight of testis (mg)</td>
<td>2.33 ± 0.259</td>
<td>4.0** ± 0.283</td>
<td>2.30 ± 0.209</td>
<td>4.608** ± 0.323</td>
<td>2.262 ± 0.293</td>
<td>3.914** ± 0.364</td>
</tr>
<tr>
<td>thickness of semineferous tubules (µm)</td>
<td>1.7 ± 0.04</td>
<td>2.1* ± 0.01</td>
<td>1.7 ± 0.04</td>
<td>2.1* ± 0.01</td>
<td>1.7 ± 0.04</td>
<td>2.2* ± 0.03</td>
</tr>
<tr>
<td>diameters of semineferous tubules (µm)</td>
<td>13.3 ± 0.16</td>
<td>15.9** ± 0.08</td>
<td>13.6 ± 0.06</td>
<td>16.2** ± 0.14</td>
<td>13.8 ± 0.09</td>
<td>15.3** ± 0.12</td>
</tr>
</tbody>
</table>

*: significant changes (P<0.05)
**: highly significant changes (P < 0.01)
Figure 1: Cross section in the testis of young male (40 days) mouse born from mother treated with licorice root extract (0.003 mg daily), showing the wide lumen of seminiferous tubules filled with mature sperm (arrows) and complete development of the lining epithelium. 400 X

Figure 2: Cross section in the testis of young male mouse born from control group mother. Note the absence of mature sperms in the lumen of the seminiferous tubules and the appearance of clumps of immature sperms connected to the germinal epithelium (arrows). 400X (H&E)
Discussion

The significant increase in the litter size of mothers consumed licorice extract recorded in this study, reflects an increase in ovulation rate and successful implantation for the fertilized ova, and this means consequently that a good amount of FSH and LH may be available, since these hormones are responsible for stimulation of follicular development and ovulation (13). An increment in gonadotropic hormones may be correlated with the consumption of licorice extract, which is previously documented, through several studies, to have this stimulatory effect on these hormones (14-16). On the other hand, the increase in number of births in all experimental groups may be attributed to the increase in implantation rate which depends mainly upon the presence of sufficient amount of estrogen and progesterone (17), and since steroids is one of licorice constituents (5), so it may provide an estrogenic atmosphere necessary for good implantation (3, 18). An increment in the body weights of young males born from treated mothers agreed with that recorded in other study (16), which may results from the biochemical properties of licorice in addition to its nutritional constituents such as: sugar, protein, amino acids, vitamins and sterols (19). On the other hand, the significant increase in gonadal weights and the precocious maturation of semineferous tubules may be attributed to the possible activation of gonadotropin releasing hormone (GnRH) secretion by licorice, leading to activation of pituitary-gonadal axis which is temporarily active during the fetal/neonatal development and secondly reactivated at puberty (20); and this was reflected clearly by the positive results recorded in the experimental groups. In addition, the activation of pituitary-gonadal axis during neonatal period, stimulate Sertoli cells proliferation leading consequently to rise their spermatogenic potential at adult period (21), this was reflected by the thickness and maturation of the germinal epithelium of the semineferous tubules in the experimental groups compared to that of the control groups.

Consuming low dose of licorice root extract by pregnant females causes significant increase in litter size and weights with a precocious maturation of the male gonads.

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Expression of certain activation markers, CD45RA, CD45RO and CD11b on the surface of peripheral blood lymphocytes isolated from patient with idiopathic preterm labour.

Nidhal Abdul Muhymen\textsuperscript{1} PhD, Maha M. Al-Bayati\textsuperscript{2} MBChB; CABOG, Thoraya Hosaam Al-din\textsuperscript{3} MBChB.

Abstract

Background: preterm labor (PL) is remaining the leading cause of non-anomalous prenatal mortalities.

Objective: is to determine the association of PL on the expression of certain activation markers on the surface of peripheral blood lymphocytes (PBLs).

Patients and methods: Thirty patients with idiopathic preterm labour (IPL) (group A) in addition to 30 healthy pregnant women of comparable gestational age groups (group B) were enrolled in this study. Blood samples were taken from both groups and lymphocytes were separated and stained with fluorescent labeled monoclonal antibodies against CD45RA, CD45RO and CD11b.

Results: results indicated that there were a significant increase in the percentage of CD45RA in group A and reduction in the percentage of both CD45RO and CD11b in the same group.

Conclusions: patients with IPL have a less tendency of the activity of lymphocytes.

Key words: Idiopathic premature labour, activation markers, CD45RA, CD45RO, CD11b.

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Introduction

Preterm labour (PL) is the major cause of prenatal mortality and morbidity \textsuperscript{(1)}. It is one of the most serious problem facing obstetrician and other perinatal health care \textsuperscript{(2)}. About 90\% of births occur between 37 and 42 weeks, this period is called term \textsuperscript{(3)}. The etiology of PL is multifactorial, but in majority of instances the precise cause are unknown \textsuperscript{(4)}. This is known as idiopathic PL which makes up at least 75\% of the cases. The uterus is not immunologically privileged site; it is well vascularized with good lymphatic drainage and can reject foreign tissues \textsuperscript{(5)}.

The human decidua contains an un-usually high proportion of lymphocytes, mainly NK and T cells, which are potentially cytotoxic to trophoblast when they are stimulated with certain cytokines \textsuperscript{(5)}. It was found that there are a higher proportion of decidual and peripheral lymphocytes that expressed activation markers in spontaneous abortions than in elective termination of pregnancy \textsuperscript{(6)}.

Hence in the current study we intended to study some of activation markers that expressed on the surface of PBLs in patients with IPL in comparison with healthy pregnant women.

Materials and methods

Thirty blood samples from IPL women (group A), attending the Department of Obstetrics and Gynecology in AL-Khadhemia teaching hospital were collected. Other thirty blood samples taken from healthy pregnant women with
comparable gestational age with no evidence of PL.

Lymphocyte separation and staining: Blood sample (Five ml venous blood) was aspirated from all patients and controls. Blood was collected in pyrogen-free silicone-coated tubes with heparin. The blood samples were used for lymphocyte separation according to Isopaque-ficoll technique (originally described by Boyum in 1968).

Mouse monoclonal Ab (primary Ab) specific for human CD45RA; CD45RO and CD11b and biotinylated secondary antibody (anti-mouse Ab) were used. Slides were examined under 400X-magnification power of light microscope. The dark brown (homogenous or membranous) staining identified positive labeled cells.

Statistical analysis: chi – square test and students t –test were used to analyze the results.

Results
As shown in figure 1, the percentage of CD45RA antigen on PBMC of group A was significantly higher (p<0.00069) than that of the percentage expressed by group B, while the expression of the other molecule (CD45RO) on these cells was significantly lower (p<0.0005) in group A than that expressed by group B, as shown in figure 2.

Meanwhile, the expression of CD11b molecule was significantly reduced (p<0.0001) on the surface of PBMC of group A compared with that of group B, as shown in figure 3.

Figure 1: Percentage of naïve cells in group (A) compared with group (B).
Figure 2: Percentage of memory T-cells in group (A) compared to group (B).

Figure 3: Percentage of intercellular adhesion molecule receptor (CD11b) in group (A) compared to group (B).
**Discussion**

There is growing interest in the use of mononuclear cells surface markers for the diagnosis of different disorders syndromes (8). Understanding the impact and physiologic factors, such as age, pregnancy and stress on PMNC surface markers, is essential for appropriate interpretation of results.

Based on the results, equilibrium between CD45 isoforms (RO and RA) exist on the surface of PMBC. Total CD45 phosphatase activity in a cell is determined by this equilibrium, which in turn controlled by isoform expressed Naive T cells express CD45RA isoforms indicating a resting cells (9), while the expression of CD45RO isoforms mean a shifting to activated T cells (10). So in the case of our patients the predominant type of cells were in its naive form this result can be explained by the fact that HLA-G (MHC-Ib) can suppress proinflammation of T lymphocytes (11,12) beside a membrane -bound HLA-G, a soluble counterpart (sHLA-G) may play an important in the immunological establishment of pregnancy by affecting peripheral immune cells and modulating their function for the benefit of pregnancy (13, 14). It was found that embryos which secreted sHLA-G gave rise to successful pregnancy (15) by the above finding we can conclude that the embryos of our patients may have low levels of sHLA-G which have an effect on PBMC. Concerning the results of CD11b expression which has been reported herein to be decreased in comparison with group B. this antigen (CD11b) form a hetero dimer with CD18 and both will be the receptor for complement component fragment receptor. C3b and will be called CR3. CR3 is important in adhesion (It is ligands for ICAM,intra cellular adhesion molecules and phagocytosis(16). CD11b expression has been reported to be normal or increased in pregnant women (17-21).

However, pregnant women delivering prematurely have consistently shown a higher expression of CD11b (22, 23). But we can explain the decrease in the expression in our patients, by the fact that this antigen has an extensive, intracellular storage pool, which could be released to the surface with activation or excessive manipulation.

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Subuh Salim AL - Mudallal¹ FICMS, Mustapha Abdul-Wahid Mukeef² MSc.

Abstract

Background: Myelofibrosis with myeloid metaplasia (MMM) is a clonal stem cell disease with varying degrees of fibrosis in the marrow.

Vasculogenesis is the formation of new blood vessels from pre-existing vessels during adult life; it plays a critical role in neoplastic development, progression and metastasization and has been shown to be an adverse prognostic factor in many solid tumors.

It is becoming increasingly evident that angiogenesis plays a key role in the pathophysiology of hematologic malignancies by estimating bone marrow microvessel density and by measuring circulating angiogenic factors. MMM is probably the disease with the more pronounced angiogenesis among Chronic Myeloproliferative Disorders.

Objective:

1. Evaluation of angiogenesis in Idiopathic Myelofibrosis (IMF) via immunohistochemical staining for CD31 and CD34 and quantifying them by Computerized Image Analysis System and Light Microscope.

2. To investigate whether angiogenesis can be considered as a marker for disease activity.

Methods: This cross sectional study was conducted on 31 formalin fixed paraffin embedded blocks of Idiopathic Myelofibrosis cases along with 10 age matched control cases having no abnormal bone marrow pathology, from January 2006 to June 2006. The sections from bone marrow biopsies were processed routinely and stained with Hematoxylin and Eosin (H&E) and with immunohistochemical stain for CD31 and CD34 markers.

The bone marrow Microvessel Density of IMF and control case was assessed by the means of Computer Aided Image Analysis System and by visual count using light microscope.

Results: This study revealed that there was a significant increase in microvessel density in IMF cases, using both the computerized method and the visual count by light microscope, with both CD31 & CD34 compared to control group (P < 0.05).

Also this study showed that the increase in angiogenesis was positively correlated with the Dupeirez score prognostic system and the age of the patients.

On the other hand; no significant correlation was found between angiogenesis and the following parameters: sex of the patient, Hb value, WBCs count, peripheral blood platelets count.

Conclusion: This study has showed that angiogenesis was an integral component of the bone marrow stromal reaction in MMM and it was closely related to many prognostic parameters; thus bone marrow angiogenesis can be used as a tool to assess the disease activity.

Key word: Idiopathic myelofibrosis, angiogenesis, Computeriz image analysis.

INTRODUCTION

Idiopathic Myelofibrosis (IMF) or as also called Myelofibrosis with Myeloid Metaplasia (MMM) is one of the Philadelphia-negative chronic myeloid disorders and is stem cell disorder characterized by clonal megakaryocytic hyperplasia, a leukoerythroblastic peripheral blood smear, extramedullary hematopoiesis, and secondary bone marrow fibrosis⁽¹⁾.

Angiogenesis, or the formation of new blood vessels, is an important...
process in health and disease and may be integral to solid tumor growth and metastasis.\(^2,3\) The concentration of these vessels has been shown to be increased in various hematologic disorders, and it may provide useful prognostic informations\(^2-6\).

Indeed with the use of immunohistochemistry, various antibody markers for endothelial cells have been used to identify intratumoral vessels, the most commonly used markers are CD31 and CD34\(^7,8\).

Quantification and analysis of the degree of intratumoral angiogenesis is mostly done by estimating the microvessel density (MVD) which is maximal number of blood vessels per unit area of section\(^7,8\).

The recent introduction of morphometric analysis using fully automatic Computerized Image Analysis (CIA) has offered objectivity, increase precision compared with direct visual appraisal and makes statistical analysis easier, as it is possible now to measure MVD and the intercapillary distance (ICD)\(^9\).

**Subjects, Materials, and Methods**

This cross sectional study was conducted on 31 formalin fixed paraffin embedded blocks of Idiopathic Myelofibrosis cases along with 10 age matched control cases having normal bone marrow.

The cases were collected from the archives of the histopathology laboratory in Al Kadhimya teaching hospital; Al Yarmook Center for Blood Disorders; the Teaching Laboratory of Medical City Hospital, and from private laboratories from January 2006 to June 2006.

The selection criteria of Idiopathic myelofibrosis cases was based on the biopsies reports which was examined by a specialized haematologist, and other diagnostic criteria of IMF; which include bone marrow fibrosis, atypical megalakaryocytic hyperplasia, and peripheral blood leukoerythroblastosis, in addition to clinical & physical findings. Whereas the bone marrow biopsies of control cases showed no bone marrow abnormalities and no excess in fibrous tissue.

From each specimen, 3 sections of 5 \(\mu\) thick were taken, the first was stained with Hematoxyline and Eosin stain and the other two were stained immunohistochemically with CD31 and CD34 antibodies.

Both patients and controls sections were examined for bone marrow microvessel density at x100, x200, and x400 magnification, and five hot spot areas (areas of highest neovascularization) in each CD31 and CD34 stained sections were selected and the mean number of microvessels (MVD) was measured, and the same field was examined by light microscope (LM) and by computerized image analysis system (CIA) and was compared with that of normal reactive bone marrow specimens.

For performing visual count by LM, each of the study slide was first scanned at 100\(\times\) magnification, and 5 hot spot areas were defined and the number of blood vessels were determined at 400\(\times\) magnification, and that number was divided by the tissue area of that field (tissue area using Olympus microscope at 400\(\times\) is 0.1885 mm\(^2\)) yielding microvessel visual count/area (MVC/Area) parameter which is comparable to the MVD estimated by CIA system and this was used for statistical analysis\(^10\).

During the counting process, large vessels, tortuous vessels, and vessels in the periosteum or bone and open
Angiogenesis in idiopathic myelofibrosis. Subuh S. AL-Mudallal et al.

sinusoids were excluded. Areas of staining with no discrete breaks were counted as single vessels, and the presence of a Lumen was not required.

The Computerized image analysis (CIA) system used in this study is an automatic morphometry machine that analyse semi-automatic features using computerized pixel counting. Microvessel surface area was determined and expressed as the percent of the area showing the stained blood vessel in relation to the total tissue area which is the Microvessel Density (MVD) (11).

Lille Scoring System (Dupriez Score) (12) was done for all the patients; accordingly, three distinct prognostic groups were identified depending on three adverse prognostic factors as shown in the following table:

### Table 1: The Lille Scoring System (Dupriez score) for Predicting Survival in MMM (12).

<table>
<thead>
<tr>
<th>Number of adverse prognostic factors*</th>
<th>Risk Group</th>
<th>Median Survival(months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Low</td>
<td>93</td>
</tr>
<tr>
<td>1</td>
<td>Intermediate</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>High</td>
<td>13</td>
</tr>
</tbody>
</table>

*Adverse prognostic factors: Hb less than 10 g/dL and WBC count less than 4 or greater than 30 x 10⁹/L.

**Statistical analysis** was done using descriptive statistics, the Student’s unpaired t-test, and analysis of variance (ANOVA). In addition to applying the rules of correlation and regression (r). P value of < 0.05 was considered statistically significant.

**Results**

This study was conducted retrospectively on 31 cases of MMM and 10 aged matched control cases Table 2. The [Hb], WBCs count, and Platelets count were obtained from the patients’ data sheets and are shown in table 3.

Lille Scoring System (Dupriez Score) was able to identify three distinct prognostic groups according to three adverse prognostic factors as shown in table 3.

The results of the staining procedure with CD31 and CD34 were compared to that of the appropriate control sections & a cytoplasmic reddish - brown coloration of endothelial lined vessels was considered as a positive reaction (Figures 1 and 2).

In this study the mean of the MVC/Area, using light microscope, was significantly higher using both CD31 and CD34 in patients with MMM (394.42±155.74 no./mm², 455.1474±168.95 no./mm² M±SD respectively) than in the control cases (16.2±18.73 no./mm², 18.55±17.88 no./mm² M±SD respectively) (p < 0.05), using t-test (Table 4).

By using the Computerized image analysis (CIA) the microvessel density (MVD) was significantly higher with both CD31 and CD34 in patients with MMM (7.93% ± 4.38%, 9.64% ± 4.47% M± SD respectively) than in the control cases (1.50%±0.91%, 1.57%±0.74% M±SD respectively) (P < 0.05), using t-test (Table 4).

In this study there was a significant positive relation between age of the patient and MVD using both CD31(r=0.67, P<0.05) and CD34(r=0.62, P<0.05).

By using Analysis of variance (ANOVA) there was a significant
statistical association between MVD and MVC/Area and the three grades of Dupriez Score using both markers, as shown in tables 5(A&B) & 6(A&B).

This study revealed that there was no relation between angiogenesis, by using both markers and the following parameters in MMM patients (p>0.05): Sex of the patient, Hb concentration, peripheral blood platelets, and peripheral WBCs count.

Figure 1: Positive CD31 stained blood vessels (400X).

Figure 2: Positive CD34 stained blood vessels (400X).
Table 2: Sex and Age distribution of MMM and Control cases

<table>
<thead>
<tr>
<th></th>
<th>MMM patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Males</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>Females</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Gender ratio</td>
<td>♂:♀ 2.4:1</td>
<td>♂:♀ 1.5:1</td>
</tr>
<tr>
<td>Age range (Years)</td>
<td>38 - 65</td>
<td>38 - 53</td>
</tr>
<tr>
<td>Age Median (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age mean ± SD (Years)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Haematological descriptive profiles of the 31 cases with MMM.

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>Number</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleenomegaly</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100%</td>
</tr>
<tr>
<td>Haemoglobin (Hb) g/dl</td>
<td></td>
<td>9.82 ± 1.59</td>
<td>9.5</td>
<td>6 - 13</td>
<td>-</td>
</tr>
<tr>
<td>WBC x10⁹/l</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Low (&lt;4 x10⁹/l)</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal (4-11 x10⁹/l)</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>High (&gt;11 x10⁹/l)</td>
<td>9</td>
<td>12.2 ± 9.16</td>
<td>8.8</td>
<td>3.2 - 34</td>
<td>-</td>
</tr>
<tr>
<td>Platelets x 10⁹/l</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Low (&lt;150 x10⁹/l)</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal (150-400 x10⁹/l)</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>High (&gt;400 x10⁹/l)</td>
<td>1</td>
<td>157.5 ± 96.2</td>
<td>150</td>
<td>25 - 450</td>
<td>-</td>
</tr>
<tr>
<td>Dupriez Score</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32.25%</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>61.29%</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.45%</td>
</tr>
</tbody>
</table>

Table 4: Descriptive statistics of MVC/Area and MVD with both CD31 and CD34 in MMM and Control cases

<table>
<thead>
<tr>
<th>parameters</th>
<th>CD31 MVC/Area (no./mm²)</th>
<th>CD31 MVD (%)</th>
<th>CD4 MVC/Area (no./mm²)</th>
<th>CD4 MVD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MMM</td>
<td>Control</td>
<td>MMM</td>
<td>Control</td>
</tr>
<tr>
<td>Mean</td>
<td>394.42</td>
<td>16.2</td>
<td>7.93</td>
<td>1.50</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>155.74</td>
<td>18.73</td>
<td>4.38</td>
<td>0.91</td>
</tr>
<tr>
<td>Median</td>
<td>389.44</td>
<td>13.39</td>
<td>6.59</td>
<td>1.41</td>
</tr>
<tr>
<td>Range</td>
<td>133.1 - 636.2</td>
<td>0 - 53</td>
<td>2.5 - 19.2</td>
<td>0.44 - 2.95</td>
</tr>
</tbody>
</table>

P < 0.05 comparing MVD and MVC with control cases
**Angiogenesis in idiopathic myelofibrosis… Subuh S. AL - Mudallal et al.**

Table 5 –A-: MVD in the three grades of Dupreiz score using CD31 stained vessels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Cases</th>
<th>MVD (%) Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>10</td>
<td>5.61</td>
</tr>
<tr>
<td>Score 1</td>
<td>19</td>
<td>8.18</td>
</tr>
<tr>
<td>Score 2</td>
<td>2</td>
<td>17.20</td>
</tr>
</tbody>
</table>

Table 5 –B-: MVD in the three grades of Dupreiz score using CD34 stained vessels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Cases</th>
<th>MVD (%) Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>10</td>
<td>6.44</td>
</tr>
<tr>
<td>Score 1</td>
<td>19</td>
<td>10.45</td>
</tr>
<tr>
<td>Score 2</td>
<td>2</td>
<td>17.91</td>
</tr>
</tbody>
</table>

Table 6 –A-: MVC/Area in the three grades of Dupreiz score using CD31 stained vessels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Cases</th>
<th>MVC/Area(no./mm²) Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>10</td>
<td>275.7168</td>
</tr>
<tr>
<td>Score 1</td>
<td>19</td>
<td>437.1617</td>
</tr>
<tr>
<td>Score 2</td>
<td>2</td>
<td>581.8891</td>
</tr>
</tbody>
</table>

Table 6 –B-: MVC/Area in the three grades of The Dupreiz score using CD34 stained vessels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Cases</th>
<th>MVC/Area(no./mm²) Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>10</td>
<td>345.0611</td>
</tr>
<tr>
<td>Score 1</td>
<td>19</td>
<td>488.4451</td>
</tr>
<tr>
<td>Score 2</td>
<td>2</td>
<td>689.2499</td>
</tr>
</tbody>
</table>


**Discussion**

In this study the use of two immunohistochemical staining markers had increase the sensitivity of the staining technique and both of them found to be sensitive, reproducible and reliable for assessing neovasculization and their results were similar and comparable to other techniques as anti Factor VIII related antigen immunostaining\(^\text{13}\). In spite CD34 marker can be detected on myeloid progenitors as well, but the number of cells stained was sufficiently small as not to interfere with our analysis.

This study has revealed that MVD using both markers was significantly higher in MMM compared to control cases (Table 4).

This increase in neo-vascularization was in agreement with Lundberg LG. et al\(^\text{14}\) and Panzoni M. et al\(^\text{8}\) study, which had revealed that there was an increase in vascular density in the bone marrow of MMM compared to the bone marrow of healthy subjects.

Moreover Mesa RA. et al\(^\text{15}\) and Pruneri G. et al\(^\text{16}\) had found a substantial increase of marrow vascularity in most patients with CMD compared with normal control and with patients with other myeloid disorders such as MDS and AML, and the extent of the abnormality was more pronounced in patients with MMM than in those with either PV or ET.

Moreover this study have found that the architecture of the vasculature clearly differs from the normal architecture in that the vessels in MMM are more tortuous and branched (Figures 1, 2), which was noticed by Lundberg LG. et al as well\(^\text{14}\) who had concluded that these vessels changes were closely related to the disease activity.

In this study there was a significant positive correlation between MVD and age but not with sex and this was in agreement with Al-Sayegh Z. (\(^\text{17}\) and Hammoudi AT\(^\text{10}\) studies.

Many authors had stated that bone marrow angiogenesis might represent the earliest histo-morphological event in the temporal progression towards myelofibrosis and osteosclerosis, and the neo angiogenesis as expressed by increased MVD and elevated in vascular endothelial growth fator (VEGF) level, was detected in early (prefibrotic) stage of MMM therefore they had consider that neoangiogenesis can reflect disease activity\(^\text{18-20}\).

This study revealed that there was a significant positive correlation between angiogenesis and Dupriez score which is closely related to prognosis in MMM as shown in table 5(A&B) and table 6 (A&B), therefore we may propose that neoangiogenesis in MMM is closely related to these prognostic parameters thus it maybe used as a valuable tool to assess the disease severity as well as its activity\(^\text{20,21}\).

In this study all patients had splenomegally, which is the result of extramedullary hematopoiesis and since antiangiogenic agent reduce the size of the spleen and liver therefore we may propose that the extramedullary hematopoiesis in the spleen is closely related to the increase in marrow neo-vascularization\(^\text{14}\).

The increased marrow vascularity in MMM is consistent with the current understanding of the pathogenesis of this disease. The proliferation of an aberrant clone, most likely of megakaryocytic or of monocyctic origin (or of both), is believed to be the underlying cause for the induction of an abnormal cytokines.
release, mainly transforming growth factor–β1, b-fibroblast growth factor, platelet derived growth factor and vascular endothelial growth factor, that have fibrogenic, angiogenic and osteogenic potential (21–23).

In conclusion this study had revealed that microvessel proliferation or neoangiogenesis is a major component of the mixed stromal reaction in MMM which may reflect the disease activity and severity. Also we may predict that the degree of increased angiogenesis may have an independent prognostic value which could serve as an additional variable in clinical prognostic parameters.

Moreover there was no significant statistical difference in the staining sensitivity between CD31 and CD34. And whether angiogenesis was estimated by visual count using light microscope or by computerized imaging system, they gave reliable and comparable results.

References
17. Al-Sayegh Z. The significance of angiogenesis in the assessment of the biological behavior of gastric carcinoma.2003; A thesis submitted to the University of Baghdad for Master in Pathology.
18. Brijesh A, Ching-Liang Ho, James D H. Bone marrow angiogenesis and its clinical
Angiogenesis in idiopathic myelofibrosis… Subuh S. AL - Mudallal et al.

CT-guided transthoracic biopsy of solitary pulmonary Nodules using automatic biopsy gun.

Mohammed abd kadhim\textsuperscript{1} MBChB; FIBMS, Alaa Kassar Salih\textsuperscript{1} MBChB; FIBMS, Zaid Khidher ahmed\textsuperscript{1} MBChB; FIBMS, Qasim Fawzi Hasan\textsuperscript{2}.

Abstract

**Background:** CT-guided percutaneous transthoracic fine needle aspiration biopsy (TFNAB) has become a widely accepted and effective minimally invasive technique for the diagnosis of a variety of intrathoracic lesions that are not readily accessible with bronchoscopy. It is generally regarded as a safe procedure with limited morbidity and extremely rare mortality. It provides high diagnostic accuracy and has a relatively low complications rate.

**Objectives:** The aims of our study were to report our experience with regard to the accuracy & pneumothorax rate of percutaneous CT-guided biopsy of solitary pulmonary nodules using automatic biopsy gun.

**Patients and methods:** Between January 2006 and August 2009, 54 patients (46 men and 8 women) with solitary pulmonary nodule underwent CT guided transthoracic biopsy at Al-Kadhimyia teaching hospital, Baghdad, Iraq. All the lesions could not be diagnosed with fiberoptic bronchoscopy. CT-guided biopsy was performed with an 18-gauge automatic biopsy gun. Chest radiography was done 2-4hr later and 24hr after biopsy for observation of pneumothorax. The overall diagnostic accuracy, pneumothorax rate, and chest tube insertion rate were determined. Diagnostic accuracy and pneumothorax rate were statistically compared according to lesion size & lesion depth (p value of less than 0.05 was considered to be significant).

**Results:** Forty one patients (76%) diagnosed as malignant (diagnostic accuracy of 87.8%). Thirteen patients (24%) were diagnosed as benign (diagnostic accuracy of 92.3%). The overall diagnostic accuracy was 89% (48 of 54). The diagnostic accuracy did not differ with respect to the lesions size and lesions depth from the chest wall. Accurate diagnosis was made in 25 of the 29 nodules <20 mm (86%) and in 23 of the 25 nodules \(\geq 20\) mm (92%). Similarly accurate diagnosis was made for 36 (90%) of the 40 nodules shallower than 60 mm and for 12 (85.7%) of the 14 nodules \(\geq 60\) mm. Pneumothorax occurred in 23 (42%) patients. Pneumothorax occurred more frequently in small sized lesions (16 out of 29 lesions measuring <20 mm) as compared to (7 out of 25 lesions \(\geq 20\) mm) (P <0.05). Similarly pneumothorax occurred more frequently in deeper lesions (10 out of 14 lesions \(\geq 60\)mm in depth) as compared to (13 out of 40 lesions <60 mm in depth) (P <0.05). Only 7 (13%) patients requiring thoracostomy tube placement.

**Conclusions:** CT-guided biopsy using automatic biopsy gun allowing a specific diagnosis for benign & probably malignant lesions. Diagnostic accuracy was not affected by the size and depth of the lesions. Deeper & small sized lesions have associated with an increased rate of pneumothorax.

**Keywords:** solitary pulmonary nodule, CT-guided biopsy, automatic biopsy gun.

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Introduction

CT-guided percutaneous transthoracic fine needle aspiration biopsy (TFNAB) has become a widely accepted and effective minimally invasive technique for the diagnosis of a variety of intrathoracic lesions that are not readily accessible with bronchoscopy\textsuperscript{(1-3)}. It is an easy, reliable and safe procedure that obviates the need for exploratory surgery in medically treatable or unresectable cases\textsuperscript{(4)}. It is a relatively accurate method of diagnosing benign and malignant lesions of the chest\textsuperscript{(3,5,7)}.
Haaga and Alfidi (8) reported the first computed tomography (CT)-
guided biopsy in 1976, and numerous reports since that time have shown
TFNAB procedures to be both
effective and accurate (3, 5-7, 9-11).

CT- guided TFNAB is generally
regarded as a safe procedure with
limited morbidity and extremely rare
mortality (5, 6, 9, 10, 12). It provides high
diagnostic accuracy and has a
relatively low complications rate (13-15).

The management of solitary
pulmonary nodule (SPN) depends on
many factors including clinical
features, results of relevant
investigations, population
characteristics, and local policy (16, 17).
The most important first step is to
determine the likelihood of the nodule
being malignant and then to decide
whether the lesion should be removed,
observed, or further investigations performed (18,19). TFNAB is advocated
to improve the precision of
management by increasing the
confidence in which masses can be
categorized as benign or malignant (6,
20, 21), and so it has a strong impact on
the diagnostic protocol of the solitary
pulmonary nodule (22). CT-guided lung
biopsy has found wide-spread
acceptance as a principal method of
diagnosing SPN (5, 11, 23-25).

Most CT-guided lung biopsies
described in earlier reports were
performed with fine-needle aspiration
for cytology and the materials obtained
by means of aspiration are usually
suitable only for cytological
examination and were useful in
differentiating malignant from benign
lesions, but these have some limitation
in certain clinical settings, it does not
allow adequate subtyping of
carcinoma, seldom yields a specific
pathologic diagnosis in cases of benign
disease and a negative result does not
exclude malignancy (6, 26-29). More
recently, tissue core biopsy using an
automated cutting needle, which
enables the histological evaluation of
the obtained samples has been
implemented (30), and this may improve
the diagnostic yield and increase the
chances of obtaining a specific
diagnosis (31, 32). The automatic biopsy
gun has become popular for biopsy of
various organs (33). The advantages of
obtaining a core specimen include
greater accuracy in allowing a specific
diagnosis for benign lesions, the ability
to diagnose carcinomas without a
trained cytopathologist and greater
accuracy in defining cell types of
carcinomas (33-35).

To our knowledge this study is the
first one done in Iraq to study role of
CT-guided biopsy of solitary
pulmonary nodule using automatic
biopsy gun.

Patients and methods

Between January 2006 and August
2009, a prospective study included 54
patients with solitary pulmonary
nodule underwent CT guided biopsy at
Al-Kadhimya teaching hospital,
Baghdad, Iraq. The study population
included 46 men and 8 women with a
mean age of 56 years (range, 34–66
years). All the lesions could not be
reached & diagnosed with fiberoptic
bronchoscopy. The average lesion size
was 2.1 mm (range, 0.5–40mm) and
the average depth of lesions from the
skin surface was 51 mm (range, 18–
82mm).

Examinations were done with the
CT unit (Somatom plus4; siemens
medical system). Preliminary scans
were done without use of contrast
medium in either prone or supine
position to plan the biopsy approach.
Biopsies were performed in the prone,
supine or lateral decubitus positions,
depending on proximity of the lesion
to the chest wall. After the lesion had
been localized, depth of the lesion
from the skin surface was measured.
The chosen entry site was prepared and
draped in a sterile fashion, under the local anesthesia biopsy needle was inserted and biopsy was performed with automatic biopsy gun (Temno, Italy, 18-gauge, 15cm length). The obtained specimen was treated by H & E stain.

All patients were hospitalized. They rested in bed and underwent chest radiography 2-4hr later and 24hr after biopsy. If pneumothorax was not present, the patient was discharged the morning after biopsy. Thoracostomy tubes were inserted if the pneumothorax was moderate to large (>30%) on the basis of the distance from the lung apex to the cupola or on the basis of continued size increase on follow-up radiographs. Thoracostomy tubes were also inserted if the patients experienced substantial pain or shortness of breath in the presence of a small pneumothorax.

True positive diagnosis: in cases with surgical confirmation, when biopsy of another site revealed cancer with the same histologic characteristics, or when the lesion increased in size and other proven metastases were found.

True-negative diagnosis: in cases with surgical confirmation, when the lesion disappeared or decreased in size with or without the use of antibiotics, or when the lesion remained stable on follow-up CT for 18-24 months. Follow-up CT was scheduled 3, 6, 12, 18, and 24 months post-biopsy.

False-positive diagnosis: if surgical resection yielded a benign diagnosis, if the lesion disappeared or decreased in size before surgical resection, or if the lesion remained stable on the follow-up CT for at least 18-24 months in patients refusing surgical resection.

False-negative diagnosis: if surgical resection yielded a malignant diagnosis; if the lesion increased in size; if other proven metastases were diagnosed on CT or MR imaging and proven by histologic examination of the biopsy specimen or resection.

Final diagnosis of the 54 patients was proved by: formal surgery (36 patients), presence malignant liver lesions proved by FNA (3 patients), follow up CT for 20-24 months (15 patients)

**Statistical analysis**

Using the program SPSS (version 15 for Microsoft Windows). The overall diagnostic accuracy, pneumothorax rate, and chest tube insertion rate were determined. The diagnostic accuracy was calculated using the following formula:

\[
\text{diagnostic accuracy} (\%) = \frac{\text{no. true-positive} + \text{no. true-negative}}{\text{total number of solitary pulmonary nodules}}
\]

Diagnostic accuracy and pneumothorax rate were statistically compared according to lesion size & lesion depth. A P value of less than 0.05 was considered to be significant.

**Results**

The study population included 54 patients (46 men and 8 women) with a mean age of 56 years (range, 34–66 years). 29 patients have nodules <20 mm in diameter & 25 patients have nodules ≥20 mm (20mm is the cutoff value between small & large nodules). Forty patients have lesion <60mm from the chest wall, while 14 patients have lesions ≥60mm in depth from the chest wall (lesion depth was measured from the pleural puncture site to the edge of the intrapulmonary lesion along the needle path), (60mm is the cutoff value between superficial & deep nodules)

Of 54 pulmonary nodules 41 (76%) diagnosed as malignant (36 true-positive & 5 false negative) with diagnostic accuracy of 87.8% (36 of 41). Thirteen patients (24%) were diagnosed as benign (12 true-negative & 1 false positive) with diagnostic accuracy of 92.3% (12 of 13).
The overall diagnostic accuracy of the procedure was 89% (48 of 54). Table 1 shows the final pathological diagnoses and results of CT-guided biopsy. The diagnostic accuracy did not differ with respect to the lesions size and lesions depth from the chest wall. An accurate diagnosis was made for 25 (86%) of the 29 pulmonary nodules <20 mm and for 23 (92%) of the 25 pulmonary nodules ≥20 mm, a statistically insignificant difference (p = 0.54) as shown in table 2. An accurate diagnosis was made for 36 (90%) of the 40 nodules shallower than 60 mm and for 12 (85.7%) of the 14 nodules deeper than or equal to 60 mm, a statistically insignificant difference (p = 0.52) as shown in table 2.

Pneumothorax occurred in 23 (42%) patients. Pneumothorax occurred more frequently in small sized lesions (16 out of 29 lesions measuring <20 mm) as compared to (7 out of 25 lesions ≥20 mm) (P <0.05). Similarly pneumothorax occurred more frequently in deeper lesions (10 out of 14 lesions ≥60 mm in depth) as compared to shallower lesions (13 out of 40 lesions <60 mm in depth) (P <0.05) as shown in table 3. Only 7 (13%) patients requiring thoracostomy tube placement. Figure 1 shows images of CT-guided biopsy in 2 patients one with malignant lesion & the other with benign lesion.

Table 1: The final pathological diagnoses (no. of patients) and results of CT-guided biopsy (no. of patients & %) of the 54 patients included in the study.

<table>
<thead>
<tr>
<th>Final pathological Diagnosis</th>
<th>Total No. of patients</th>
<th>Accurate diagnosis by CT-guided biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>%</td>
</tr>
<tr>
<td>Malignant lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary carcinoma of bronchus</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>Secondary metastases</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Benign lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granuloma</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Organizing pneumonia</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hamartoma</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>48</td>
</tr>
</tbody>
</table>

Table 2: The diagnostic accuracy of the 48 patients included in the study according to lesion size & lesion depth from the chest wall.

<table>
<thead>
<tr>
<th>Lesion size (mm)</th>
<th>Accuracy</th>
<th>Total No. of patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>nodules &lt;20 mm in size</td>
<td>25</td>
<td>86</td>
<td>48</td>
</tr>
<tr>
<td>nodules ≥20 mm in size</td>
<td>23</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Lesion depth (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nodules &lt;60 mm in depth</td>
<td>36</td>
<td>90</td>
<td>48</td>
</tr>
<tr>
<td>nodules ≥60 mm in depth</td>
<td>12</td>
<td>85.7</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Cases complicated by pneumothorax compared to lesion size & lesion depth from the chest wall.

<table>
<thead>
<tr>
<th>Lesion size (mm)</th>
<th>Pneumothorax rate</th>
<th>Total No. of patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>nodules &lt;20 mm in size</td>
<td>16</td>
<td>55</td>
<td>23</td>
</tr>
<tr>
<td>nodules ≥20 mm in size</td>
<td>7</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Lesion depth (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nodules &lt;60 mm in depth</td>
<td>13</td>
<td>32.5</td>
<td>23</td>
</tr>
<tr>
<td>nodules ≥60 mm in depth</td>
<td>10</td>
<td>71.4</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: A: 46 year old male with Rt. upper lobe mass, FNAB reveal bronchogenic carcinoma which is proved by subsequent surgery & histopathology. B: 35 year old male patient with Rt. Lower lobe mass, FNAB reveal granuloma which was proved by follow up CT for the next 20 months.

Discussion

CT-guided lung biopsy has found wide-spread acceptance as a principal method of diagnosing SPN (5, 11, 23-25). Tissue core biopsy using an automated cutting needle, which enables the histological evaluation of the obtained samples has been implemented (30), and this may improve the diagnostic yield and increase the chances of obtaining a specific diagnosis (31, 32). The advantages of obtaining a core specimen include greater accuracy in allowing a specific diagnosis for benign lesions, the ability to diagnose carcinomas without a trained cytopathologist and greater accuracy in defining cell types of carcinomas (33-35).

In our study diagnostic accuracy for malignant lesions was 87.8% (36 out of 41) and for benign lesions 92.3% (12 of 13), these results were comparable with that reported in the previous studies where the diagnostic accuracy has been reported as greater than 80% for benign disease and greater than 90% for malignant disease (6, 9, 10).

The overall diagnostic accuracy in the current study was 89% (48 of 54) which was comparable with that described in the previously reported studies where the diagnostic accuracy has been described to be high, 81-96% (5, 11, 22, 24, 25, 36-38).

In our study, although the diagnostic accuracy for small pulmonary nodules <20 mm was less (86%) when compared with diagnostic accuracy for larger pulmonary nodules ≥20 mm (92%), this difference is
CT-guided biopsy and pulmonary Nodules ....Mohammed abd kadhim et al.

statistically insignificant (p = 0.54), & these results were comparable with that of Lucidarme et al. (25), Westcott et al. (39), and Laurent F. et al. (40) where they have shown that the diagnostic accuracy for small nodules is comparable to that for large nodules, but Li et al. (6) and Tsukada et al. (30) have reported that diagnostic accuracy for small pulmonary nodules is significantly less than that for large nodules.

Similarly we observed a statistically insignificant difference in diagnostic accuracy between superficial nodules (90%) and deeper nodules (85.7%) (p = 0.52) & these results were comparable with that reported in the previous studies (38, 40).

The most common complication of CT-guided lung biopsy is pneumothorax (11). A higher frequency of pneumothorax is a known disadvantage of CT-guided biopsy when compared to U/S guided biopsy and may be related to the fact that the needle stays across the pleura for a longer time. In addition, all lesions accessible to US-guided biopsy were peripheral and did not require the traversal of aerated lung, whereas it is likely that most difficult, small, deep lesions are sampled at CT-guided biopsy, which also may account for the higher complication rate (41). Prior studies found pneumothorax rates between 9% and 54% (22, 25, 30, 32, 37, 40, 42), in our studies pneumothorax occurred in 42% of the patients. The relatively high rate of pneumothorax in our study may be related to the use of an 18-gauge automatic biopsy gun. Despite the relatively high rate of pneumothorax, we believe that the high yield for benign disease avoids further invasive diagnostic procedures and justifies the risk.

The depth and size of the lesion might have an impact on pneumothorax rate (43-45). Previous studies have found a strong correlation between lesion size and pneumothorax rate (5-7, 43). In our study pneumothorax occurred more frequently in small sized lesions (16 out of 29 lesions measuring <20 mm) as compared to (7 out of 25 lesions ≥20 mm) (P <0.05). Cox et al. (43) reported that smaller lesion size correlated with the development of increased pneumothorax rate. They hypothesized that when the lesion is relatively small, the up-and-down movement of the needle tip results in more tearing of adjacent lung parenchyma.

Many authors have reported that greater lesion depth caused the pneumothorax rate to increase (5, 7, 42, 43). In our study pneumothorax occurred more frequently in deeper lesions (10 out of 14 lesions ≥60 mm in depth) as compared to (13 out of 40 lesions <60 mm in depth) (P <0.05). It would be reasonable to hypothesize that a longer needle path may have a greater chance to tear the pleura and normal lung tissue as patients breathe during the procedure (2). On the other hand, Yeow et al. (44) showed that subpleural lesions that were 2 cm from the pleural surface correlated with a higher pneumothorax rate than those farther from the pleura because shallow anchoring made dislodgement of the needle to the pleural cavity easy, causing air ingress. In the present study only 7 patients (13%) requiring thoracostomy tube placement & theses results are roughly comparable with that shown by Golfieri R. et. al. (22) which required thoracic drainage in 10% of cases.

In conclusions CT guided biopsy using automatic biopsy gun allowing a specific diagnosis for benign & probably malignant lesions. Diagnostic accuracy was not affected by the size and depth of the lesions. Deeper & small sized lesions have associated
with an increased rate of pneumothorax.

References
Detection of B1 gene of *Toxoplasma gondii* in blood of pregnant and abortive women infected with this parasite.

Souhaila Haiawi Mahmood PhD, Hayfa Hadi Hassani PhD, Khawla Hori Zghair PhD.

**Abstract**

**Background:** Primary maternal infection with toxoplasmosis during gestation and its transmission to the fetus continue to be the cause of tragic yet preventable disease in offspring.

**Objective:** This study was aimed to investigate the utility of nested PCR (nPCR) technique for detection recent infection with *Toxoplasma gondii* in blood of pregnant and abortive women.

**Methods:** One hundred twenty women were included in this study with a history of single or repeated abortion and thirty women with normal pregnancy were used as a control. Blood samples were tested for specific anti-*Toxoplasma* IgM and IgG antibodies by an enzyme-linked immunosorbent assay (ELISA) and detection of B1 gene of *T. gondii* by nPCR.

**Results:** The results indicated that 43.33% of abortive women were exposed positive for anti-*Toxoplasma* antibodies, 25.83% had IgG, and 13.33% had both IgM and IgG, and 56.55% had no antibodies. Subsequently, nested PCR analysis was used to detecting *T. gondii* DNA in blood of abortive women. It was found that 15.83% of abortive women exposed positive result for B1 gene of *T. gondii*, those abortive women involved 10.52% of them with IgM, 31.57% with IgG, and 26.31% with both IgM and IgG, and 31.57% of them had none anti-*Toxoplasma* antibodies.

**Conclusion:** It can be concluded that nPCR assay in blood has advantage in detection of recent and active toxoplasmosis.

**Key Words:** *Toxoplasma gondii*, nested PCR, toxoplasmosis.

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**Introduction**

*Toxoplasma gondii* is an obligate intracellular protozoan responsible for common parasitic infections throughout the world; it can invade and multiply in the nucleated cells of virtually all worm blooded animals including humans, mammals and birds, with members of the cat family being the only known hosts for the sexual stage of *T. gondii* infection (1).

In general, *T. gondii* infections are asymptomatic and self-limiting especially among healthy immunocompetent hosts; however the infection may cause severe complications in pregnant women and immunocompromised patients (2,3). Fetal toxoplasmosis, particularly in early pregnancy can cause miscarriage, stillbirth, and birth defect. Infected babies may not develop any disease, or they may experience serious damage to the brain and eyes, to degree depending on the gestational age (4).

Direct methods to demonstrate the presence of the parasite in tissues or body fluids, are more useful. Whereas, radiological findings, histology, tissue culture, or inoculation into mice are difficult, time consuming, or impractical assays.

Diagnosis of *T. gondii* is routinely based on serological tests. Polymerase chain reaction (PCR) is used for direct detection of parasites in the tissues or other fluids. Lamoril et al. (1996) (5) used this technique to diagnose toxoplasmosis...
in immunocompromised and pregnant women.

Recently, researchers (6, 7) illustrated that the serological diagnosis of recent infection in early pregnancy could be confirmed by a positive *Toxoplasma* specific PCR result from blood samples in the first half of pregnancy. However, the reliability of the PCR is a crucial technique to evaluate the prevalence of *Toxoplasma* reactivation when the detection of circulating DNA is the only clue to its reactivation (8, 9).

*B1* gene is highly conserved in all *T. gondii* strains (10, 11). Approximately 35 copies of this gene are presented in the *T. gondii* genome, but absent in the other mammalian cells (10).

Thus, the aim of the present study was to determination the relationship between serological and molecular methods for detection of *T. gondii* in blood of abortive women.

**Methods**

- **Study groups:**
  The cases were collected during the period from 1st April to the end of November 2008 in the central Health Laboratory in Baghdad. They were included 120 women, who had spontaneous abortion; whom referred with a physician report for TORCH tests to determine the final diagnosis of pregnancy loss. In addition, 30 healthy women with a history of a normal pregnancy were attended to the outpatient clinics for routine gynecologic checking was considered as control. Relevant information about the cases and the possible risk factors were recorded for each woman. Any woman undergoing therapy against the disease was excluded from the study.

- **Serological test**
  The sera of all cases were tested for the presence of specific IgM and IgG anti-*Toxoplasma* antibodies via ELISA kits (Biokit Diagnostics Company, Spain) according to the manufacture’s instructions.

- **Isolation of genomic DNA from whole blood:**
  DNA was extracted from the whole blood samples of the study groups using a commercial purification system (Wizard Genomic DNA purification kit, Promega, Madison, WI) following the manufacture’s instruction for DNA purification from blood. Purified DNA molecules were resuspended in 100 µl of DNA rehydration solution (TE buffer) (10 mM Tris-HCL, 1mM EDTA, pH=8) and stored at -80°C, after estimation of DNA concentration and purity, thereafter they were separated by agarose gel electrophoresis.

- **Amplification and detection of *T. gondii* DNA by Nested PCR (nPCR) assay.**
  According to Burge et al. (1989) (10) method, nested PCR was performed for all DNA samples to amplify a fragment of *B1* gene by two steps with different primer pairs as follows:
  
  **First round**
  The primers used in the first round correspond to nucleotides 694-714 and 887-868 with the sequences: 5'-GGAACTGCATCCGTTCATGAG-3’ and 5’-TCTTTAAAGCGTTCGTGGTC-3’ (Promega, USA) respectively. These primers used to amplify 193bp of *B1* gene. 3µl of DNA template were added to a final reaction volume equal to 25 µl consisting of 2.5 µl PCR buffer (10 mM Tris-HCL, 50 mM KCL), 1 µl MgCl2, 1 µl dNTPs mix, 2 µl for each primer, 0.25 µl Taq polymerase (5000 U/ml) and 13.25 µl of H2O (nuclease free). A negative control reaction in each experiment was set up containing all components of the reaction except
template DNA. The cycling condition for the first round were 94°C for 2 minutes, followed by 40 cycles at 94°C for 10 seconds, 57°C for 10 seconds and 72°C for 30 seconds and a final extension at 72°C for 5 minutes.

**Second round**

Nested reaction was performed to amplify 96 bp of B1 gene by using the primers which correspond to nucleotides 757-776 and 853-831 with the sequences 5’-TGCATAGGTTGCAGTCACTG-3’, 5’-GGCGACCAATCTGCGAATACACC-3’ respectively.

Three microliters of the first round products were used as a template for the second round PCR with the same components of the master mix in a total volume of 25 µl. Negative controls of sterile water were included in the nested reaction. The cycling condition for nPCR were 94°C for 2 minutes, followed by 40 cycles of 93°C for 10 seconds, 62.5°C for 10 seconds and 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. PCR product was analysed on 2% agarose gel at 5 v/cm for 2 hours and stained with ethidium bromide.

**Statistical analysis**

Data were analysed with chi-square and P value ≤ 0.05 was considered statistically significant.

**Results**

Figure (1) indicate that 43.3% of abortive women were significantly (P ≤ 0.05) exposed positive for anti-Toxoplasma antibodies (IgM and IgG) in comparison with normal pregnant women 20%.

The level of specific anti- Toxoplasma antibodies was determinate. Figure (2) shows that 4.16% of abortive women revealed a positive result for IgM in comparison with non IgM in sera of normal pregnancy. IgG antibodies were seen in 25.83% of abortive women in comparison with 20% IgG in normal groups. While, IgM and IgG levels were formed 13.33% and 0% in abortive women and normal pregnancy respectively. Whereas, no antibodies were detected in 56.55% of abortive women and 80% of normal group.

Besides the serological diagnosis of *T. gondii* nested PCR (nPCR) technique was used to confirm the infection with *T. gondii* by detection of *T. gondii* DNA in the blood of abortive women.

Toxoplasma gondii DNA was successfully extracted and analyzed by nPCR. It was noticed that 15.83% of abortive women had *T. gondii* DNA in their blood if compared with normal group who showed no toxoplasmosis (0%). Whereas, the negative nPCR analysis was significantly (P ≤ 0.05) formed 84.16% in abortive women when compared with control group (100%).

Moreover, the DNA products from the first and second round of nPCR analysis indicated that the abortive women had B1 gene of *T. gondii* with 193 bp and 96 bp in the first and second round of nPCR respectively (Figure 4).

On the other hand, 15.83% of abortive women who gave positive in molecular diagnosis were serologically detected. The results were significantly (P ≤ 0.05) showed that 10.52% of positive nPCR had IgM, 31.57% had IgG, 26.31% had IgM and IgG, and 31.57% had no antibodies in comparison with negative nPcR analysis (Figure 5).
B1 gene of Toxoplasma gondii in pregnant and abortive women ....Souhaila Haiawi Mahmood

Figure 1: Seroprevalence of anti-Toxoplasma antibodies by ELISA in the abortive women and normal pregnancy.

Figure 2: level of IgM and IgG antibodies detected by ELISA in abortive women and normal pregnancy.

Figure 3: nested PCR analysis in the abortive and normal pregnancy groups.
Figure 4: Amplification of \textit{B1} gene of \textit{Toxoplasma gondii} DNA from the blood of the infected abortive women. Lane-M, molecular weight marker (100 bp ladder), Lanes-NC negative control, Lanes 1-8 positive samples at 193bp in the first round of n-PCR, Lanes 9-16 positive samples at 96 bp in the second round of n-PCR. Running conditions: Agarose gel (2%), 5 v/cm for 2 hrs, stained with ethidium bromide.

Figure 5: Nested PCR analysis distributed according to anti-\textit{Toxoplasma} antibodies in abortive women.


Discussion

The most likely sources of human toxoplasmosis are ingestion of lightly cooked meat containing live *T. gondii* tissues cysts, ingestion of vegetable or fruits contaminated with oocysts derived from cat faeces that may be encountered in gardens. Toxoplasmosis is also recognized to be a water-borne zoonosis (12, 13).

High prevalence of *Toxoplasma* infection in women may be attributed to the socio-economic status, the unhygienic environment and low level of education in which they resides (14, 15).

It’s rare that a woman who got toxoplasmosis before getting pregnant will pass the infection to her fetus, because she will have built up immunity to the infection. It can occur through, if a pregnant woman who’s had a previous infection becomes immunocompromised and her infection is reactivated (4). In the current study the presence of signification rates of anti-*Toxoplasma* antibodies are considered as indirect evidence of the organism being the cause of bad obstetric history in women of reproductive age group. These results are in agreement with (16, 17).

Although serological testing has been one of the major diagnostic for toxoplasmosis, it has many limitations; it may fail to detect specific anti-*Toxoplasma* IgM or IgG during the active phase of infection, because these antibodies may not be produced until after several weeks of parasitemia. Furthermore, the test may fail to detect *T. gondii* infection in certain immunocompromised patients due to the fact that the titers of specific anti-*Toxoplasma* antibodies may fail to rise in this type of patient.

Indeed detection of *T. gondii* DNA using nPCR minimizes the problems faced when using serodiagnostic assays and facilitates diagnosis in difficult cases.

Therefore, the negative results obtained by both PCR and ELISA rule out an infection in abortive women.

The fact that could explain the proportion of 2.97% of abortive women that revealed negative PCR results and positive IgM is the probability to find patients with residual IgM detected during prolonged periods of time. Also false positive IgM tests results have been reported previously (18).

*Toxoplasma* DNA was found in 31.57% abortive women with positive IgG indicates chronic infection since it is knows that patients with latent toxoplasmosis present intermittent parasitemia with low parasite burden.

*Toxoplasma* DNA was detected in 31.57% seronegative abortive women; it could correspond to a very recent infection at the time of serological leading to an insufficient production of immunoglobulin not detected by serology, or other explanation that those patients are not able to produce specific antibodies, representing a state of immunodeficiency.

Molecular tests that could detect the presence of circulating parasites would be of extreme application in this scenario. A positive serological result is only indicative of infection, whereas direct detection of *T. gondii* in blood or other clinical samples categorically confirms the parasite presence leading to the diagnosis of primary, reactivated or chronic toxoplasmosis (19).

Acknowledgment

This work was financial supported by Research and Development unit in the Ministry of High Education and Scientific Research in Iraq.
B1 gene of Toxoplasma gondii in pregnant and abortive women ....Souhaila Haiawi Mahmood

References
The possible protective role of BCL-2 in recurrent abortion.

Haider Sabah Kadhim MBChB, MSc, PhD.

Abstract

Background: Recurrent abortion is a worldwide problem, with undefined causes. Apoptosis could play a major role in the process. Objective: Detect the expression of Bcl-2 protein at the materno-fetal interface in patients with recurrent pregnancy loss (RPL). Methods: Immunohistochemistry analysis of Bcl-2 protein using paraffin embedded sections of curet samples obtained from 40 women divided into three groups: 24 women with recurrent abortion, 10 women with abortion for the first time, and 6 women with induced abortion. Results: The mean value of the expression of Bcl-2 protein was (57.9±1.4), which is significantly higher than that of the second group (39±1.9), and the third group (47.5±2.4). Conclusion: High expression of Bcl-2 protein in women with recurrent abortion may have a protective role in preventing placental apoptosis that leads to failure of pregnancy. Keywords: Bcl-2, recurrent pregnancy loss (RPL).

Introduction

Apoptosis plays important roles in placentation and embryonic development (1). The cells undergoing apoptosis have characteristic structural changes in the nucleus and cytoplasm. Expressions of apoptotic regulatory molecules, such as Fas, Fas ligand, P53, and the proteins of Bcl-2 family, have been reported in human placenta (2, 3). Bcl-2 and P53 are two of the key players in the apoptotic signaling cascades. Bel-2, a proto-oncogene first discovered in human follicular lymphoma (4), is involved in the inhibition of apoptosis and the survival of a variety of cell types (5). Bcl-2 protein is located in the membranes of endoplasmic reticulum, nuclear envelope, and mitochondria. Over-expression of Bel-2 suppresses apoptosis by preventing the activation of caspases that carry out the process. P53 is well known as a tumor suppressor. It is a transcription factor that induces apoptosis mainly through inducing the expression of a batch of redox-related genes (6) and the down-regulating Bcl-2 (7).

The expression of Bcl-2 in human placenta has been studied (1, 8). However, the cellular distribution in the implantation site at early stage of pregnancy has not been reported. Apoptosis is the physiological process by which excess or dysfunctional cells are eliminated during development or normal tissue homeostasis, and nowhere is it more dramatic than in the reproductive system. For example, apoptosis occurs cyclically in human nonpregnant endometrium, throughout pregnancy in mouse (9) and human deciduas (1), human placenta (10), amnion epithelial cells (11, 12), rat cervical SMCs during pregnancy (13), and mammary glands during weaning (14, 15). At least two pathways activate apoptosis (16). The first is a mechanism that involves activation of a group of tumor necrosis...
factor receptors, such as Fas (ligand-receptor pathway). The second (exogenous stimulus pathway) is a parallel, mitochondria-dependent route activated by physiological stimuli (lack of growth factors, changes in hormonal environment, hypoxia, and hypoglycemia) and/or environmental stimuli (exposure to cytotoxic compounds, radiation, and viral infection) that is transmitted independently of surface membrane receptors and is governed by BCL2 family proteins (17).

Despite the remarkable nature of uterine growth during pregnancy, little information is available regarding the mechanisms that initiate and regulate this growth. Furthermore, no data precisely maps the contribution of hyperplasia, hypertrophy, and apoptosis to uterine growth from early to late pregnancy. The role of cell apoptosis in myometrial growth is largely unknown (17). Therefore, the goal of the present study was to gain insight regarding the role of Bcl-2 in early pregnancy, whether this role is protective or not.

**Patients and methods**

In this study, forty (40) patients were collected from Al-Kadhimya teaching hospital and Al-Elwiya teaching hospital, and then divided into three groups:

**Group A**: 24 pregnant ladies presented with abortion during the first trimester, with history of previous 3-6 consecutive first trimester abortions, with no medical diseases (like autoimmune diseases), nor family history of genetic diseases or uterine anomaly, also all of them were confirmed by laboratory tests to be negative for acute infection with CMV, rubella and toxoplasmosis.

**Group B**: 10 pregnant ladies presented with abortion during the first trimester and had at least three previous normal pregnancies with no previous abortion, and no history of any other medical illness.

**Group C**: 6 pregnant ladies with elective termination of pregnancy in the first trimester for maternal diseases (illness not related to apoptosis) under approved consent of two senior gynecologists and a physician. This group considered as control group. Curate samples of the maternal-fetal interface were taken from all these women at the end of evacuation curate operation then embedded in paraffin and confirmed by a pathologist, and then subjected for immunohistochemistry technique using DAKO cytomation detection kit (Denmark).

Detection of Bcl2 done by Immunohistochemistry, the procedure includes briefly; 5µm thickness tissue sections on positively charged slides were deparafinized in xylene then rehydrated in a series of ethanol concentrations. And then, 2-3 drops of peroxidase block were applied onto the tissue sections a step which is followed by application of the primary antibody (anti-Bcl2 protein) (DAKO Denmark), then the secondary antibody was added, followed by application of the hoarse reddish peroxidase (HRP) conjugate, and then its substrate DAB chromogen. Sections were counterstained with hematoxyline, sections dehydrated and mounted to be finally examined under the microscope. The expression of Bcl2 was measured by counting the number of positive decidual and trophoblastic cells, which gave a brown cytoplasmic staining under the light microscope (figure 1). The extent of the immunohistochemistry signal in the villi was determined in 10 fields (X100 magnification). In each field the total
number of villi were counted and the extent of nuclear staining of the cytotrophoblast and syncytiotrophoblast in a given villous was counted and simplified as percent, the percentage of positively stained villi was calculated for each case by taking the mean of the percentages of the positively stained villi in the 10 fields. The scorer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers.

Negative controls were obtained by omitting the monoclonal antibody (Anti-Bcl2) and using phosphate buffer saline to verify the signal specificity.

Statistical Analyses

The program SPSS was used to determine the difference in the expression of Bcl2 protein among the three groups. Values of $p < 0.001$ were considered as statistically significant. A correlation had been done by using Pearson correlation depending on frequency of abortion as group A more than 1 abortion, and group B one abortion.

Results

The results of immunohistochemistry for Bcl-2 in group A, those with recurrent abortion in 1st trimester, had a mean reading of 57.9% with standard deviation 1.4 see table (1). It is significantly higher than the mean results of group C the group considered as control that was 47.5% with standard deviation 2.411. The mean results of group B, those with one abortion only, was 39% with standard deviation of 1.94 and it was significantly lower than group C and A.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Bcl-2 Mean ± STD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>24</td>
<td>57.9 ± 1.416</td>
<td>0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>10</td>
<td>39.0 ± 1.944</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>6</td>
<td>47.5 ± 2.411</td>
<td></td>
</tr>
</tbody>
</table>

Group A: Recurrent abortion.
Group B: One abortion.
Group C: Control.

The correlation was done by using Pearson correlation depending on frequency of abortion as group A had more than 1 abortion and group B had only one abortion, and results showed a significant correlation as in table 2.

<table>
<thead>
<tr>
<th>Bcl2 expression</th>
<th>Frequency of Abortion</th>
<th>Pearson Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A N=24</td>
<td>&gt;1</td>
<td>0.602</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group B N=10</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Detection of Bcl-2 protein by immunohistochemistry in women with pregnancy loss. The expression was diffuse heterogeneous brown cytoplasmic staining involving the trophoblasts, both cyto- and synsytiotrophoblasts in the three groups of women but the percentage of villi involved is higher and more diffusely stained in the recurrent loss group. Light microscope magnification power (X400).

The results are summarized in figure 2 as it shows mean of the 3 groups.

Figure 2: The mean percentage of Bcl-2 expression in the three groups under study.
Discussion

In the present study, we found a significant increase in the expression of BCL2 protein at recurrent abortion as it may be a protective measure to counteract a probable increase of apoptotic proteins. It is reasonable to speculate that BCL2 proteins could be protecting myometrial SMCs from premature termination of the proliferative phase of uterine growth by preventing the development of true apoptosis in myometrial tissue, which is incompatible with the function of this organ. In group A, this was of no benefit as pregnancy loss took place recurrently. In the 2nd group, namely B, the level of Bcl-2 expression was low as the process of loss took place for the 1st time, hence the level was not as high as in the recurrent group. In the group C the condition represents the normal level of expression as it is an elective termination and no role of any of the pro- or anti-apoptotic proteins in it.

Despite the clear transformation that takes place in the myometrium and the possible importance of the different phases of uterine growth throughout pregnancy and labor, little is known about the causes and mechanisms of this transition from myometrial hyperplasia to hypertrophy. We believe that such transformation is triggered by the process called uterine conversion, an adaptive mechanism to accommodate the growing fetus (19). During early pregnancy, the shape of the fetus is spherical, and maternal blood supply is abundant. The process of fetal growth continues until a critical time, specific to each species, when the conceptus reaches a maximum spherical radius and the uterine tissue is stretched. Tension is so great that it creates ischemia, resulting in circulatory stasis, which is detrimental to maternal blood flow. The conversion of embryo shape from a sphere to a cylinder, which requires only a few hours, causes a release of uterine tissue tension and reestablishment of the maternal blood supply throughout the uterus (20). Notably, late gestation is accompanied by rapid growth of the fetus, and it also is marked by a second period of mechanical stretch, which ends at parturition. It is a reasonable hypothesis that uterine conversion can cause transient ischemia in a stretched myometrium that can lead to a hypoxic response in this tissue and activation of the intrinsic apoptotic-pathway (13).

In conclusion, our data may demonstrate that the myometrium undergoes an increase in the anti-apoptotic proteins from early pregnancy to prevent pregnancy loss. These changes could decide the fate of the pregnancy.

References

Down's syndrome a new maternal Iraqi risk.

Mohammed Sh. Tawfik MBChB; PhD.

Abstract

**Background:** The occurrence of Down's syndrome is usually associated with advancing maternal age and increased parity.

**Objectives:** This study explores the risk factor associated with the occurrence of Down's syndrome for different age groups.

**Methods:** Blood sample from suspected Down's syndrome babies were prepared for chromosomal preparation. A hundred and fifty four blood samples from patients suspected of being Down's syndrome baby came with variable features which raise the suspicion of being Down's.

**Results:** The study groups were 65 male and 89 female. Seventy seven (77) were the first member of the family, 68 were born for young mothers with an age between 15-34 years. Only nine were born for old mothers aged between 35-43 years. Seventy three were members of the family other than the first. Twenty six were born for mothers aged between 35-43 years, while the other 47 were born for mothers aged between 15-34 years. Chromosomal study for 4 cases revealed normal chromosomal findings.

**Conclusion:** This study may show that young Iraqi mothers (under 35 year) carry high risk of having down's baby both in multi as well as primigravida.

**Key words:** Down's syndrome, trisomy, primigravida, multigravida, Chromosome.

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Introduction

Down's syndrome was diagnosed by Lejeune et. al (1) in 1959 and since then it continued to be the first and the most common of the clinical chromosomal syndromes known in human.

A lot of studies and researches (2) explore the nature of the disease, its clinical feature, the various types of the disease and the risk factors. Most of the studies explore high risk group with advancing maternal age (3-5) and increase parity as well as with very young age mothers below 18. In this study we explore the occurrence of the large number of Down's syndrome for young mothers (mostly primigravida as well as multigravida young mother).

Patients and methods

A total of one hundred and fifty four baby aged 5 days- 5 years were referred from different pediatricians and pediatric hospitals to Dr. M. Tawfik private cytogenetic lab in Baghdad for the period 1997 -2003.

Full clinical examination was carried out for every part to explore the features raise the suspicion of being Down's patient such as: Depressed nasal bridge, Epicanthic fold, Slanting eyes, Simian's line, Separated big toe, Flat face, High arched palate, Congenital heart disease, Protruded tongue, Hypotonic muscle and Broad hand.

The maternal age of every patient at the time of her pregnancy for the affected baby was recorded, as well as the gravity whether she was primi- or multigravida.

The procedure of chromosomal analysis and Down's syndrome diagnosis was done as follow: 2ml of blood were aspirated from each patient aseptically using heparin coated
Down's syndrome a new maternal Iraqi risk...Mohammed Sh. Tawfik

A total of one hundred and fifty four Iraqi babies came with variable features which raise the suspicion of being Down's. Chromosomal study were performed for all the cases, Trisomy 21 was the result of 150 babies while only 4 babies gave normal 46 chromosomal karyotype (Figure 1). 65 were males and 89 were females (Figure 2). From the one hundred fifty patients 77 were the first member of the family while 73 were members of the family other than first (Figure 3). Sixty eight of those who were the first member of the family were born for younger mothers with an age between 15-34, only 9 were born for old mothers with an age between 35-43 years (Figure 4). The patients who were other than first in the family can be divided into: 47 were born for young aged mothers between ages 15-34 years, while the rest 26 were born for old age mothers 35-43 years (Figure 5).

**Figure 1:** The normal and abnormal distribution of the children included in this study.
Male: 65 (42%)  
Female: 89 (58%)  

Figure 2: The sex distribution of the children included in this study.

The first member: 77 (51%)  
Other than 1st: 73 (49%)  

Figure 3: The order in family of the Down's patient included in this study.

Children born for Young Maternal Age: 68 (88%)  
Children born for Old Maternal Age: 9 (12%)  

Figure 4: The distribution of the patients who were the first member of the family and the maternal age included in this study.
Figure 5: The distribution of the patients who were other than first in the family and the maternal age included in this study.

Table 1 showing the age and sex distribution of the children in which the females were more than the males and mostly at the age of 1 month - 11 months.

Table 1: The age & sex distribution of the children included in this study

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day - 6 day</td>
<td>10</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>7 day - 29 day</td>
<td>22</td>
<td>20</td>
<td>42</td>
</tr>
<tr>
<td>1 month - 11 month</td>
<td>15</td>
<td>32</td>
<td>47</td>
</tr>
<tr>
<td>1 year - 5 year</td>
<td>18</td>
<td>23</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>89</td>
<td>154</td>
</tr>
</tbody>
</table>

Table 2 showing the age distribution of the young mothers (under 35) in relation to the number of the Down's baby and their order in family in which we obtain that the most affected age was between 25-29 years old which were (35 years) woman from a total of 115 (the young mothers only), also this table showing that the first member of the family were more than the other than first (primi were mostly affected). In general the young primi women were mostly affected.

Table 2: The age distribution of the young maternal age in relation to the number of the Down's baby & their order in family included in this study.

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>Number of Down's</th>
<th>Order in family</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First</td>
</tr>
<tr>
<td>15-19</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>20-24</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>25-29</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>30-34</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>68</td>
</tr>
</tbody>
</table>
Table 3 showing the age distribution of the old mothers (above 35 years) in relation to the number of the Down's baby and their order in family. In which we obtain that the old mothers were less than the young woman from a total of 150, also this table showing that the old multigravida are more than the old primi mothers.

Table 3: The age distribution of the old maternal age in relation to the number of the Down's baby & their order in family included in this study.

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>Number of Down's</th>
<th>Order in family</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First</td>
</tr>
<tr>
<td>35-39</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>40-44</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>9</td>
</tr>
</tbody>
</table>

Discussion

Down's syndrome as a disease can affect any pregnancy. The disease increases in both extremes of age, it is more for young mothers below 18 years old and those over 35 (2). As it is documented in most studies all over the world (6). All the patients were Down's of trisomy type and this indicate that young mothers are at more risk of having abnormal baby in our country than outside. We run our risk which differs from others (7), both young primi and multigravida give birth to down's baby.

The data obtained from this study shows that young Iraqi mothers (under 35 year) carry high risk of having Down's baby both in multi as well as primigravida. This reflects that Iraqi people have exposed to different risks of not only Down's but could be other diseases due to the exposure during the last 30 years because of wars and sanction.

References
2- Little BB, Ramin SM., Cambrige BB ,Schcider NR, Cohen DS,Snell LM, risk of chromosomal abnormalities ; with emphasis an live born off spring of young mother Hum – Genet.1959 Nov; 57; 1178-85
Clinical Characteristic and In –Patients Mortality among 100 Patients with Heart Failure Admitted to Ibn Seena Central Hospital, Mukalla, Hadhramout- Yemen.

Ahmed Ali Bahaj MBPP, MB (Medicine).

Abstract

Background: The rate of heart failure in Hadhramout is steadily increasing.

Objective: To determine the clinical characteristic and the predictors of in –hospital mortality of heart failure patients.

Method: The first 100 consecutive patients with heart failure admitted to Ibn Seena central hospital in Mukalla for whom clinical history, physical examination and the basic investigations( including hemogram, blood sugar, chest X-Ray. Renal function test, serum cholesterol, electrocardiogram and echocardiogram) were performed during the period from October 2007 to June 2008.

Results: In this study, we found male were more affected than female (65% versus 35%). The mean age of the patients was 57 ±12.1 years for males and 59 ±12.2 years for females. The most common underlying causes in all the patients were IHD in 52% followed by AHT 25%. IHD was more in males and AHT was more in females, while valvular heart diseases was the cause of HF in only 7%. The most common associated co-morbidities were renal dysfunction, anemia, dyslipidemia, diabetes mellitus and stroke in 43%, 41%, 35%, 25% and 12% respectively. Patients of heart failure with impaired left ventricular function were 67.8%. And it was more in males than in females (52.7% &15.1%). The most common presenting symptoms was dyspnea of different classes in all of the patients and the most common sign was cardiomegaly in 89% of the patients followed by pulmonary crackles in 82%. And the most common arrhythmia were ventricular ectopic in 28% and atrial fibrillation in 21%. During the admission period 9 out of 100 patients died. The most common underlying causes were IHD, AHT and DCM and the most common co-morbidities of death were elder age of the patients, male sex, anemia, renal failure, DM, Stroke, impaired systolic ventricular function, and class IV NYHA dyspnea.

Conclusion: This study revealed that HF is common in our community and it is recommended that early detection and management of the underlying etiology and associated co-morbidities could reduce the morbidity and mortality of HF

Key words: Heart failure, clinical presentation, predictors, outcome. Mukalla, Yemen

Introduction

Heart failure (HF) is the end stage of many cardiovascular disorders (1). It is a global serious health problem especially in people aged above 65 years (2). It is estimated that nearly 6.5 million people in Europe, five million people in the USA, and 2.4 million people in Japan suffer from HF. Overall; it appears that HF affects 1–3% of the general population and 10% of the elderly.

In developing countries its incidence is steadily increasing (3).

HF is a broad clinical syndrome with many different underlying etiologies. In some studies ischemic heart disease (IHD) was the commonest cause and in other studies arterial hypertension (AHT) was the most common (4, 5). However, the etiology of HF varies with different population, in developed countries it is usually due to IHD and AHT. In developing countries viral and bacterial infection as streptococcal infection and its role in the etiology of rheumatic heart disease play an important role (6).
Recently, evidence-based pharmacological and device therapies indicated the decrease in heart failure symptoms, hospitalization, mortality and improve quality of life of patients with HF, still many patients treated with these regimens often remain burdened by dyspnea and fatigue, diminished exercise tolerance, reduced quality of life, recurrent hospitalizations and early mortality, and if underlying cause is not corrected, more than half of the patients with HF will die within 1-4 years depending on the severity of the disease (7).

Several indicators were developed for prediction of risk and prognosis and to determine the guideline for management of patients with HF. The three most predictors were elder age of the patient, low left ventricular ejection fraction, and presence of diabetes mellitus. Other risk predictors include high class dyspnea of New York Heart Association (NYHA), male sex, low diastolic blood pressure (8).

In Yemen, few studies were conducted regarding cases of HF and in Hadhramout this is the first study aimed to identify the clinical characteristics and mortality among patients admitted to Ibn Seena central hospital in Al-Mukalla city in Hadhramout governorate, Yemen, during the period from October 2007 to June 2008.

**Patients & methods**

**Study design:** This is a hospital-based, prospective study.

**Study population:** 100 patients who met the modified Framingham criteria of diagnosis of HF (9) were admitted to the Intensive care unit (ICU) and medical wards from the period from October 2007 and their investigations were completed including (CBC, blood sugar, blood urea, serum creatinine, serum cholesterol, Chest X-ray, echocardiography, electrocardiogram). Readmitted patients were included in their first admission.

**Settings:** This study was conducted in Ibn Seena central hospital in Mukalla. It is the central hospital in Hadhramout governorate. However in this hospital there is no specialized cardiac unit (CCU) and all the patients of heart diseases are admitted in ICU or general medical wards.

**Exclusion criteria:** All the patient who due to any reason dropped from follow up before complete his investigations (died, transferred, discharged)

**Data collection:** Data were collected by direct interviewing of the patients or their relatives and from the medical records of the patients using closed-end questionnaire.

**Ethical consideration:** The purpose and the method of the study were explained to the patients and their relatives and oral consent was given.

**Study variables:**

*Ischemic heart disease:* was considered if the patient is diagnosed before as IHD, presence of ischemic ECG changes or having wall motion abnormality in echocardiography.

*Arterial hypertension:* was considered if patient has history of hypertension and on anti-hypertension treatment or if there is more than three readings of systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg during admission period (10).

*Valvular heart disease:* was considered if there is clinical and echocardiography evidence of valvular disease.

*Dilated cardiomyopathy (DCM):* was diagnosed when echocardiography shows heart chambers enlargement and diastolic dysfunction and no evidence of other underlying cause.

*Anemia:* was considered when hemoglobin concentration was < 11 g/dl.

*Diabetes Mellitus:* was considered when the patient was diagnosed as
Mortality among Patients with Heart Failure in Hadhramout- Yemen…Ahmed Ali Bahaj

diabetic case and on regular treatment or repeated fasting blood sugar was more than 126mg/dl during admission period. 
**Renal insufficiency:** was considered if serum creatinine >2mg/dl.  
**Hypercholesterolemia:** was considered if a patient with fasting total cholesterol more than 240mg/dl according to guidelines of the third report of the American national cholesterol education program (11).  
**Stroke:** referred to clinical syndrome of vascular origin manifested by rapid development of signs of focal or global disturbance of cerebral function, lasting for more than 24hours.  
**Cardiomegaly:** in chest X-ray is defined when C/t ratio is more than > 0.5.  
**Data analysis:**  
Was performed using SPSS-15 statistical program. P value < 0.05 was considered statically significant. Qualitative data were tested by Chi-square test, while quantitative data were tested by student t-test

**Results**

Table 1: Distribution of the patients according to age &sex

<table>
<thead>
<tr>
<th>Age group in Years</th>
<th>male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>&lt;35</td>
<td>3</td>
<td>4.6</td>
<td>1</td>
</tr>
<tr>
<td>35-44</td>
<td>5</td>
<td>7.7</td>
<td>2</td>
</tr>
<tr>
<td>45-54</td>
<td>9</td>
<td>13.9</td>
<td>4</td>
</tr>
<tr>
<td>55-64</td>
<td>22</td>
<td>33.8</td>
<td>8</td>
</tr>
<tr>
<td>&gt;65</td>
<td>26</td>
<td>40.0</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>100</td>
<td>35</td>
</tr>
</tbody>
</table>

• Percentage is calculated in relation to total in the columns.  
• Mean age of the patients 58 ±12.3 years.  
(male 57.6 ±12.1 years & Female 59±12.2 years)  
(P = 0.08)

Table 1 shows that 65 of the admitted patients with HF during this period were male and 35 were female, with male to female ratio of 1.86 : 1. The frequency of HF cases increasing with age, the most common age group were > 65 years representing 46% of the patients followed by age group of 55-64 years in 30% of cases. This table also shows that in the age group of more than 65 years, 26 were males and 20 were female indicating that male were affected more than female (56.5% &43.5%) respectively.

Our study showed that, the most common symptom among our patients of HF was dyspnea in 100% of the patients, 63% had dyspnea class IV according to NYHA classification and 37% had class III. Fatigability, cough and palpitation were present in 81%, 65%, and 20% respectively, while 18% had chest pain and/or tightness.

Regarding physical findings, cardiomegaly was the most common in 89%, followed by crackles of different types, basal, mid-chest and diffuse was found in (82%) of the patients (82%), while other findings like peripheral or generalized edema was present in 62%, Gallop rhythm in 39%, high jugular venous pressure in 31% hepatomegaly in 20% and ascitis in10%. There is a significant number of patients of HF had arrhythmias, and the most common arrhythmias were ventricular ectopic beats in 28% and Atrial fibrillation in 21%.
Table 2: Distribution of the underlying causes according to age & sex

<table>
<thead>
<tr>
<th>Underlying cause</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Ischemic heart disease (IHD)</td>
<td>37</td>
<td>71.2</td>
<td>15</td>
</tr>
<tr>
<td>Arterial hypertension (AHT)</td>
<td>13</td>
<td>52</td>
<td>12</td>
</tr>
<tr>
<td>Idiopathic dilated cardiomyopathy (IDCM)</td>
<td>7</td>
<td>63.6</td>
<td>4</td>
</tr>
<tr>
<td>Valvular heart disease (VHD)</td>
<td>4</td>
<td>57.2</td>
<td>3</td>
</tr>
<tr>
<td>Corpulmonale</td>
<td>4</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>100</td>
<td>35</td>
</tr>
</tbody>
</table>

*Percentage were calculated in relation to total of the column while the rest to total of rows. The difference between causes of HF in both sexes was statically not significant (P = 0.22).

Table 2 shows that the most common underlying cause of HF in all the patients was IHD in 52% followed by AHT in 25%, IDCM (11%), VHD (7%) and pulmonary heart disease in 5%. Distribution of the underlying causes of HF among male and female showed that male have higher prevalence in all the causes. The difference between causes of HF regarding sexes was not statically significant (P value > 0.05).

Regarding left ventricle function, 63 out of 93 patients had HF with impaired ventricular function (ILVSF) and 30 patients had preserved left ventricular systolic function (PLVSF) representing 67.8% & 32.2% respectively, that was statically significant (P = < 0.05 and $X^2 = 18.69$). In 7 of our patients, HF was due to valvular heart disease (VHD), so LVSF was not calculated because most of the valvular lesion out of aortic valve lesions; it interferes with measurement of LVSF.

Regarding sex distribution, male patients with ILVSF was higher than in female (80.9% & 19.1% respectively). Whereas PLVSF was higher in female patients than male (56.7% & 43.3% respectively).

Table 3: Distribution of associated co-morbidities among patients with HF (n = 100)

<table>
<thead>
<tr>
<th>Associated co-morbidity</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal dysfunction</td>
<td>43</td>
<td>43%</td>
</tr>
<tr>
<td>Anemia</td>
<td>41</td>
<td>41%</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>35</td>
<td>35%</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>25</td>
<td>25%</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>12</td>
<td>12%</td>
</tr>
</tbody>
</table>

Table 3 shows that the most common co-morbidities among our patients with HF were renal impairment (43%) followed by anemia (41%), Dyslipidemia (35%), DM (25%) and ischemic stroke (12%).

Table 4: Distribution of dead patients (n = 9) according to age & sex:

<table>
<thead>
<tr>
<th>Sex</th>
<th>45-54</th>
<th>55-64</th>
<th>&gt;65</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>11.1%</td>
<td>1</td>
<td>11.1%</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>11.1%</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>11.1%</td>
<td>2</td>
<td>22.2%</td>
</tr>
</tbody>
</table>

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Table 4 shows that 9 of 100 patients died during their stay in the hospital and 91 patients discharged alive, giving overall mortality rate of 9%. The dead patients were 7 out 65 male (10.8%) and 2 out 35 female (5.7%). Among dead patients, males were more than female representing 77.8% and 22.2% respectively. Most of the dead patients (66.7%) were in the elder age group (> 65 years).

Table 5: distribution of the dead patients (n =9) according to clinical features underlying cause, co-morbidities and left ventricular systolic function

<table>
<thead>
<tr>
<th>Clinical features:</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA class:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>88.9</td>
</tr>
<tr>
<td>Edema:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalized</td>
<td>4</td>
<td>44.4</td>
</tr>
<tr>
<td>Peripheral</td>
<td>8</td>
<td>88.9</td>
</tr>
<tr>
<td>Gallop rhythm</td>
<td>5</td>
<td>55.6</td>
</tr>
<tr>
<td>Underlying cause:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHD</td>
<td>6</td>
<td>66.7</td>
</tr>
<tr>
<td>AHT</td>
<td>2</td>
<td>22.2</td>
</tr>
<tr>
<td>DCM</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>Co-morbidity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>6</td>
<td>66.7</td>
</tr>
<tr>
<td>Renal impairment</td>
<td>5</td>
<td>55.6</td>
</tr>
<tr>
<td>DM</td>
<td>4</td>
<td>44.4</td>
</tr>
<tr>
<td>Stroke</td>
<td>3</td>
<td>33.3</td>
</tr>
<tr>
<td>LVSF:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired</td>
<td>7</td>
<td>77.8</td>
</tr>
<tr>
<td>Preserved</td>
<td>2</td>
<td>22.2</td>
</tr>
</tbody>
</table>

Table 5 shows that the main symptoms in the dead patients was dyspnea class IV in 88.9% and the main signs were peripheral/ general edema in all the patients followed by gallop rhythm in 55.6%. The most common underlying cause of HF among of dead patients was IHD representing 66.7% followed by AHT in 22.2% and DCM in 11.1%.

Regarding co- morbidities, anemia was the commonest findings in 66.7% followed by renal impairment, DM and stroke in 55.6%, 44.4% and 33.3% respectively. In regard to left ventricular systolic function, it was impaired in 77.8% and preserved in 22.2%.

**Discussion**

This is a hospital – based study and does not reflect the epidemiology of HF in the community because there are many patients with heart diseases don't come to our hospital and are treated in other private and governmental hospitals.

In our studied HF patients, males were more than female (65% versus...
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35%) and this was consistent with several local, regional and international studies. In a study conducted by Ba-Ishen in Aden/ Yemen, he found that 70% of HF were male and 30% were female (12). and in other study from a neighboring country, Oman, A.K. Agarwal found that 62.3% of HF were males and 38.7% were females (13). In Framingham study and other studies from Singapore and Malaysia indicated that HF is lower in females than in males (14-16). The preponderance of HF in male may be attribute to high incidence of IHD in male and the effect of estrogen protection against cardiovascular diseases in female.

In regards to age of the patients, the mean age of the patients in our study was 58 ± 12.3 years and this was similar to that is studies from Yemen (12); Pakistan (17) and Nigeria (18) which it was 57.1 ± 13.3 years, 61 years and 57.6 ± 15.9 years respectively, but less than those from Japan where it was 70.0 ± 12 years (19).

Our study showed that most of the patients with HF were the age groups of 55-64 years and > 65 years and this support the high prevalence of this condition in the older population. With advance of age, the sex ratio is narrowed and in the age of more than 65 years in our study 56.5% were male indicating that male were affected more than female and this close to study by Stromberg et al on 2003 (20) and this may be due to the increasing number of postmenopausal females with AHT and IHD.

The underlying cause of HF in our study was IHD (52%) AHT (25%), DCM (11%), VHD (7%) and Corpulmonale (5%). This was close to the study of Agarwal et al from Oman who reported IHD (51.7%), AHT(24.9%), DCM (8.3%), VHD (4.0%) and Corpulmonale (4.3%) (13), Framingham study and other studies by Chong A, et al in Malaysia and Leong KTG, et al in Singapore which showed that IHD was the most common underlying cause of HF followed by AHT (14-16).

In our study, the patients had HF with impaired systolic function (HF-ISF) was more than HF with preserved systolic function (HF-PSF) with 67.8% and 30 % respectively. This was consistent with study from Aden/Yemen by Al-khameri et al on 2005 who reported HF-ISF in 66.4% versus 33.6% of HF-PSF (17) and a study by Ibrahim et al on 2003 from Egypt who found HF- ISF in 66% versus 34% had HF- PSF (22).

Our study showed that HF-ISF was more in male than in female (52.7% versus 15.1%) and this may be attributed to high prevalence of IHD in males than in females. On the other hand HF-PSF was more in females (19.3% & 12.9% respectively) and this was consistent with other studies and this presumed to be due to difference in ventricular remodeling in response to pressure overload and post-infarction remodeling between males and females(23,24).

The most common associated co-morbidity in our patients of HF was renal impairment which was found among 43% and this was the same as in the study by Smith, et al. (2006) who found that renal impairment was present in 43% of his patients (25) .Renal impairment in the patients with HF is due to hemodynamic changes and renovascular abnormalities occurred secondary to heart failure.

Anemia was found in 41% of our patients, and this is consistent with other study who found that anemia is a common associated co-morbidity in patients with HF (26). Presence of anemia in HF patients is because of hemodilution due to fluid retention, depressed erythropoisis due renal dysfunction, anorexia and malnutrition and the old age of most of the patients.
In this study Diabetes mellitus (DM) was present in 25% of our patients and this was similarly observed by Berry et al who reported DM in 24% of his HF cases. DM is known to accelerate coronary atherosclerosis and increase of myocardial infarction, besides it is now recognized as independent risk factor for development of HF by inducing specific cardiomyopathy characterized by myocardial hypertrophy, fibrosis and microangiopathy. Also this study revealed that stroke is associated in 12% of our cases, all of them were of ischemic type and this was more than 5.3% reported by barsheshet A et al.

Regarding the clinical manifestations, Dyspnea was the most distressing symptoms. NYHA class III dyspnea was present in 37% and 63% was having dyspnea class IV and the same was reported was in some other studies.

Fatigue was the second most common symptoms; it was present in 81% of the case and this close to 83% and 84% reported by other studies. Signs of congestion (rales, peripheral edema, high jugular pressure, hepatomegaly and Ascitis) was present in 82%, 62%, 31%, 20% and 10% respectively and this almost the same as reported by others.

During the study period 9 out of 100 patients died in the hospital and this represents overall mortality 9% which is higher than 6.4% reported by Baishen from Aden/Yemen and close to that by Lee, et al who reported mortality rate of 8.6%. Most of the dead were males (77%) and 22.2% were females and the mortality rate was higher among older age patients, 66.7% of them were aged 65 years or more, while 22.2% were in the age group of 55-64 years and this could be related to complex interaction between advancing age and high frequency of non cardiac co-morbidity in elderly than in younger patients. The most common underlying cause of death in all patients was IHD which was present in 66.7%.

It is well known that the presence of non cardiac co-morbid conditions in HF increase hospitalization and death. The most common co-morbidity was anemia which was present in 66.7% of dead patients. It is documented that when it is present in patients with HF lead to poor prognosis and increase mortality. In the current study, 5 of 9 dead HF patients had evidence of renal dysfunction which is reported that it is associated with adverse outcome in HF and this may be due to increased retention of fluid and salts, effect of HF on peripheral vessels and because of limited use of life-saving intervention in HF like use of ACE inhibitors which were stated to induce renal impairment.

4 of 9 dead patients (44.4%) detected to have DM which is reported to influence survival and a predictor of adverse outcome in HF with ischemic etiology.

Also in this study 7 of 9 dead patients had impairment systolic function representing 77.8% and only 2 patients (22.2%) were with preserved systolic function. McCarthy, et al. identified impaired LSVF as a predictor of increase mortality in HF. In DIAMOND study survival decreased with decreasing LVSF. This can be attributed to many reasons, patients with impaired LVSF are predominantly male, the majority with ischemic etiology and significantly associated with renal impairment. All of these factors carry poor prognosis with HF.

In our patients, dyspnea class IV NYHA, advanced edema and presence of gallop rhythm were present in 88.9%, 66.7% and 55.6% respectively, this was mentioned in several studies.
to be associated with increasing mortality among patients of HF \(^{(12,39)}\).

**Conclusion & Recommendations:**

This hospital-based study of heart failure patients in central Mukalla hospital revealed that male patients were affected more than females and the frequency increased with advancing age. IHD was the most common cause of heart disease in HF-ISF while AHT was the other significant etiology in HF-PSF. The most common presenting symptom was dyspnea and the most common sign was cardiomegaly followed by pulmonary crackles and edema. The most common co-morbidities were renal dysfunction; anemia and DM. Mortality rate during admission period was 9% and was associated with old age, male sex, impaired systolic function, high HYHA class, anemia, DM and renal impairment. It is recommended that increase the awareness of the patient's early detection and management of the underlying causes and morbidities associated with HF could decrease the burden of morbidity and mortality of HF.

**Acknowledgement**

Deep thanks and gratitude to my students, Maysoon, Wisam, Yaser, Saeed, Ali and others who helped me in collection of the data and to Mr. Ahmed Muhdar from medical statistical unit in Ibn Seena Hospital for his keen cooperation.

**References**

17. Vim S, Ahmadi H. Factors influencing the length of hospital staying the patients with
Treatment of congenital undescended scapula with Woodward operation, the functional and cosmetic outcomes

Abd Ali Muhsin FICMS.

Abstract
Background: Although congenitally undescended scapula (Sprengel’s deformity) reported to be rare, yet many families still seek medical help for their children. Cases with congenitally undescended scapula were collected, evaluated, and the majority subjected to surgical treatment with Woodward operation.

Objective: Is to demonstrate the functional in addition to cosmetic improvements obtained with Woodward procedure used for Sprengel’s deformity.

Methods: Between December 2007 and November 2009, fifteen patients with Sprengel deformity were evaluated (all have unilateral deformity) and treated surgically with Woodward procedure. The study included 9 males and 6 females, their age range from 5 to 11 years; right side is affected in 8 patients and left side in 7 patients. The preoperative grade of the deformity (according to Cavendish classification) and the maximum shoulder abduction were reported to assess severity and to be compared with the postoperative results.

Results: All the patients get improvement in scapular elevation by about 2 grades and the increase in the maximum combined abduction was 20.33° in average. Two patients developed transient brachial plexus palsy. An omo-vertebral connection was found in 10 cases, in 3 cases the connection was bony, another 3 cases was cartilaginous, and 4 cases were fibrous.

Conclusions: children with grade 2 and more and especially if abduction is much affected need to be treated with surgery, Woodward operation seems to be effective in handling both the cosmetic concern and the functional impairment.

Keywords: Sprengel deformity, undescended scapula, Woodward.

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Introduction

Congenital Undescended Scapula is also widely known as Sprengel’s deformity, it is uncommon congenital anomaly. In this deformity, the scapula is elevated, hypoplastic, and usually somewhat adducted. Almost without exception, there are associated anomalies of the cervical and thoracic spine, as well as the ribs. Attention was first directed to this deformity by Eulenberg, in Germany, in 1863. In 1891, Sprengel, in Germany again, drew attention to this deformity by describing another 4 cases. Kolliker, who also described 4 cases in 1891, gave the condition its eponym, Sprengel deformity. Recently a historical study on a mummified fetus, from ancient Egyptian civilization believed to be the daughter of king Tutankhamun, have shown to have scoliosis, spina bifida, and Sprengel deformity.

Frequency

Although Sprengel deformity is the most common congenital malformation of the shoulder girdle and some refers to prevalence of <1 per 10,000, the exact prevalence is not known. The male to female ratio is variable with different studies but most of these refer to equal involvement, however some attribute the slight female predominance to their parents’ cosmetic concerns. Right and left sides affection is of...
variable figures in different articles, and bilateral involvement occurs in 10% to 30% of the cases\(^{[16-18]}\).

**Etiology**

The exact causation is not known but some hypotheses are available like: The exogenous theory, the bleb theory, and Subclavian artery supply disruption sequence hypothesis. The condition is sporadic. Rarely, it may run in families (autosomal dominant pattern of inheritance)\(^{[8, 9, 18, 21, 22]}\).

**Presentation**

The hallmarks of the Sprengel deformity are shoulder asymmetry and restriction of shoulder abduction. Clinically, the affected scapula is usually elevated 2-10 cm and is adducted, and its inferior pole is medially rotated. Due to this rotation, the glenoid faces inferiorly. A prominence in the suprascapular region is characteristic due to the upwardly rotated superomedial angle of the scapula, which causes the ipsilateral side of the neck to appear fuller and its normal contour to be lost. The scapula is hypoplastic, and the length of the vertebral border is decreased. Occasionally, some anterior bending of the supraspinous portion is present. The condition may sometimes be bilateral, in which case, although it is cosmetically much more acceptable, functionally, it is more disabling\(^{[23, 24]}\).

**Classification**

More than one classification has been introduced for Sprengel deformity but the Cavendish classification is the most currently used. Sprengel deformity can be classified as follows:

- **Grade 1:** very mild. The shoulder joints are level and the deformity is invisible, or almost so, when the patient is dressed.
- **Grade 2:** mild. The shoulder joints are level or almost level but the deformity is visible when the patient is dressed, as a lump in the web of the neck.
- **Grade 3:** moderate. The shoulder joint is elevated 2 to 5 centimetres. The deformity is easily visible.
- **Grade 4:** severe. The shoulder is much elevated, so that the superior angle of the scapula is near the occiput, with or without neck webbing or brevicollis\(^{[9]}\).

**Patients and methods**

Between December 2007 and November 2009, fifteen cases of congenital undescended scapula treated with Woodward procedure for correction of the deformity, 2 cases from Al-Kadhemiyyah Teaching Hospital and another 13 cases from Al-Wasity Teaching Hospital for Reconstructive Surgeries. Table 1 show the distribution of the patients and their age, sex, and side of involvement.
Table 1: Distribution of the patients and their age, sex, and side of involvement. The numbering of the cases was according to the alphabetical order of their first names. M for male, F for female, R for right, and L for left.

<table>
<thead>
<tr>
<th>Case №</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Side</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>F</td>
<td>L</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>F</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>M</td>
<td>L</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>M</td>
<td>L</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>M</td>
<td>L</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>F</td>
<td>R</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>M</td>
<td>R</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>F</td>
<td>L</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>M</td>
<td>L</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>M</td>
<td>R</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>M</td>
<td>R</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>M</td>
<td>R</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>F</td>
<td>L</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>F</td>
<td>R</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>M</td>
<td>R</td>
</tr>
</tbody>
</table>

Preoperative evaluation
Preoperatively the patients were evaluated by detailed history and examination, taking in consideration the family history of similar problem and other skeletal anomalies and any problems during gestation. Examination of the shoulders and both upper limbs, spines, and lower limbs were made giving attention for scapular bony prominences, scapular winging, scoliosis, chest asymmetry, or any associated morphological abnormalities. All the patients were examined for the grade of the deformity. Movements of the shoulders were examined as well as of elbow, wrist, and hand; looking for range of motion especially of the scapulo-thoracic motion, whether the scapula is anchored to the spine or not. Full neurological examination of both upper limbs was performed, comparing the sensation and muscle power of both sides. All of the patients subjected to anteroposterior view of both shoulders or Chest X-Ray, some needs radiograph of the cervical, thoracic, and lumbar spines. Most of the patients have associated anomalies (Table 2).
Table 2: The associated deformities recorded in the 15 patients.

<table>
<thead>
<tr>
<th>Associated deformity</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rib anomalies</td>
<td>11</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>5</td>
</tr>
<tr>
<td>Toricollis</td>
<td>3</td>
</tr>
<tr>
<td>Spina bifida</td>
<td>2</td>
</tr>
<tr>
<td>Cervical spine fusion</td>
<td>2</td>
</tr>
<tr>
<td>Accessory nipple</td>
<td>1</td>
</tr>
<tr>
<td>Umbilical hernia</td>
<td>1</td>
</tr>
</tbody>
</table>

Results

All the patients have improvement of their shoulder deformities ranging from partial to complete correction in regard to shoulder level or bony prominence with an average of improvement by 2 grades, (Table 3). Most of the patients get significant improvement in abduction by an average of 20.33 degrees (0 – 45 degrees). Patients who had the omo-vertebral connection and especially if it is not fibrous were associated with much abduction limitation. This connection found to be 66.67 % of our patients, the bony and cartilaginous bar was 40 % (Table 4). We asked the parents to put their satisfaction regarding the improvement of their children after surgery in one of 4 levels (excellent, good, fair, and poor), (Table 5). The excellent and good satisfaction was 86, 67%; fair satisfaction was 13.33%, and no poor contentment.

Regarding the complications, one patient (case № 13) developed loss of active abduction and loss of sensation over C5 dermatome in the involved side with preservation of sensation and motor activity of other regions of the same limb. This neurological deficit resolved spontaneously and completely within 6 weeks. Another case (case № 4) developed complete brachial plexus palsy, necessitate urgent clavicular morcellization (morcellation) next day. The patient have full recovery in 7 months during which vigorous physiotherapy was underwent, the resolution started proximally and proceeded distally. Nine patients have had wide scars with no keloid or hypertrophy. One case developed multiple discrete superficial infections treated with oral cefalexin and changing dressings. Some of the patients included in the study are shown below with their postoperative results (Figure 1, 2, 3, and 4).
Table 3: The preoperative and postoperative values for elevation grade and maximum abduction (and the obtained gain) in addition to the average of these values.

<table>
<thead>
<tr>
<th>Case №</th>
<th>Omo-vertebral connection</th>
<th>Grade of undescending</th>
<th>Abduction (in degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>1</td>
<td>Fibrous</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Fibrous</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>cartilaginous</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Fibrous</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Fibrous</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Cartilaginous</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>cartilaginous</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Bony</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Bony</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>Bony</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Bony</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>Bony</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Bony</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>Fibrous</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>Fibrous</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Average</td>
<td>-</td>
<td>3.13</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Table 4: The percentages of all omo-vertebral connections and of their types.

<table>
<thead>
<tr>
<th>Type of connection</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bony</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Cartilaginous</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Fibrous</td>
<td>4</td>
<td>26.66</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>66.66</td>
</tr>
</tbody>
</table>

Table 5: table of the parent satisfaction in 4 levels with no poor contentment revealed.

<table>
<thead>
<tr>
<th>Level of satisfaction</th>
<th>Number of parents</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>4</td>
<td>26.67</td>
</tr>
<tr>
<td>Good</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>Fair</td>
<td>2</td>
<td>13.33</td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 1: A, a preoperative X-ray of a patient with grade 3 deformity, note the level of the left scapula and the superomedial portion of the scapula before operation. B, 14 months postoperatively with correction of the elevation and rotation of scapula. Note also the left clavicle after morcellation (in B).

Figure 2: Case with left side elevated scapula reaching up to the occiput with bony prominence; B. postoperative correction of the shoulder level, note the widening of the scar in its upper half; C. abduction of left arm is limited just preoperatively; D. the postoperative improvement is obvious.

Figure 3: A and B showing the pre and postoperative photographs respectively with correction of the elevation, the dotted lines drown on the patient refer to the level of the scapular spines which are parallel.
Discussion

Most of the studied cases were from Baghdad. Although all references refer to the deformity of being rare, most of them did not mention its prevalence in community. Because the diagnosis is clinical in the first step and the unawareness of the mild cases and because of the many and may be more attracting associated anomalies, its exact prevalence is difficult to be estimated. Many articles refer to different figures of predominant side of affection and of gender mostly affected. Most said that right to left ratio is 1 – 2:1(13, 16, 21) and Aydinli et al said it is 1:2, also most said that the females are affected more. This study showed nearly equal side involvement (R: L ratio 1.1:1) and more affection of males with a ratio 1.5:1. We have not found any family history of same problem even in the 2nd and 3rd degree relatives. Also no gynaecological problems are reported for the mothers of the patients during gestation. The Woodward procedure has been praised in having the ability to provide better both cosmetic and functional results (13, 25, 26). Woodward operation is usually straight forward (taking in consideration the regional anatomy) in cases of minimal complexity. In many severely affected cases it could be difficult and dangerous, especially when ribs are deficient and the field of surgery is separated from the pleura by thin sheath of delicate muscles. When there are deficient or deformed ribs, the scapula is the only protecting bony structure of the posterior upper chest and it is seated in a relatively deep bed making its lower down positioning more difficult, more bulging outward, and less congruent with the new rib cage articulation, and so less smooth and smaller range of scapula – thoracic movement. Much lowering of the scapula is not necessary associated with good function or good appearance as Cavendish noticed, so we did not rely on the mere radiological lowering of the scapula in assessing cosmetic appearance postoperatively and we think that Cavendish classification is still valid. Woodward, Grogan et al, Carson et al, and Dendane et al report a gain in abduction postoperatively ranging from 32.2 to 40 degrees. This study showed...
20.3 degrees gain in abduction. This may be attributed in part to the short follow up period in our study. Even in achieving good scapular descent (clinical and radiological) there may be little improvement in function due to associated anomalies or due to less remodeling reserve in older children. The best results obtained in patients aged 5 – 7 years while patients above 10 have less improvement. The patients who are in greater expectancy of obtaining more functional improvement are those with more severe affection and those with omo-vertebral bars. Obviously the follow up period of 1 to 24 months is not sufficient to evaluate long term results and remote complications. During the follow up period no worsening of the obtained improvement and no recurrences were occurred. The reviewed available articles with long time follow up showed no recurrence or worsening over long follow up. Robinson et al and Carson et al advice routine clavicular osteotomy while Grogan et al not recommend routine osteotomy. Our data cannot either support routine clavicular osteotomy nor predict vulnerable patient to neurovascular compromise. Good closure technique of the skin using non absorbable synthetic sutures can greatly affect the scar formation and even better results were obtained by subcuticular skin closure. Apart from the case with complete brachial plexus palsy and the case of transient focal neurological deficit, the complications in general are within the expected range.

In conclusions:
1. The primary indication for correction is cosmetic concerns and the surgery is justified further for the more severe cases.
2. The improvement in abduction can be expected in and especially needed for the more severely affected children.
3. Woodward procedure seems to be especially effective in obtaining both the cosmetic and functional gain at the same time.
4. The procedure needs good deal of attention in the handling of the delicate muscles and tissues of the child with good experience and anatomical knowledge.
5. The family satisfaction about the operative results can be encouraged further by reporting the grade and function preoperatively and comparing them with the post operative reports especially by photographs. Accordingly we recommend the following:
1. Longer period of follow-up and more patients are needed to be included in such a study to assess the results and whether relapse or other complications can occur later in life.
2. We advice to operate patients before the age of 10 years.
3. Our study used Woodward operation for Sprengel deformity but other available procedures need to be evaluated by further studies to compare results and reveal the best procedure.
4. Most of the patients with congenital undescended scapula have other congenital malformations and especially skeletal malformations which may need further assessment and management.
5. It seems to be beneficial to found pediatric orthopaedic centers to offer greater care and better evaluation for congenital and developmental disorders including data collection, management, and conducting researches.

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قائمة المحتويات
المقالات

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المستشارين المعتمدين للمجلة العراقية للعلوم الطبية

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أهمية استعمال محلول بيروكسيد الهاييدروجين موضعياً على الجرح بعد استئصال الثدي لمرضى سرطان الثدي في تقليل نسبة رجوع السرطان موضعياً.

الخلاصة

خلفية الدراسة:
يروكسيد الهاييدروجين يتكون بصورة طبيعية داخل بعض خلايا الجسم الخلايا السرطانية بصورة عامة تستهلك أوكسجين أقل من الخلايا الطبيعية لذلك فهي تنمو بصورة أفضل في محيط لاهاوي وإن وجود الأوكسجين حولها يسبب تأخير نموها أو حتى قتلها.

تقييم أهمية استعمال محلول بيروكسيد الهاييدروجين موضعياً على الجرح بعد استئصال الثدي لمرضى سرطان الثدي في تقليل نسبة رجوع السرطان موضعياً، وهل يتسبب ذلك بآية مضادات للمرضى.

المرضى وطريقة العمل، Алексدت 100 مريضة مصابة بسرطان الثدي للاحصت وقد أجريت لهن عملية استئصال الثدي. قسمت المريضات إلى مجموعتين متساويتين وتملائمتين من حيث الفئة العمرية ومرحلة المرض ونوعه التنسيجي ونوع العملية الجراحية. عضمت المريضات في المجموعة الأولى بمحول بيروكسيد الهاييدروجين على الجرح بعد استئصال الثدي، وأيضاً كل نقاط النزيف بينما لم يستعمل محلول بيروكسيد الهاييدروجين في المجموعة الثانية.

تمتابة جميع المريضات لمدة 15 سنة.

النتائج، لا يوجد أثر لرجع السرطان موضعياً في مرضى المجموعة الأولى بينما نسبة رجوع السرطان في المجموعة الثانية هي 14%.

نسبة المرضى الذين عاشوا خمسة سنوات وعشرة سنوات وخمسة عشر سنة في المجموعة الأولى هي 60%، و14% و6% على التوالي. ولم تلاحظ مضادات على المرضى من جراء استعمال محلول بيروكسيد الهاييدروجين على الجرح على الرغم من متابعتهم بصورة دورية لمدة 15 سنة بينما نسبة المرضى الذين عاشوا خمسة سنوات في المجموعة الثانية هي 24% ولم يش بقية المرضى لأكثر من 7 سنوات.

الاستنتاج، استعمال محلول بيروكسيد الهاييدروجين موضعياً على الجرح بعد استئصال الثدي لمرضى سرطان الثدي مؤثر في تقليل نسبة رجوع السرطان موضعياً وتقليل نسبة الوفيات، و لايسبب ذلك في آية مضادات.

مفتاح الكلمات: سرطان الثدي، بيروكسيد الهاييدروجين، رجوع السرطان موضعياً.

ه生存 الجراحة [ كلية الطب – جامعة النهرين]

المجلة العراقية للعلوم الطبية 2010 م المجلد 8 العدد 3 ص 3-13.
التفاوتات النمائية في الأعضاء التناسلية لدى الفئران الذكور المرتبطة بالتناول مستخلص عرق السوس من قبل الأمهات قبل واثناء الحمل

باسم محمد الجبوري، نسرين خزعل فليح

الخلاصة

خلفية الدراسة:

Glycrrhiza glabra

هدء الدراسة:

 وكذلك وفقاً للأمثل، وقبل واثناء الحمل، عند 30 طرخ ملحوظة تم قياس الوزن ووزن الجسم بعد ما تغذى اثناء الحمل.

الطريقه العملية:

امتدت لسلسلة اختبارات على (60) فارما يبلغ عدد الانتان عند 30، تم قياس كل مجموعة إلى ثلاث مجموعات متساوية

النتائج:

ازدادت ملحوظة في حجم الولادات لجميع الحيوانات التجريبية.

المستنتاج:

وقد يعود ذلك إلى حصول زيادة مضاعفة في اعداد ووزن الولادات. وذلك نجوم مبكر في مناسيل الذكور والإناث.

مفتاح الكلمات:

Glycrrhiza glabra، الخصوبة، الحمل، عرق السوس، مناسيل الذكور والإناث.
ظهور بعض من معلمات التنشيط على سطح الخلايا اللمفاوية للدم المحيطي والمعزولة من مريضات الولادات المبكرة المتعة الجينية مجمعة الأسباب

نماذج عبد المنعم 1، مما محمد البياتي 2، فريدة حماه الدين 3

الخلايا

خلفية الدراسة
تتضمن الولادات المبكرة السبب الأساسي للوفيات قبل الولادة. تهدف الدراسة، تحديد مسؤولية الولادة المبكرة على التعبير عن بعض معلمات التنشيط على سطح الخلايا اللمفاوية للدم المحيطي.

المريض ومراقبة العمل
أجريت الدراسة على 30 مريضة تعاني من الولادة المبكرة (المجموعة A) بالإضافة إلى 30 إمرأة حامل بنفس رطبة الحمل (مجموعة B). أخذت عينات دم من كل النساء وفصلت الخلايا اللمفاوية بعدفا صبغتها بالخلايا بالجسم المضاد وحيدة النسلية الخاصة CD45RA, CD45RO إضافة إلى المعلم CD11b البالغ. أوضحت النتائج أن هناك زيادة معنوية بنسبة الخلايا التي تحمل المعلم CD45RA في المجموعة (A) وانخفاض في نسبة التعبير عن المعلم CD45RO وCD11b في تلك المجموعة.

النتائج
في الولادات المبكرة هناك قلة في نشاط الخلايا اللمفاوية بدلالة التعبير عن بعض معلمات التنشيط على سطح الخلايا اللمفاوية.

الاستنتاج
النتائج، الولادات المبكرة، معلمات التنشيط،

مفتاح الكلمات
ولادة المبكرة، معلمات التنشيط، CD45RA, CD45RO, CD11b.

1 قسم الأحياء المجمعة [كلية الطب، جامعة النهرين]
2 قسم النساء والتوليد [كلية الطب، جامعة النهرين]
3 قسم النساء والتوليد [مستشفى الكاظمية التعليمي]

المجلة العراقية للعلوم الطبية 2010 م المجلد 8 العدد 3 ص 20-24
تقييم تكوين الأوعية الدموية في متلازمة تصلب نخاع العظم التلقائي المصبوغ بواسطة CD34 وCD31

الملخص

الخلفية الدراسة: لقد أُدرِكت الملاحظات الأخيرة للصحة الحيوية لتكون الأوعية الدموية داخل الورم وتأثيرها المحتمل على تطور الورم. لقد تبين أن زيادة في تكوين أوّية نّخاع العظم موجودة في مختلف أورام امراض الدم، بضمن ذلك متلازمة أورام نّخاع العظم المتعددة.

الهدف: تقييم تكوين الأوعية الدموية في متلازمة تصلب النخاع الابتدائي.

المواضيع والأهداف: هذه الدراسة الاستقصائية أجريت على 31 قلبًا لبارايفين مثبتة بالفورمالين لمرضى مصابون بتصلب نّخاع العظم التلقائي، بالإضافة إلى مجموعة سيطرة من 10 حالات منتظمة العمر بدون علّة مرضية في نّخاع العظم. لكلّ حالة تمّ اخذ 3 أقسام ذوّ سمك 5μm لكلّ قسم. صبغ الأول بواسطة صبغة هيماتوكسيلين ويوسين، والأحمر صبّاً منعاً باستخدام الأجسام المضادة ضدّ CD34 وCD31 على التوالي.

نتيجة الدراسة: لقد درست جميع المعلومات السريرية بخصوص عمر وجنس المريض، مقياس الدّم (قّيّمة الهرمون)، عدد كرّات الدم البيضاء، عدد الصفيحات الدموية، بالإضافة إلى تحلل عينة نّخاع العظم. لقد قُدرّت كثافة الأوعية الدموية في نّخاع العظم بواسطة تحليل الصورة الإلكترون. تمّ تحسين النّسب المضبوط بالهيماتوكسيلين واليوسين بواسطة المجهر الضوئي لتقييم كمية الخلايا المكونة للنسيج الدموي، وتأتيّ نّخاع العظم بالإضافة إلى عدد الخلايا المولدة للصفائح الدموية.

النتائج: كشفت هذه الدراسة أنّ هناك زيادة في كثافة الأوعية الدموية في حالات تصلب نّخاع العظم التلقائي باستخدام الحاسب، وواصلت باقي الأجسام المضادة CD34 (4.47±9.64) % (p<0.05). هذه الزيادة كانت هامة جدًا مقارنةً إلى CD34 (0.91±1.57) % على الوالد CD31 وCD34 (0.74±1.57) % باستخدام (p<0.05) (0.05).

لقد أظهرت هذه الدراسة أيضاً بأنّ زيادة في تكوين الأوعية ارتبطت ارتباطاً إيجابياً مع نتيجة Dupreiz، بينما كانت مستفيدة عن تأثير نّخاع الدم، بينما كانت هناك زيادة هامة في الخلايا المكونة للنسيج الدموي. ومع ذلك، فإنّ هذه الدراسة أنّ هناك زيادة هامة في الخلايا المكونة للنسيج الدموي في الحالات القائمة (5.5% ± 8.31) % بالنسبة لحالات تصلب نّخاع العظم التلقائي (60.96 ± 50.93) %، بينما كانت هناك زيادة هامة في تأثير نقي (2mm /n 19.78±43.6 13.53±36.5 (15.93 % ± 17.50) % بالنسبة إلى الحالات القائمة، 17.50 % على (2mm /n 5.03 ± 8.34±19.7) (p<0.05) (0.5).
الملخصات العربية

معتاج العلامة، تصلب نخاع العظام التلقائي، تكون الأوعية الدموية.

فرع البيانولوجي والطب العدلي [حقيقية الطبيب، جامعة النهرين]

مستشفى الكاظمية التعليمي

المجلة العراقية للعلوم الطبية

أخذ الخزعات النسيجية من العقد الرئوية المنفردة باستخدام بندقية الخزعة الآلية بمساعدة المفجري

المجلة العراقية للعلوم الطبية 2010 م المجلد 8 العدد 3 ص 25-33

أخذ الخزعات النسيجية من العقد الرئوية المنفردة باستخدام بندقية الخزعة الآلية بمساعدة المفجري.
الملخصات العربية

المؤلف:

محمد عبد غاطس، علاء علي صالح، ريد عمر أحمد، حسام فوزي، حسن.

العنوان:

الدراسة، اخذ العينات النسجية باستخدام الأبرة النحيفة بمساعدة المفراس أظهرت من التقنيات المقبولة جدا، وقليلة التداخل لتشخيص العديد من اللافات الموجودة داخل الصدر، والتي ليست سهلة الوصول بهدوء بتنظير القصبات، وتعتبر عموماً إجراء أمر مع تمارس محدود ونافذ نادر جداً. هذه التقنية ذات مستوى عالي من الدقة التشخيصية ولها نسبة تعقيدات منخفضة جداً.

الهدف:

كانت تشير الدراسة فيما يتعلق بالدقة التشخيصية ونسبة حدوث الاستروال الهوائي اخذ الخرازات النسجية من العدد الرئوية المفرادة باستخدام بندقية الخصعة الآلية بمساعدة المفراس.

المراجع:

المرسوم: 49 من 54 حالة (96%)، أعطت نماذج كافية من الأفراد للتشخيص النسجي.

المرضى: الخمسة الذين لم يعطوا نماذج كافية للتشخيص تم اعادة العملية عليهم بعد اسبوع. 41 مريض (76%) شخّصوا حالات خبيثة (الدقة التشخيصية: 87.8%). 13 مريض (24%) شخّصوا حالات حميدة (الدقة التشخيصية العامة: 89% من اصل 54). الدقة التشخيصية لم تختلف فيما يتعلق بحجم الأفراد وعمقها من جدار الصدر. الدقيقة الدقيقة كان في 25 من العدد 29 لكل من 20 مليمتراً (86%) و 23 من العدد 25 أكثر من أو مساوياً 20 مليمتراً (92%). بنفس الطريقة التشخيص الدقيقة كان في 36 (90%) من العدد 40 كل من 60 مليمتراً و 12 (85.7) من العدد 14 أعمق من أو مساوياً 60 مليمتراً. الاستروال الهوائي حدث في 23 مريض (42%).

الاستنتاجات:

الدراسة، اخذ الخرازات النسجية من العدد الرئوية المفرادة باستخدام بندقية الخصعة الآلية بمساعدة المفراس تعطي تفاصيل دقيقة في اللافات الحمية والمتصلة الخبيثة. الدقة التشخيصية لم تتأثر بحجم وعمق الأفراد. الآفات العمق وذات الحجم أصغر إرتباطاً بتراúa ل معدل حدوث الاستروال.

المجلة العراقية للعلوم الطبية
الملخصات العربية

شوخ العربية

المجلة العراقية للعلوم الطبية

7

فرع الجراحة [كلية الطب. جامعة النهرين]

قسم التصوير الشعاعي . مستشفى المظفري التعليمي

1

2

التحري عن الجين B1 في حمض النيميا المواء والليوميادمواد التفاعلية للمعتدلي الصناعي

المجلة العراقية للعلوم الطبية

2010 م المجلد 8 العدد 3 ص 34-41

التحري عن الجين B1 في حمض النيميا المواء والليوميادمواد التفاعلية للمعتدلي الصناعي
الملخص

العنوان: دور المحتمل لبروتين BCL-2 في الحماية من الإجهاض المتكرر.

المؤلفين: عمهلة محمود، نورا حاذق، رفعت يوسف.

الملخص:

الجهاز التنميمي: في الدراسات السابقة، تم التعرف على دور بروتين BCL-2 في الحماية من الإجهاض المتكرر.

الغرض من الدراسة: هذه الدراسة هدفها التأكد من وجود بروتين BCL-2 في المرضى الذين خففت تطور الإجهاض.

الرياضيات: تضمنت الدراسة 200 حالة، من نساء حاملات إجهاض، تم استخدام فحص ELISA لقياس مستويات البروتين BCL-2 في الدم.

النتائج: أظهرت النتائج أن 40% من النساء الحاملات كانت لديهن حساسية مرتفعة للبروتين BCL-2.

الاستنتاج: يمكننا الاستنتاج من النتائج أن البروتين BCL-2 يلعب دورًا حيويًا في الحماية من الإجهاض المتكرر.

البحث العلمي، المجلة العراقية للعلوم الطبية، المجلد 8، العدد 3، ص 42-48.
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явية الدراسة، الإجهاض المتكرر عند النساء الحوامل يعتبر معضلة طبية في كل إنحاء العالم.

تحديد الدراسة: تعتمد التصنيع الكيميائي النسيجي المناعي لبروتين BCL-2 في حالات فقدان الحمل المتكرر.

المرضى وطريقة الدراسة: استخدمت تقنية التصنيع الكيميائي المناعي لبروتين BCL-2 في عينات الجرب الرحمي والتي تم الحصول عليها من 40 امرأة تم تقسيمهم إلى ثلاث مجموعات المجموعة A وتشمل 24 امرأة حصل لها فقدان حمل متكرر، المجموعة B وتشمل 10 نساء حصل لهن إجهاض طفلي للمرة الأولى، والمجموعة C وتشمل ستة نساء أجريت عملية إنهاء حمل علاج.

النتائج: أظهرت النتائج أن مستويات التعبير الموضوعي لبروتين (BCL-2) في حالات فقدان الحمل المتكرر ذات زيادة ملحوظة مقارنة مع المجموعات الثانية والثالثة.

المستنتاج: إن ارتفاع نسبة التعبير الموضوعي لبروتين BCL-2 قد يلعب دور مهم في الحماية من موت الخلايا المبرمج في الرحم مما يؤدي إلى فشل الحمل.

مفتاح الكلمات: BCL-2، الإجهاض، بروتين BCL-2، الإجهاض المتكرر

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الخلاصة

خلفية الدراسة:
إن متلازمة داون محتملة الحدوث في الأطفال الذين يولدون بنساء حوامل متقدمات بالسن أو نساء لديهن عدد كثير من الولادات.

المريض وطريقة العمل:
استخدم الفحص الكروموسومي لمجموع 154 طفل أخيلوا من أطباء اختصاص كونهم يحملون صفات تدل على احتمالية كونهم مصابين بمتلازمة داون.

النتائج:
تضاعف الكروموسوم رقم 21 كانت النتيجة التي ظهرت لـ 150 طفل بينما أظهرت النتائج وجود 4 حالات فقط من الأطفال المحالين يحملون 46 كروموسوم بواقع (3 حالات يحملون xx)بينما حالات الرابعة أظهرت وجود (46xy).

المصادر:
بينت الدراسة بأن تقدم العمر للنساء الحوامل قد لا يلعب دور بزيادة احتمالية حدوث متلازمة داون.

مفتاح الكلمات:
متلازمة داون, فحص كروموسوم.

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الخلاصة
خلفية الدراسة: ازداد معدل حالات مضاعف فشل القلب في حضروموت، التي يتم إدخالها إلى مستشفى ابن سيناء المركزي بالملعلا/ حضروموت باضطراد.

مقدمة الدراسة: تهدف هذه الدراسة إلى تحديد الخصائص الإكلينيكية للمرضي وتحديد أسباب توفر الوقائع بينهم.

طريقة الدراسة: وقد شملت هذه الدراسة مادة مريض بفشل القلب ممن تم إدخالهم للمستشفى خلال الفترة من أكتوبر 2007 م إلى يوليو 2008 م من معرفة التاريخ المرضا لهم وتم فحصهم سريرياً. وأجريت لهم الفحوصات الضرورية والتي شملت فحص الهيموغلوبين والسكر والكولسترول ووظيفة الكلى والأشعة السينية للصدر وتطبيقات القلب والأكوب. ولم تشتمل الدراسة المرضى الذين لم تعمل أو تكلمت لهم الفحوصات بسبب الوفاة أو التحويل إلى مستشفى آخر أو أي أسباب أخرى.

النتائج: وأظهرت الدراسة ارتفاع معدل المصابين بنبوط القلب بين الذكور أكثر من النساء بنسبة 65% إلى 35% وكان متوسط العمر للذكور 57.1 ± 12.1 سنة بينما كان 59 ± 12.2 سنة وكان أهم أسباب الوفاة القلبية في 52% متبوعاً بارتفاع ضغط الدم في 25% وكان مسنة. وارتفاع ضغط الدم الأكبر بين النساء بينما كانت أعراض الدم الساميات القلبية في السبب في 7% . كانت أعلى ألوان المرض الإكلينيية في القلب اكتمال الكلى، فقر الدم، السكري، اختلال الكولسترول والصدمة الدماغية في 43%، 41%، 25% و12%. وكان المرضى المصابين بالضعف الانقباطي لي بطين الأرض للقلب الأكبر في الذكور من النساء (52.7%) مقابل 15.1%. وكانت صعوبة التنفس أكثر الأعراض بنسبة 100% وكان ألوان المرض الإكلينيية في عضلة القلب في 98% ووجود الشخيص النروية في 82%. خلال فترة الدراسة توفي 9 من 100 مريض أثناء وجودهم في المستشفى وكان أكثر الأسباب المؤدية للوفاة هي الوفاة القلبية، فوق الدم، اضطراب الكلى، ضعف الوظيفة الانقباطية للقلب ووجود صعوبة في التنفس من الدرجة الرابعة بقياس جمعية القلب في نيويورك.

الاستنتاج: خصصت الدراسة إلى أن حالة نبوط القلب في مجتمعنا في ازدياد وان الانتشار المكسيك المальный إلى فشل القلب واستخدام العلاج المناسب للأمراض المسببة والمضادة يمكن أن يساعد في التقليل من معدلات المرض الإكلينيية والوقائع.

مفتاح الكلمات: عطق القلب الباطني}
النتائج الوظيفية والمظهرية لمعالجة عاهة عدم هبوط لوح الكتف الخلفية بطريقة "وود ورد" الجراحية

عبد علي محسن

الخلاصة

خلفية الدراسة

قد لا يتطابق الجانبين الأيمن والأيسر للانسان مطابقة تامة، لكن عندما يكون أحد كتفي الطفل وخاصة لوح الكتف (أو اللوحين معاً) أعلى من الطبيعي وفي مستوى الرقبة فهذا قد يكون عاهة عدم هبوط لوح الكتف الخلفية (عاهة "سيربنجل") وبخاصة حينما تكون حركة الكتف متحركة بشكل غير طبيعي.

وبرغم أن هذه العاهة غير شائعة إلا أن هناك من الأطفال وذويهم من يعاني منها ويطلبون العلاج لها. مجموعة من هؤلاء الأطفال قمت حالاتهم وأكثرهم تمت معالجتهم بطريقة "وود ورد" الجراحية ومتابعتهم في مستشفى الكاظمية التعليمي ومستشفى الوسطي التعليمي للجراحات التقويمية.
مقدمة الدراسة: إظهار النتائج والتحسن على المستويين الوظيفي والمظهري المتحصلتين من العلاج بطريقة "ورد وورد" الجراحية للعاج "سبرنجل".

طريقة العمل: تم تقسيم (20) طفل مصابين بالعاج، منهم (3) علوجو تحضورياً و (17) أطفالاً رفعت عليهم إجراء عملية طفليهما، وقد خضع (15) طفلًا المتبقين لجراحة "ورد وورد"، وكلهم يعانون من العاج لفترة طويلة وقد تم تدريبهم وبدأت العلاج الجراحي ببداية 40٪ من الذكور و75٪ من الإناث. كان من الأطفال 45٪ مصابين بعاج يمنى ومثل كلاً 40٪ و25٪ كانت العاج بالجانب الأيسر، وقد نظرت علاج الأطفال بين 5 - 11 عاماً. وقد تم توثيق شدة أو درجة العاج حسب تصنيف "كافندش" وقيست اقصى مباعد للطرف العلوي قبل وبعد العملية لتحديد شدة تأثر الطفل والمقارنة بعد العملية لتبين النتائج.

النتائج: كل الأطفال تحسن مظهرهم وانخفضت شدة ارتفاع لوح الكتف بدرجتين تقريبًا حسب تصنيف "كافندش" وكانت الزيادة في مدى حركة المباعدة للكتف من صفر إلى حد 45 درجة (بمعدل 20 درجة). إذ أنهم من المرضى حصل لهم شلل مؤقت في الطرف العضدي بعد العملية. إذ أنهم كان شلل الظهرة جزئياً والآخر كلياً. العديد من المرضى كانت نبضة جرح العملية عريضة نسبياً لكن لميحصل لأي منهم ندبة تورم. وجد في (16, 80٪) (10 أطفال) أن هناك ارتباط بين الفقرات و لوح الكتف.

قيمت قراءة ذوي الأطفال عن نتائج العملية الجراحية وكان الورضا ممتازاً وأيضاً في 14 حالة (7, 6٪) وعقولاً في 3 حالات (0, 0٪) ولم يكن منهم من هم مسئول.

الاستنتاج: إن العملية الجراحية هي الطريقة العلاجية الفضلي لعلاج العاهات من الدرجة الثانية وصعودًا وبالذات حينما يكون مدى مباعدة الطرف العلوي محدودًا كثيراً (خاصة حينما يكون لوح الكتف مرتبطاً بالفقرات عظم أو عضو). قد تتبين أن طريقة "ورد وورد" الجراحية فعالة في إصلاح الخلل الوظيفي والمظهري معاً. ويمكن التقليل من المضاعفات بعد العملية بإتباع الأساليب الجراحية الدقيقة.

مختصر الكلمات: طفلي، "ورد وورد"، لوح الكتف غير الناهض، "ورد وورد".

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