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Drugs Use for Diarrhoea In Children At Home.

Luay Al-Nouri* , Numan Nafie Hameed* , Abdul-Salam Dawood, Ahmad Hatem****

ABSTRACT:

BACKGROUND :

Children's acute diarrhea is an extensive global problem .It has increased in Iraq seventeen fold after wars and economic sanctions. Proper management is important in saving lives and prevention of complications. We wanted to see what drugs were used, how often and how it compared with oral rehydration use.

METHOD:

The mothers of 175 children who presented with diarrhoea of acute onset and admitted to the Children Welfare Hospital, Baghdad, over a period of 8 months were interviewed.

RESULTS:

Apart from 39 children, who had parenteral infections, two had celiac disease and one accidentally ingested a laxative, the others were diagnosed as gastroenteritis at hospital. Of the 102 children diagnosed as gastroenteritis before admission, 29(28%) had their stools microscopically examined and a parasite was found in 12. Seventy- three (75%) of those who did not have stools examined, and (59%) of those who had no parasites demonstrated in stool were given antibacterials (metronidazole, gentamicin, amoxycilin, +/- cloxacilin, co-trimoxazole). Many had antiemetics and anti spasmodics. Oral rehydration solution (ORS- WHO) was given to (46%) of patients.

CONCLUSION:

Seventy-Four (73%) of children with gastroenteritis were given anti bacterials at home and less than half had ORS.

KEY WORDS: Drugs, Diarrhoea , Children .

INTRODUCTION :

Diarrhea remains the leading cause of death of children in the world (1). In developing countries children may have an average of 2.2 – 3.3 episodes per year, with an annual death of 3-4 millions (1) (2). This means that (1- 4%) of the billion episodes of diarrhoea worldwide each year are fatal. In developed countries like USA and Canada, the death from diarrhoea is estimated to be 325-425 a year (1). An estimated hospital admission for diarrhoea in USA is almost 50 per 10 000 children every year (1), and approximately (9 %) of all hospitalization of children under 5 years of age was because of diarrhoea. This makes a direct loss of 2 billion dollars a year .In Iraq after the first Gulf War and during economic sanctions, the death from diarrhoea increased from 1950 in 1989 to 21000 in 1998 i.e. 14 fold increase. Among the reasons were unsafe water supply, defective sanitation and malnutrition (3, 4). In Children Welfare Hospital (Al-Mansoor), Baghdad, in the year 2004, there were 2430 children admitted for diarrhoea out of the total admission of 10515 i.e. (23%). The lowest admission was during January (15 `/.) . (5) Drugs are commonly used for diarrhoea often with no measures for rehydration. We wanted to see how common drugs were used at home for diarrhoea, what type of drugs and how often oral rehydration fluids were given.

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PATIENTS AND METHOD :

The mothers of children admitted for acute diarrhoea in certain wards of the Children Welfare Hospital , Medical City , Baghdad were interviewed and forms filled by two of us AD and AH , for the period June 26 , 2004 to March 2 , 2005 . The mothers were interviewed about the causes of diarrhoea before admission , and the results of microscopic stool examination, what drugs were given at home and whether oral rehydration solution was used.

RESULTS :

The patients studied were 175; 116(66%) were males and 59(34%) were females. The male to female ratio was 1.96: 1. The age of children ranged from 24 days to 8 years; 137 (78 `/.) were infants and only 8 (5 `/.) were older than 2 years. Of the 102 who had gastroenteritis diagnosed before admission 76(77.5%) were infants.

Fifteen of these infants (20 `/.) were breast fed and 61 (80`/.) were bottle fed. The age of the mothers ranged from 16 to 44 years.

Their education level varied, as (14`/.) of them were illiterate, while (7`/.) were college graduates. Ninety-six` /. Of the mothers were full time housewives, and with exception of one who was a student, all the others were government employees. Half of the mothers were primiparous. Of the children who had diarrhoea 39(22`/.) had parenteral infection, 26(67`/.) had respiratory infections, 4 had meningitis, 5 had urinary tract infections and one had septicemia. Two children were diagnosed as having celiac disease. Of the remaining 132 children , 102 were considered to have gastroenteritis , 14 (8`/.) as diarrhoea due to exposure to a cold environment, 11(6`/.) as due to teething, 5(3`/.) ascribed to the use of unsuitable milk formula, and one considered to have had fear as a cause of diarrhoea(table1). Of the 14 children whose diarrhoea was thought to be due to exposure to a cold environment (mistabred) 10(71`/.) of them were given antibacterial drugs , and of the eleven who were considered to have diarrhoea as a result of teething , 6(55`/.) took antibacterials, while 4 out of the 5(80`/.) of children whose diarrhoea was considered to result from the use of unsuitable milk formula were given antibacterials(table2) . There were 102 children diagnosed as having gastroenteritis before hospital admission: 73(75%) of them had no stool examination done and 55(75`/.) of these had antibacterials given prior to admission to hospital. Those who had their stools microscopically examined were 29(28%), and 17(58.6%) of them had no pathogens demonstrated, yet 10(59`/.)Of them received antibacterials.

Of the twelve who had parasites demonstrated in stool, 6(50%) had Entamoeba histolytica cysts, one had trophzoites and two had Giardia duodenalis cysts, and two gardia duodenalis trophzoite (table 3). The antibacterial drugs used most commonly were: metronidazole 29 times, gentamicin 19 times, ampicillin, +/-cloxacillin 12 times, cefotaxime 8 times, amikacin once and on three occasions the antibacterial was not identified. Single antibacterial was used 40 times (39`/.), while multiple antibacterials were used 34 times (33`/.), and only 28 (27`/.)Children were given no antibacterials (table 4). Of the total 133 children who were found to have gastroenteritis at hospital, 94(71`/.) received antibacterial drugs at home before admission.

Table 1– causes of diarrhea

Prehospital diagnosis	Number	Hospital diagnosis
Gastroenteritis	102 (58 %)	Gastroenteritis
Parenteral infection	39 (22 %)	Parenteral infection
Cold exposure	14 (8 %)	Gastroenteritis
Teething	11 (6 %)	Gastroenteritis
Unsuitable formula	5 (3 %)	Gastroenteritis
Drug effect	1 (0.5 %)	Drug effect
Celiac disease	2 (1 %)	Celiac disease
Emotional (fear)	1 (0.5 %)	Gastroenteritis
total	175	

Hospital diagnosed Gastroenteritis = 133
Percentages are approximated

Table 2 – Diarrhoeal cases not diagnosed as gastroenteritis Before admission and were given antibiotics

Prehospital	Total number	Cases received antibacterial
Cold exposure	14	10 (71 %)
teething	11	6 (55 %)
unsuitable formula	5	4 (80 %)

Table 3 – Stool examination results and the use of antibacterials In pre hospital diagnosed gastroenteritis .

Direct stool Examination	Cases received antibacterial	Total
Not done Stools examined :- No pathogen	55 (75 %) 19 (66%) 10(59%)	73 (71.5 %) 29 (28%) 17(16.6%)
Entamoeba H . cyst	6 (85%)	7(6.8%)
Entamoeba H .trophozoite	1(100%)	1(o.98%)
(Giardia D.cyst	1(50%)	2(1.9%) 12(11.7%)
(Giardia D.trophozoite	1(50%)	2(1.9%)
	74 (73%)	102(100%)

Table 4- Antibacterials used for gastroenteritis cases(Prehospital diagnosis) .

Antibacterial agent	How often used	Single or multiple Antibacterials
Metronidazole	29	Single
Gentamicin	19	40(39%)
Ampicillin +/- cloxacillin	12	Multiple
Cefotaxime	8	34(33%)
Amikacin	1	No drug
Unidentified	3	28(27%)

Total = 102 cases

Table 5- Oral dehydration salt use (ORS).

Prehospital Diagnosis	Total No.	Patients received ORS
Gastroenteritis	102	48(47%)
Cold exposure	14	8 (57%)
Teething	11	2(18%)
Unsuitable formula	5	3 (60%)
fear	1	0(0%)
	133	61(46%)

DISCUSSION :

In a community with high incidence of diarrhoea, proper management is expected to reduce morbidity and mortality. It may reduce suffering, financial loss , unnecessary hospitalization and increased malnutrition. In spite of the fact that diarrhoea is often a self-limited condition, its consequences can be largely prevented (4). Gastroenteritis , which is the commonest cause is largely due to viruses like Rota virus which causes at least half of all cases of acute diarrhoea, and less commonly to adenoviruses, caliciviruses(1)(6). Bacterial agents are less common, and in developed countries accounts to(2-10`/.) , the most important of which is the Shigella group (1).Of the parasitic causes the most important are Entamoeba histolytica and Giardia duodinalis (lamblia) which account for (1-8`/.) in developed countries(1). The mainstay of the management of diarrhoea is fluid and electrolyte replacement. The oral rehydration salts (ORS) formulated and advocated by the World Health Organization (WHO) had saved the lives of many children and reduced the suffering (7, 8). Of the 102 children who were diagnosed before admission to have gastroenteritis, 48(47`/.) were given ORS, of those 14 children who were diagnosed to have diarrhoea as a result of exposure to a cold environment 8(57`/.) Had ORS, and those 14 diagnosed as having teething as the cause of diarrhoea 2(18`/.) had ORS and of those 5 who had unsuitable milk formula 3(60`/.) had ORS (i.e. only 61 (46`/.) of children with diarrhoea were given ORS).(table 5). Cold environment exposure is not a cause of diarrhoea; in fact moderate hypothermia may lead to intestinal hypo- motility (9). Many mothers in our community are advised to keep the child warm when having diarrhoea which may lead to increased fluid loss from the skin, make the child uncomfortable, fretful and may possibly delay seeking medical advice. Teething on the other hand is not a cause of diarrhoea (10), and this again may be a reason for delaying proper management (11). Children whose diarrhoea was

ascribed to unsuitable milk formula were all diagnosed as gastroenteritis at hospital.

ORS solution is to be used for children with diarrhoea except those who may need parenteral fluid therapy i.e. those with severe dehydration, shock, and moderate dehydration with persistent vomiting, unconscious patient or the patient with ileus (4). Children who are dehydrated rarely refuse ORS solution, however those who are not dehydrated may refuse the solution because of the salty taste. Children with mild diarrhoea and no dehydration should be fed regular diet and do not require glucose and electrolyte solutions (4).

In the past, mothers were advised to stop feeding for a variable period of time (12).This might contribute to under nutrition and impair resistance to infection. The trend now is to continue breast feeding during the diarrhoeal episode, while the bottle fed baby may resume it after the rehydration process completed, Some four hours afterwards (7). Early refeeding with milk or food after rehydration does not prolong diarrhoea.

There is evidence that it may reduce the duration of diarrhoea by approximately half a day and is recommended to restore nutritional balance as soon as possible (4). Infants fed human milk can be nursed safely during episodes of diarrhea. Unrestricted diets do not worsen the course or symptoms of mild diarrhoea as can decrease the stool output compared with ORT or IV fluids alone. Children who require rehydration should be fed age appropriate diets as soon as they have been dehydrated. Clinical experiences based on controlled clinical trials suggest that certain foods including complex carbohydrates (rice, wheat, potatoes, bread, cereals, lean meat, yogurt, fruit, and vegetables) are better tolerated.

Fatty food or food with high simple sugars including juices, soft drinks should be avoided (4).

DRUGS:

Antiemetics : Metoclopramide was given to 11 (6`/.) of our patients. Others that were not used for our patients include prochlorperazine and chlorpromazine. They may cause sedation that interfere with oral rehydration therapy, and extra pyramidal signs like stiffness of neck and limps, which are more pronounced with metoclopramide . For these reasons antiemetics should not be given to children with diarrhoea. Almost all children who have vomiting and dehydration can be treated with ORS. The key to therapy is to administer small volumes of the glucose – electrolytes solution frequently e.g. 5ml every 1-2 minutes (4), moreover vomiting stops when the child is dehydrated (4, 6).

Antispasmotics: They were used for 19(10.8`/.) of our patients including homatropine methyl bromide (Antispasmine-SDI) and pipenzolate(Piptal-SDI). These and other anticholinergic (parasympatholytic) agents like hyoscyamin are commenly used in acute gastroenteritis to reduce abdominal cramping by decreasing motility and reducing tone of smooth muscles. There are little data on their safety and efficiency (1). Infants and young children are especially susceptible to the toxic effect of anti-cholinergic drugs such as coma, respiratory depression and paradoxical hyper-excitability. Anti-cholinergic agents are not recommended in the management of diarrhoea in children (4). ***Drugs for the control of diarrhoea:***

Adsorbents: Adsorbents like kaolin , pectin , attapulgite , smectite , activated charcoal and cholestyramine are promoted to bind and inactivate bacterial toxins, and other substances that cause diarrhoea .None have been of practical value in the management of acute diarrhoea in children(7). There is no conclusive evidence that these agents reduce the duration of diarrhoea , stool frequency or stool fluid loss(4).Disadvantages include adsorption of nutrients , enzymes and antibiotics(4). They should not be used for treatment of diarrhoea (13). Kaolin was used for one of our patients in a mixture (Enterosept-SDI).

Antimotility Drugs: They include loperamide, diphenoxylate with atropine, tincture of opium, camphorated tincture of opium, paregoric and codeine. These drugs reduce the frequency of stool passage in adults, but they do not appreciably decrease volume of stool in young children. They can cause paralytic ileus which can be fatal and may prolong infection by delaying elimination of the causative organism. Sedation may occur at the usual therapeutic dose and fatal central nervous system toxicity has been reported for some agents. None of these should be given to infants or children with diarrhoea (7), as side effects also include lethargy, ileus, respiratory depression, coma and death (4). Opiates have been shown to worsen the course of diarrhoea in patients with Shigellosis, antimicrobial associated colitis and diarrhea caused by E.Coli (4).

Drugs altering secretions:

Bismuth subsalicylate , bismuth subgallate and bismuth subnitrate are examples. Bismuth subsalicylate has been found to inhibit intestinal secretions caused by E.Coli and cholera toxin. Trials in children demonstrated decrease in duration and frequency of stool. However the beneficial affect has been modest, and the treatment regimen involves a dose every 4 hours for 5 days. This treatment is rarely practical (4, 7).

Antibacterials: Of the 102 cases diagnosed as gastroenteritis before admission, 74 (73`/.) Received antibacterial drugs. This included 73 (75%) who had no stool examination done, but 55 (75`/.) of them were given antibacterials.

Of the 29 (28`/.) who had their stools examined 12 (11.7`/.) only had parasites demonstrated, yet even in the absence of a pathogen 10(59`/.) had antibacterials given. (Table 3). Single antibacterials were given on 40 occasions (39`/.), and more than one antibacterial given on 34 occasions (33`/.) (Table 3).

Even those considered to have diarrhoea due to cold environment exposure, teething or unsuitable formula, (46`/.) Of them had antibiotics.

The recent evaluation of these antibacterial agents use in diarrhoea reveals:

Streptomycin and dihydrostreptomycin have no proved value in the treatment of diarrhoea. There is some evidence that they may actually increase severity and prolong the course of diarrhoea and may promote bacterial resistance. Oral preparations containing these drugs should not be used.(6) .Neomycin is of no proved efficiency, may be associated with gastrointestinal toxicity and may exacerbate and prolong the diarrhoeal episode, and promote antibacterial resistance, so its use cannot be justified (13). Hydroxyquinolines have not been shown to be effective. Optic neuritis and subacute myeloptic neuropathy have been associated with their use. Their use in acute diarrhoea cannot be justified (13). One of our patients received a preparation containing iodochlorhydroxyquinolene (Enterosept-SDI). Nonabsorbable sulphonamides include sulphaguanidine, succinylsulphathiazide and phthalylsulphathiazole , lack efficiency , and concern about their toxicity make their use unjustified (13). Other antibacterials should not be used routinely (7). They are really helpful only for children with bloody diarrhoea (probably Shigellosis), suspected cholera with severe dehydration, and non-intestinal infections such as pneumonia. Antibacterials that are ineffective for treatment of shigellosis are metronidazole , streptomycin , tetracycline, chloramphenicol, Sulphonamides , amoxicillin , nitrofurantoin, Gentamicin , kanamycin, and first and second generation cephalsporins. WHO experts recommend for Shigella – ciprofloxacin or pivmecillinam, for cholera – tetracycline or erythromycin, for amoebiasis-metronidazole, for giardiasis – metronidazole (7).

Treatment of amoebiasis should be considered only when microscopic examination of fresh stool done by a reliable laboratory reveals trophozoite of E. histolytica (EH).

Five of our patients had E.H cyst in stool and all were given metronidazole.

Treatment for giardiasis should be given only when the child with persistant diarrhoea have cysts or trophozoites of Giardia duodenalis seen in faeces or small bowel aspirate.

Children with acute diarrhoea should not be treated for giardiasis (7). Two of our patients had giardia cyst and two had trophozoites in stool.

As for lactobacillus containing preparations, they are used to alter intestinal flora to reduce stool pH that produce short chain fatty acids and deter intestinal pathogens. Short chain fatty acids are absorbed through colonic mucosa and facilitate water absorption. They are not recommended for acute diarrhoea in children (4).

Zinc supplement 10-20 mg/ day as soon as diarrhoea starts significantly reduce the severity and duration of diarrhoea as well as the risk of dehydration will be reduced. It is now recommended that zinc 10-20 mg/day given for 10-14 days to all children with diarrhoea (7), although a recent study from Turkey has shown that it did not have much effect in children with good nutrition (14). The new antidiarrhoeal agents like berberine , nicotinic acid , chloride channel blockers, calmodulin inhibiters , octreotide actate nonsteroidal anti-inflammatory drugs , all were considered experimental (AAP- 1996) (4) , and none is recommended by WHO manual of 2005 (7). Misuse of drugs for diarrhoea has been reported in other countries (15, 16).

RECOMMENDATIONS:

As doctors we should remind ourselves that diarrhoea is a self-limited disorder , and the commonest agents are viruses , so drugs are of limited value and often unnecessary.

ORS is by far the most important measure and can be lifesaving. Breast feeding, cleanliness and sterilization are the important preventive measures. (17,18,19,20). Breast feeding should continue uninterrupted during diarrhoeal episodes while milk formula and food are to be given as soon as rehydration is done i.e. some four hours later, and to be given undiluted (7).

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L-1α and IL-8 levels in leukopenic leukemic patients with bacteremia

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ABSTRACT:

BACKGROUND:

Interleukin-1α and Interleukin-8 are an inflammatory cytokines. They are a heterogeneous group of humeral mediators of the inflammatory response. In leukemic patient's leukopenia developed as a result of cytotoxic chemotherapy and/ or disease itself. Therefore, those patients were suffering from low number of leukocytes in addition to defects in the function of these cells. Normally leukocytes are the main source of cytokines production.

METHODS:

IL-1α and IL-8 were studied in (84) adult patients, males and females and more than 15 years old. The leukemic patients were suffering from leukopenia and bacteremia. The study including (20) healthy. Interleukin-1α and interleukin-8 concentrations were measured by using a commercially available enzyme-linked immunosorbent assay (ELISA).

RESULTS:

Statistical analysis shows significant increase in the levels of IL-1α and IL-8 between leukopenic leukemic patients with bacteremia and healthy control group.

CONCLUSION:

Leukopenic leukemia patients strikingly show distinct increases in plasma IL-1α and IL-8 levels during bacteremia.

KEYWORDS: Bacteremia, Leukemia.

INTRODUCTION:

Leukemias are a heterogeneous group of neoplasms arising from the malignant transformation of haemopoietic cells. Leukemia cells proliferate primarily in the bone marrow and lymphoid tissues where they interfere with the normal haemopoiesis and immunity; then emigrate into peripheral blood and infiltrate other tissue.Leukemias are classified according to the cell types primarily involved (lymphoid or myeloid) and as acute or chronic based upon the natural history of the disease [1-3].The specific drug which is used for treatment of leukemia generally aggressive. Their major antitumor effects are on actively dividing cells, so normal tissues with a high rate of cells proliferation are also affected by these agents [4, 5]. Decreased immunity is on the top of these effects caused by these drugs [6]. Leukopenia may occur as a result to this treatment. Leukopenia is defined as circulating leukocytes count is less than $4x10^9$ cell/litter [7].

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Leukocytes are normally the main producers of inflammatory cytokines [8, 9].Cytokines are produced by lymphocytes, monocytes, macrophages, and, for some cytokines, also fibroblast, neutrophils, endothelial cells, or mast cells [10]. The major functional activities of cytokines are concerned with the regulation of the development and behavior of the immune effector cells. Interleukin-1α is rapidly synthesized by mononuclear cells, primarily monocytic phagocytes that have been stimulated by microbial products or inflammation. The molecular weight of the mature forms is 17,500 [11, 12]. Among cytokines, only IL-1 and tumor necrosis factor (TNF) can induce IL-8 gene expression at the transcriptional level [13]. IL-8 is produced by many different cell types such as monocytes, macrophages, endothelial cells, fibroblasts and neutrophils. Interleukin-8 may play major roles in the in flammatory process by recruiting neutrophils and T-cells into inflammatory sites. Another important function of IL-8 is its ability to activate neutrophils following their attachment to vascular endothelium. IL-8 is one of the chemokines which are 8-to-10 kd proteins with 20 to 70 percent homology in amino acid sequences [14].

Because of the fact that the patients included in our study have disturbances in their innate immune system this immunological study was conducted to detect the role of IL-1α and IL-8 in the circulation of those patients during bacteremia.

MATERIALS AND METHODS:

A total of 28 adult patients (more than 15 years of age). Those leukemic patients suffering from leukopenia and bacteremia (16 males, 14 females). They were admitted to Baghdad teaching hospital. Those patients with (Acute myeloid leukemia =19, Chronic lymphoid leukemia =5, Acute lymphoid leukemia =2, Chronic myeloid leukemia=2). They were bacteremic patients. 20 apparently health adult individuals. Males and females.

Two milliliter of blood was taken from each patients and healthy controls. The blood was injected into a plane tube with no anticoagulant, left to clot at room temperature then centrifuged. Serum was collected in two separated tubes and stored at (-40°c) until used for investigation (Estimation of serum IL-1α and IL-8 levels) (18). The IL-1α and IL-8 concentrations were measured by using a commercially available enzyme-linked immunosorbent assay (ELISA).

The principle of the test was carried out according to the assay procedure given by manufacturing company Immunotech. Sample results are calculated by interpolation from a standard curve that is performed on the same assay as that of the sample. Data have been analyzed statically using SPSS program version 10. Analysis of quantitative data was done using ANOVA. Acceptable level of significant was considered to be below 0.05.

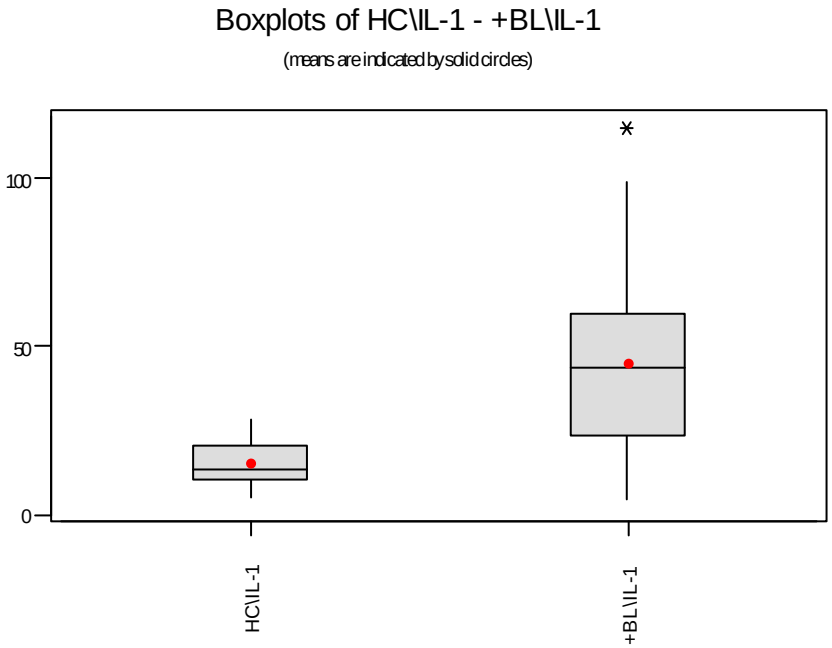
RESULTS:

Figure (1) shows the distribution of ELISA reading of IL-1α of the healthy control group and leukopenic leukemia patients with bacteremia.

The mean reading of healthy control group was 15049 pg/ml a SD of ±6.89, while the mean reading of leukopenic leukemia patients was (45.04 pg/ml a SD of ±26.66).

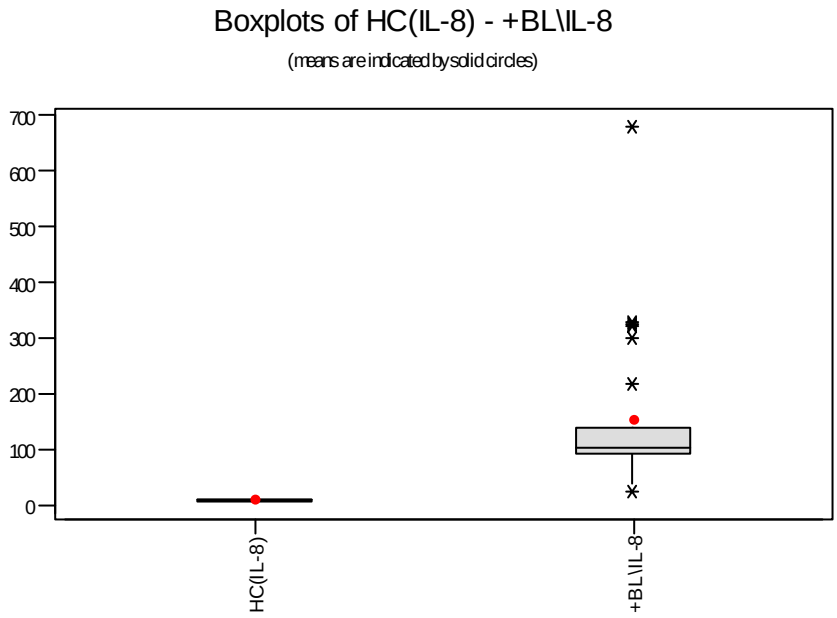
Figure (2) show the distribution of the ELISA reading of IL-8 of the healthy control and leukopenic patients with bacteremia.

The mean reading of healthy control group was (9.9 pg/ ml SD of ± 2.7). While the mean reading of the leukopenic patients with bacteremia was (152 pg/ml SD of ± 133.5).



HC\IL-1: IL-1α level in healthy control group
+BL\IL-1: IL-1α level in leukopenic leukemia patients with positive blood culture

Figure 1: The distribution of the ELISA reading of IL-1α of the healthy control, leukopenic patients with positive blood culture



HC (IL-8): IL-8 level in healthy control group
+BL\IL-8: IL-8 level in level in leukopenic patients with positive blood culture

Figure 2: The distribution of the ELISA reading of IL-8 of the healthy control, leukopenic patients with positive blood culture

DISCUSSION:

Statistical analysis shows increase in serum IL-1α in the plasma of leukopenic patients during bacteremia figure (1). There is significant difference between healthy control group and leukopenic leukemia patients with bacteremia (P=0.002). This is an expected result since it is well known that Gram negative bacteria and their endotoxins (lipopolysaccharide), as well as the cell wall components of Gram positive bacteria (peptidoglycans, teichoic acid) can activate the inflammatory cascades. Those molecules bind to membrane-bound and soluble receptors (CD14, mannose binding protein, toll-like receptors/ TLRs) inducing excessive production and release of pro-inflammatory mediators which include IL-1 and others [17]. this cytokine is mainly secreted by leukocytes, especially monocytes. However, bone marrow toxicity as the result of chemotherapy and the disease itself lead to reduced number of leukocytes in leukemic patients; this factor may be lead us to cytokine concentrations were unrelated to leukocyte counts. We conclude cytokine release in leukopenic leukemia patients during bacteremia dose not depend on circulating leukocyte [18]. This increase may be indicating source other than leukocytes for cytokines production in leukopenic leukemia patients during leukopenia [19]. Statistical analysis shows increase in the level of IL-8. There was significant difference between healthy control group and leukopenic patients with bacteremia.

This results was explained by that the high levels of IL-8 in leukopenic patients with bacteremia is a part of an effectors phase characterized by the production of IL-1 and tumor necrosis factor alpha (TNF-α) and then the production of IL-6 and IL-8 as result of blood stream invasion by Gram negative as well as Gram positive bacteria [20, 21]. This finding is in agreement with the finding by Schonbohn et al. (1995) during a study of plasma levels of IL-8 in patients undergoing chemotherapy for acute myelogenous leukemia. This result also confirms results of an analysis of serum IL-8 concentration in neutropenic cancer patients with Gram negative bacteremia. This result may be due to that endothelial cells instead of leukocytes become the most important producers of IL-8 during bacteremia in patients with chemotherapy induced leukopenia. The TLRs on endothelial cells act as pattern recognition receptors that induce the production of IL-8 upon binding of bacterial cell wall components [19]. Endothelial cells become important producers of IL-8 during the inflammatory response against bacteria through TLR-2 and TLR-4 signaling. Recently, the involvement of Toll-like receptors (TLRs) as pattern recognition receptors in the innate immune response was demonstrated. The TLRs are characterized by an extracellular domain contain leucine-rich repeats and intracellular domain sharing a high degree of similarity with the IL-1 receptor [22].

CONCLUSION:

The exact mechanism of the inflammatory response in leukemic patients with disturbed innate immunity is not completely clear. Leukopenic leukemia patients strikingly show distinct increases in plasma IL-1α and levels during bacteremia, suggesting that there may be source other than leukocytes for IL-1α and IL-8. because of cytokines seems to be promising diagnostic parameter, further studies about the role of IL-1α and IL-8 levels in an inflammatory response in leukopenic leukemia patients with bacteremia may be recommended.

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Clinical Profile and OutComeOf Respiratory Failure In Iraqi Children

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ABSTRACT:

BACKGROUND:

The frequency of acute respiratory failure is higher in infants and young children than in adults.Acute respiratory failure remains a significant cause of morbidity and mortality for children .

PATIENTS AND METHODS:

One hundred and twenty children under the age of 15 years presented with respiratory failure and admitted to RICU in Children Welfare Teaching Hospital and Surgical Specialty Hospital in Medical City - Baghdad in the period from the 1st of May -2003 to the 30th of June 2005 were enrolled in a descriptive study.

RESULTS:

Seventy nine (65.83%) cases were males and 41 (34.17%) were females. Male / female ratio was 1.93:1.The mean age was 30.21 months, 35 (29.16%) cases were neonates. Sixty eight (56.7%) cases were from urban areas and 52 (43.3%) were from rural areas. Seventy eight (65%) children were admitted for medical diseases and 42 (35%) were admitted for surgical problems. The most common medical causes were respiratory (50%) followed by neurological (37. 17%).The most common respiratory cases were bronchiolitis (28.2%) and most common neurological cases were Guillain- Barre Syndrome (58.6%).The majority (90.47%) of surgical cases were admitted post-operatively. The average duration of stay in RICU was 9.71 days. Fifty three patients (44.17%) survived and 67 (55.83%) died.

CONCLUSIONS:

The most common age group admitted to RICU is infancy, medical cases are more commonly admitted than surgical cases, the most common medical causes of admission are respiratory followed by neurological causes, the most common respiratory cause of admission is acute bronchiolitis.

KEY WORDS: children,respiratory failure; Iraq.

INTRODUCTION:

Respiratory failure develops when the rate of gas exchange between the atmosphere and blood is unable to match the body's metabolic demands [(1)]. It is diagnosed when the patient is unable to adequately ventilate, which leads to hypercarbia and hypoxemia, or when the patient loses the ability to provide sufficient oxygen to the blood which leads to hypoxemia [(2)]. Acute respiratory failure remains a significant cause of morbidity and mortality for children [(3)]. Cardiac arrests in children frequently result from respiratory failure.

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Respiratory failure can be classified on the basis of pathophysiologic mechanisms that lead to hypoxemia and /or hypercarbia [(4)]. Acute respiratory failure can occur when hypoxemia is caused by alveolar ventilation and pulmonary perfusion (V/Q) mismatch, intrapulmonary shunt, hypoventilation, abnormal diffusion of gases at the alveolar-capillary interface, reduction in inspired oxygen concentration, increased venous desaturation with cardiac dysfunction plus one or more of the above 5 factors [(5)]. The frequency of acute respiratory failure is higher in infants and young children than in adults for several reasons [(6)]. The neonates are obligate nose breathers. This nose breathing occurs until the age of 2-6 months. Because of the close proximity of the epiglottis to the nasopharynx, nasal congestion can lead to significant distress in this age group [(7)].The airway size is smaller. Size is one of the primary differences in infants and children younger than 8 years when compared with older patients .Infants and young children have a large tongue that fills a

small oropharynx .Infants and young children have a cephalad larynx. The cephalad larynx and large epiglottis makes laryngoscopy more challenging [4].Infants and young children have a narrow subglottic area which is cone-shaped with the narrowest area at the cricoid ring. A small amount of subglottic edema can lead to significant narrowing, increased airway resistance, and increased work of breathing [2]. In slightly older children, adenoidal and tonsillar lymphoid tissue is prominent and can contribute to airway obstruction [8].Infants and young children have fewer alveoli therefore they have less area for gas exchange [3].The alveolus is smaller. Collateral ventilation is less developed, making atelactasis more common. During childhood, anatomic channels form to provide collateral ventilation to alveoli. This important feature allows alveoli to participate in gas exchange in the presence of an obstructed distal air-way [7].Smaller intrathoracic airways are more easily obstructed. With age, the airway enlarges in diameter and length [9].Infants and young children have less cartilaginous support of the airway. As cartilaginous support increases, dynamic compression during high expiratory flow rates is prevented [10].The respiratory pump includes the nervous system with central control respiratory muscles, and chest wall [11].The respiratory center is immature in infants and young children, which leads to irregular respirations and the risk of apnea [2].The ribs are horizontally oriented. During inspiration, less volume is displaced, and the capacity to increase tidal volume is limited when compared with that in older people [12, 13]. The musculature is less developed. The slow-twitch fatigue resistant muscle fibers in the infant are under developed [14, 15].

Management of acute respiratory failure begins with the determination of the underlying etiology. While supporting the respiratory system and ensuring adequate oxygen delivery to the tissues, an intervention specifically defined to correct the underlying condition is instituted [16 Conventional mechanical ventilation optimizes lung recruitment, increases mean airway pressure and functional residual capacity, and reduces atelactasis between breaths [2]

PATIENTS AND METHODS:

This descriptive study was conducted on 120 children under the age of 15 years presented with respiratory failure and admitted to RICU in Children Welfare Teaching Hospital and Surgical Speciality Hospital, Medical City-Baghdad in the period from the 1st of May -2003 to the 30th of June 2005. The RICU in Children Welfare Teaching Hospital consists of an average of 4 beds with a nurse to patient ratio is 1.5:1 and Surgical Speciality Hospital consists of 12 beds with nurse to patient ratio of 1:1. The unit is staffed by a pediatrician, an anesthetist, pediatric residents, nurses, and supported by pediatric subspecialists. Ventilators are handled by the pediatrician or the anesthetist with back up support of the medical engineers for any technical faults. Suction and nebulizer therapy are routinely handled by RICU nurses. Physiotherapists and nurses provide chest physiotherapy as instructed by the attending pediatrician. These two hospitals do not have a separate laboratory for the RICU but a central laboratory. Results of arterial blood gases analysis, electrolytes are not available on immediate basis round the clock. Children with all types of diseases are admitted if they have impending or manifest respiratory failure, patients with a high risk of organ dysfunction and failure due to general pediatric surgery and patients whose organ function needs to be closely monitored independent on the underlying disease.

The diagnosis of respiratory failure was based on clinical findings plus oximetry results. Arterial blood gases analyses were recorded in 25 cases (20.8%). Data including age, sex, residence, and diagnosis of the disease classified by systems, duration of stay in RICU and over all outcomes were recorded.

RESULTS:

The age rang of admitted children was from birth–15 years with a mean age of 30.21 months. The majority 41 cases (34.2%) were infants followed by neonates 35 cases (29.16%) (Table -1). From these neonates 30 cases (85.7%) were full term and 5 cases (14.3%) were preterm babies, 25 (71.4%) were males and 10 (28.6%) were females and 4 newborns (11.4%) were admitted on the first day of life. The minority were (5.83%) above the age of 12 years. Seventy nine (65.83%) were males and 41 (34.17%) were females with a male to female ratio of 1.93:1. Sixty eight (56.7%) cases were from urban areas and fifty two (43.3%) were from rural areas.

Out of 120 children who were admitted, 78 (65%) were medical cases, 42 (35%) were surgical. The mean duration of stay in RICU of the medical and surgical cases was 12.97 days and 4.07 days respectively. From the medical cases, 37 (47.44%) survived, and 41 (52.56%) died. Regarding the

surgical cases, 16 (38.1%)) survived and 26 (61.9%) died (table-2).

Thirty nine (50%) of medical cases were respiratory, with mean duration of stay in RICU of 12.23 days, 22 (56.4%) survived and 17 (43.6%) died.

Twenty nine (37.17%) were neurological cases with mean duration of stay in RICU 17.21 days, 14 (48.3%) survived, and 15 (51.7%) died. 7 (8.97%) cases were admitted to RICU due to development of sepsis with mean duration of stay, in RICU of 3.71 days and all of them died. one case was chronic renal failure, 1 case was fulminant hepatic failure and 1 case was heart failure (1.28% each) as shown in table -3.

Thirty nine (50%) respiratory cases were admitted to RICU. 11 (28.2%) were bronchiolitis, 10 (25.64%) were respiratory distress syndrome.

Nine (23.07%) were pneumonia and 4 (10.25%) were asthma, 2 (5.128%) were whooping cough, 2 (5.128) were laryngomalacia and 1 (2.56%) was bronchiectasis. From the 39 respiratory cases, 22 (56.4%) survived and 17 (43.6%) died (table-4).

Twenty nine (37.17%) neurological cases were admitted to RICU, 17 (58.6%) were Guillian Barre Syndrome, 8 (27.6) were meningitis, 2 (6.9%) were encephalitis, and 2 (6.9%) were status epilepticus. From these 29 neurological cases, 14 (48.3%) survived, and 15 (51.7%) died (table-5).

Forty two (35%) surgical cases were admitted to RICU, 26 (61.9%) died, and 16 (38.1%) survived. Details about these surgical cases are noted in table -6.

Thirty five neonates (29.16%) were admitted to RICU, 21 (60%) were surgical cases, and 14 (40%) were medical cases. The mean duration of stay in RICU was 4.18 days. 25 (71.4%) were males, and 10 (28.6%) were females. From those neonates 7 (20%) survived and 28 (80%) died as shown in table -7.

For all types of cases the average duration of stay in RICU was 9.71 days.

The overall outcome of one hundred twenty children included in this study was 53 (44.17%) survived and 67 (55.83%) died.

Table -1:Age distribution of children with respiratory failure admitted to RICU

Age	No.	%
0-4 weeks	35	29.16
1-12 months	41	34.2
> 1 yr – 3 year	17	14.16
> 3 yr – 5 year	9	7.5
> 5 yr – 12 year	11	9.2
> 12	7	5.83
Total	120	100%

Table -2:General causes of admission to RICU with the mean duration of stay and outcome.

General causes	No.	%	Duration of stay (days)	Surviva		Death	
				No.	%	No.	%
Medical	78	65%	12.97	37	30.83	41	34.16
Surgical	42	35%	4.07	16	13.34	26	21.67
Total	120	100%		53	44.17	67	55.83

Table -3:Medical cases who were admitted to RICU with the mean duration of stay and outcome

Medical causes	No.	%	Duration of stay (days)	Survival		Death	
				No.	%	No.	%
Respiratory	39	50%	12.23	22	28.21	17	21.8
Neurological	29	37.17	17.21	14	17.95	15	19.23
Sepsis	7	8.97	3.71	0	0	7	8.97
Renal	1	1.28	3	1	1.28	0	0
Hepatic	1	1.28	7	0		1	1.28
Cardiac	1	1.28	1	0		1	1.28
Total	78	100%		37	47.44	41	52.56

Table -4:Respiratory causes of admission to RICU with the mean duration of stay and outcome

Respiratory causes	No.	%	Duration of stay/days	Survival		Death	
				No.	%	No.	%
Broncholitis	11	28.2	15.45	6	15.38	5	12.82
RDS	10	25.64	3.1	1	2.564	9	23.07
Pneumonia	9	23.07	4.55	7	17.94	2	5.128
Asthma	4	10.25	3.75	4	10.25	0	0
Laryngomalacia	2	5.128	36	1	2.569	1	2.564
Whooping cough	2	5.128	70	2	5.128	0	0
Bronchiectasis	1	2.56	2	1	2.564	0	0
Total	39	100		22	56.4	17	43.6

Table -5:Neurological causes of admission to RICU with the mean duration of stay and outcome

Neurological causes	No.	%	Duration of stay (days)	Survival		Death	
				No.	%	No.	%
Guillian Barre Syndrome	17	58.6	24	12	41.4	5	17.24
Meningitis	8	27.6	9.25	1	3.45	7	24.14
Encephalitis	2	6.9	2	0		2	6.9
Status epilepticus	2	6.9	1.5	1	3.45	1	3.45
Total	29	100%		14	48.3	15	51.7

Table -6:Surgical cases which are admitted to RICU with the mean duration of stay and outcome

Surgical cases	No.	%	Duration of stay (days)	Survival		Death	
				No.	%	No.	%
Post-operative TOF	10	23.81	2.4	1	2.38	9	21.42
Post-operative diophragmatic hernia	8	19.04	5.12	1	2.38	7	16.66
Post-operative congenital lobar emphysema	7	16.7	6	4	9.52	3	7.14
Post-operative Intestinal obstruction	3	7.14	7	0		3	7.14
Head injury	3	7.14	3.66	0		3	7.14
Bullet injury in the chest	2	4.76	1.5	1	2.4	1	2.38
Post-operative hydatid cyst in the lung	2	4.76	2	2	4.76	0	0
Post-operative duodenal atresia	2	4.76	4.5	2	4.76	0	0
Post-operative intussusception	1	2.38	3	1	2.38	0	0
Post-operative mass in the left jaw	1	2.38	2	1	2.38	0	0
Post-operative achalasia	1	2.38	1	1	2.38	0	0
Post-operative mediastinal tumor	1	2.38	1	1	2.38	0	0
Post-operative bronchoscopy for suspected foreign body	1	2.38	2	1	2.38	0	0
Total	42	100		16	38.1	26	61.9

Table -7:Neonatal cases admitted to RICU with the mean duration of stay and outcome

Neonatal cases	No.	%	Duration of stay/days	Survival		Death	
				No.	%	No.	%
Post-operative TOF	10	28.8	2.4	1	2.85	9	25.7
RDS	10	28.6	3.1	1	2.85	9	25.7
Post-operative diaphragmatic hernia	7	20	5.42	1	2.85	6	17.14
Post-operative Intestinal obstruction	2	5.71	9.5	0		2	5.7
Post-operative duodenal atresia	2	5.71	4.5	2	5.7	0	0
Sepsis	2	5.71	1	0		2	5.7
Broncholitis	1	2.85	3	1	2.85	0	0
Laryngomalacia	1	2.85	6	1	2.85	0	0
Total	35	100		7	20%	28	80%

DISCUSSION:

As the published data on respiratory failure in Iraqi children are scanty, an adequate comparison of epidemio-logical, diagnostic profiles and outcome is not possible within the country. In this study which included 120 patients, 79 (65.83%) were males and 41 (34.17%) were females and males/females ratio was 1.93:1. In a study done by Praveen K. [17] interestingly more male children were admitted to RICU than female children, and the male / female ratio was 2.9:1.In addition Randolph A.G. [18] recorded that the majority of patients were males (55%).In this study the mean age was 30.21 months, which is lower than Praveen K.study which was 41.48 months.

In the present study the most common age group admitted to RICU were infants (34.2%) followed by children between 1-12 years 30.86%, then neonates (29.16%) and (5.83%) were children above 12 years, this agrees with Randolph A.G. who found that the most common age group affected were infants (40%), followed by children between 1-12 years (34%), then neonates (16%) and the least common is children above 12 years (14%).

Medical causes accounted for 65% of cases and surgical causes accounted for 35%. In Praveen K. study surgical causes were less common than medical causes (20.5%).In this study respiratory causes accounted for majority of medical cases (50%) followed by neurological causes (37.17), then by sepsis (8.97%).In Praveen K. study respiratory cases accounted for (19.7%) followed by neurological causes (17.9%) then by infectious causes (12.5%) and these results are comparable in sequence to results in this study. Karandes [19] also reported pulmonary disease to account for (68%) of cases followed by neurological cases (22%) and 10% were miscellaneous cases. Randolph A.G.found that 62.4% of patients had pulmonary disease, 14.2% had neurological disease and 8.9% had cardiac disease.

The most common respiratory cause is bronchiolitis reported in 11 (28.2%) cases. In Randolph A.G study the most common acute diagnosis was bronchiolitis in infants (43.6%) and pneumonia in children 1 year old and older (24.5%). Karandes found that bronchopneumonia was the most common cause of acute respiratory failure (31.6%).

The average duration of stay in RICU was 9.71 days which is higher than average duration of stay of RICU in Praveen K. study which was 7.12 days.

In this study, out of 120 cases who were enrolled 53 (44.17%) survived and 67 (55.83%) died. In Karandes study the over all mortality was 58%.

From the present study conclusions include; male children are more likely to be admitted to RICU than female children, the most common age group admitted to RICU is during infancy, medical cases are more commonly admitted than surgical cases, the most common medical causes of admission are respiratory followed by neurological causes, the most common respiratory cause of admission is acute bronchiolitis.

Acute respiratory failure has varied etiology and high mortality rate so proper laboratory investigations may prevent death since all children with sepsis died and early diagnosis with proper management of surgical cases may decrease mortality.

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Spiral Computed Tomography Findings In Clinically Suspected ACute Pulmonary Embolism

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ABSTRACT:

OBJECTIVES:

To show the role of enhanced spiral computed tomography in clinically suspected acute pulmonary embolism(PE) patients .

PATIENTS AND METHODS:

From October 2003 to October 2004, Fourty two patients with clinically suspected acute pulmonary embolism ,were examined by thin slices contrast enhanced spiral computed tomography as the primary diagnostic test to role out or confirm the diagnosis of pulmonary embolism. Patients were examined in the Radiology Departments of (Al-Kadymia, Al-Yarmook & Ibn Al-Bittar Teaching Hospitals).

RESULTS:

Pulmonary embolism diagnosed in 43% of the patients by showing clot within pulmonary arteries with or without non specific signs like effusion , wedge infarct & dilated pulmonary artery .CT was normal or gave an alternative diagnosis that could explain the patient's signs & symptoms in 52%. Inconclusive findings were seen in 5%.

CONCLUSION:

Computed tomography can be used safely as the primary diagnostic tool in clinically suspected acute pulmonary embolism patients, to confirm or role out the diagnosis.

INTRODUCTION:

Pulmonary embolism is a common and potentially fatal disorder [1] often missed because more than 70% of them are not suspected clinically [2, 3]. Laboratory investigation like D-dimer test misses 10% of patients, but could confirm the diagnosis in 30% . Simple investigation like chest radiograph is not specific[3]. The result of V/Q lung scan is non-diagnostic (non specific) in 40-70% of cases [4]. Pulmonary angiogram is considered the diagnostic standard examination for PE, but it is invasive, expensive and is not widely available or accessible [5]. The ideal test should be accurate, safe, readily available, costly effective and should have widespread acceptance. The contrast enhanced spiral CT is an ideal single test in the diagnosis of PE because it shows emboli directly like the pulmonary angiogram, it is less invasive, cheap and widely available like V/Q scintigraphy [2,5] , the radiation dose exposure of the spiral CT is approximately 5 times smaller than angiography [2], and it is the only test that can provide significant additional information or an alternative diagnosis[4,5].

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PATIENTS AND METHODS:

This prospective study done in the Radiology Departments of three participating hospitals (AL-Kadymia, AL-Yarmook & Ibn AL-Bittar Teaching Hospitals) from October 2003 to October 2004. During which fourty two patients with clinically suspected acute PE were examined by contrast enhanced spiral CT, most of them examined immediately using Somatom plus 4 unit (Siemens medical system). Three months follow up of patients was done [2,6], Recurring symptoms, death during the study and during follow up were recorded .All patients were examined in supine position in caudo-cranial direction during breath hold of 20-30 second. CT protocols (5 mm collimation, pitch of 1, KV 120-140, mAs 100-200) both native and enhanced CT were done. Enhancement was achieved by giving 350-525mg/kg of intravenous non-ionic low osmolar contrast media (Omnipaque 350mg/ml) which injected manually over 30-40 sec through two I.V lines using 18 G cannula (the injection

method was modified because of the non availability of automatic injector in the CT units) scanning start immediately after finishing the injection. CT image analysis done by the consultant radiologist at the CT unit of each hospital.

RESULTS:

In the first group (52%) , CT scan was either normal (no findings) or gave an alternative diagnosis like pneumonia or atelactesis which explain patient's signs & symptoms. On three months clinical follow up, most of them were improved after treatment without occurrence of PE, which give 100% negative predictive value for CT.

In the second group (43%) , CT scan findings were specific for PE i.e. the presence of clot within the pulmonary arteries with additional non-specific signs such as effusion, wedge infarct and dilated pulmonary artery. They were furtherlly classified according to occlusion (partial or complete) depending on the effect of the clot within the vessels. Totally occlusive clot or emboli producing the sign of complete vessel cut-off was seen in (39%),while partially occlusive emboli giving the sign of tram track when viewed in longitudinal axis (on reformatted image) or giving the rim sign or the signet ring sign when viewed on axial section was seen in (61%) (Fig1).

In the third group (5%) CT findings were negative or inconclusive although there was high clinical suspicion of pulmonary embolism .

Another classification of CT findings in PE could be made according to the location and multiplicity (Fig 2) . Bilateral emboli were found in 11 patients (61%) including 4 cases (22%) revealed saddle embolism (embolus lodged at the bifurcation of pulmonary trunk with extension to the right and left pulmonary arteries) (Fig 3). Right Side pulmonary emboli were seen in (28%) . Left Side emboli were seen in (11%) .

Further classification of CT scan findings in PE could be made according to the site of emboli within the pulmonary arteries , this include emboli in pulmonary trunk, Rt and Lt main pulmonary arteries and lobar arteries were seen in 16 patients (89%). Emboli within segmental arteries were seen in 2 patients (11%) . No subsegmental emboli were identified in this study.

Additional important CT scan findings in PE were the non specific findings , the presence of those in addition to the specific findings confirms the diagnosis of PE (Table 1).

DISCUSSION:

Pulmonary embolism is a common & potentially fatal disorder [(1)], it is fatal especially when untreated, thus the need for an accurate diagnosis is essential, for which CT is safely used as the main diagnostic test [(4)]. The CT-scan findings in this study were compared with the others ,and appeared to be approximately similar. (Table. 2)

Negative C.T findings were seen in 52% of patients, it is one of the advantages of CT to provide alternative diagnosis which explain patient's symptoms like pneumonia [(2,4,5)].three months clinical follow up for those patients is acceptable to differentiate missed PE from new episode of PE [(2)] The negative predictive value of CT in this study is 100% (no occurrence of PE in those patients with negative CT) , this value is higher than what was seen by Bourriot K.(2003) [(8)] This slightly higher negative predictive value is mostly related to low number of patients in this study .

Positive CT findings may show both specific & nonspecific signs. The direct specific signs include the demonstration of vascular intra luminal filling defect (thrombus) which is considered a very specific diagnostic sign. This filling defect may produce different signs that include complete filling defect (vessel cutoff sign) (Figure 3), which is caused by thrombus completely obstructing vessel cross-section, this was seen in 7 patients (39%).Rim sign (polo mint sign) which was seen in 11 patients (61%), is produced by partially occlusive intra luminal thrombus surrounded by contrast medium when viewed in axial section,(Figure 4). The railway track (train track sign) is seen when the partially occluding thrombus is viewed sagittaly (reformatted image) (Figure 5). Saddle embolus sign seen in 4 patients (22%) in which the obstructing clot or thrombus lodged at the bifurcation of pulmonary trunk with limb extension to both right & left pulmonary arteries (Figure 6). Other positive findings depend on the site of emboli, Bilateral emboli were seen in (61%), Right lung emboli were seen in (28%) & Left lung in (11%), this show slight differences in comparison with Dahnert W. (1999) [(9)] & Quandli J.D (2000) [(7)] , where bilateral emboli, RT. Lung & LT. Lung seen in 45%, 36% &18 % respectively. Multiple emboli seen in 13 patients (72%) those are within the range of findings in other studies (65-81%)

Other positive findings seen in this study depend on the locations of the thrombi within the pulmonary circulation and this may be central

involving main pulmonary arteries seen in (89%),or may be involving the segmental arteries seen in (11%).When both are considered the main arteries involvement (collectively) is 100%. This in comparison with (Quandli J.D 2000) [7] was (94%),is slightly higher incidence of thrombi in the main pulmonary arteries and this may be related to the fact that sub segmental emboli were not identified in this study. The low incidence of sub segmental emboli in this study is related to the fact that in most of other studies automatic injector for the IV contrast administration was used , while in this study the incidence of emboli in the main pulmonary artery was higher since we use manual injection of contrast which miss many of subsegmental empoli as compared with the automatic injector. However, the clinical importance of isolated sub segmental PE is uncertain, and depends on both the incidence and significance of that emboli . Episodes of small sub segmental embolism may be common but of little clinical relevance in otherwise healthy individuals. A burden of small emboli, however, may be more significant if they occur, in patients with underlying cardio-respiratory disease, when they are multiple and recurrent caused by underlying silent DVT [4].

The indirect signs are not specific and may occur in a variety of other conditions and their absence does not role out PE [2,3,4]. CT is sensitive for their detection and their presence in addition to the specific direct signs confirms the diagnosis of P.E [2]. These signs include Pleural effusion (56%) representing the most common secondary associated finding which is in agreement with its prevalence in other series (50%)[10]. The pleural effusion in patients with PE is commonly unilateral and result from haemodynamic consequences of sudden pulmonary artery occlusion [10,11] . Dilated pulmonary artery occured in 4 patients (22%). That are especially seen in cases of completely occlusive emboli causing sudden occlusion of the pulmonary artery resulting in cessation or reduction of blood flow distal to the embolus and increasing the pressure proximal to it [10]. Pulmonary infarct (Hampton's sign) was seen in 6 patients (33%) which is in agreement with the findings in other studies (25-61%). This pulmonary infarct is a wedge-shaped, pleural-based consolidation which is classically not enhancing & easily differentiated from atelactesis or pneumonic consolidation which enhance after contrast (Fig 7). Atelectasis identified in 5 patients (27.78%). This indirect sign is frequently seen but not specific [2,12]. The underlying cause of atelectasis in patients with PE is due the physiological consequences of pulmonary artery occlusion leading to creation of an alveolar dead space resulting in pneumoconstriction. Another physiological consequence of PE is depletion of the alveolar surfactant resulting in atelectasis [10]. Mosaic attenuation (perfusion) seen in 2 patients (11%), this sign represents the differential perfusion of lung parenchyma after contrast administration (areas of low attenuation lung containing attenuated vessels, contrast with higher attenuation area of relatively over perfused lung) when the CT images viewed at lung window [2,10].

In the third category of CT findings (2 patients 5%) in whom there was high clinical suspicion of P.E but CT findings were inconclusive (CT showed wedge-shaped consolidation with central necrosis & associated pleural effusion but no definite intra vascular clot could be identified) in comparison with other series in which CT results were indeterminate in 2-13% [2,6], The main causes of these inconclusive findings are incomplete opacification of the pulmonary vessels due to the use of manual injection, tachypnea related motion artifact and the use of single slice spiral CT which is affected more by this artifact because of relatively longer scan time when compared with dual slice or multi slice spiral CT but should keep in mind, that this incidence of inconclusive findings is in the same range of inconclusive pulmonary angiography (0-17%) , and much better than the range of inconclusive V/Q scan (30-80%)[2] . On considering this group of findings, the sensitivity of CT in this study was 90% which is approximate to the findings of other studies in which the sensitivity of CT in detecting PE was 53-100% .The sensitivity is as high as 100% when only the main pulmonary arteries evaluated , but decreases to its lower limit when the sub segmental arteries were included[1,2,3,5,11] . Those two patients in this study underwent pulmonary angiography at IBN AL-BITTAR hospital, and showed evidence of segmental P.E, the CT underestimation of those patients may be related to that the CT done at time when the emboli were at the sub segmental level (in which CT sensitivity for detection of emboli decreases down to 60%) while angiography done within 24-48 hours after CT, at this time interval further emboli may be built up causing these discrepant findings.

CONCLUSION:

In conclusion when CT findings were specific, this establish the diagnosis of PE and urgent

treatment should be started without further need for confirmative diagnostic tests, when CT findings are completely normal or give an alternative diagnosis that explains the patients signs and symptoms , no further investigative steps are needed. In some patients the CT findings were inconclusive. In these group of patients further work up by angiography should be the next step to establish or exclude the diagnosis of PE.

The results of this study recommend to use contrast enhanced spiral CT as the primary diagnostic tools in clinically suspected acute PE patients, the use of automatic injector of contrast media & the dual slice or multi slice spiral CT will improve the sensitivity of diagnosing PE.

Table 1: The frequency and percentage of PE non-specific CT signs detected in patients with positive CT scan for PE .

*The non specific(indirect) signs of PE	No. of patients	Percentage
Pulmonary infarct (Hampton's sign).	6	33%
Pleural effusion	10	56%
Large pulmonary artery	7	39%
Atelactesis	5	28%
Mosaic attenuation	2	11%

* Patient with PE may show one, two or more of those signs.

Table 2: CT findings in this study as compared with other studies.

Author	CT findings		
	Negative for PE	Positive for PE	Inconclusive
Quandli.J.D(2000)[7]	59%	37%	4%
Perrier A. (2001)[1]	47%	39%	4%
Vanstrijen (2003)[6]	74%	24%	2%
This study	52%	42%	5%

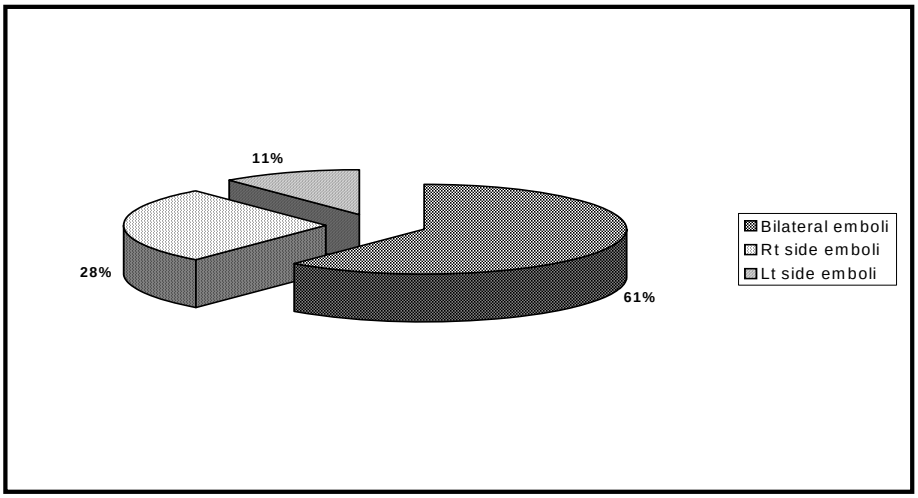

Figure 1 : The frequency of positive CT scan findings of pulmonary emboli according to the number and location of emboli detected in 18 patients.

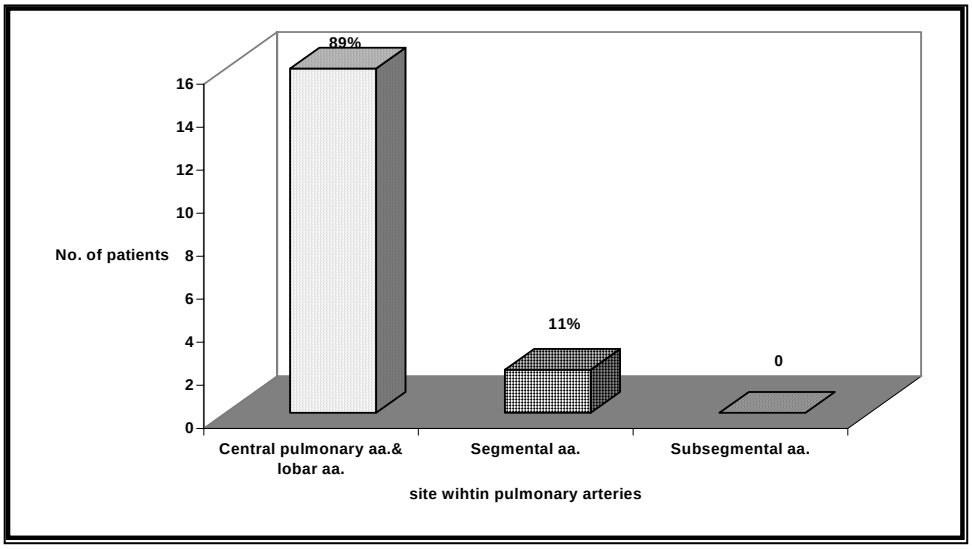

Figure 2: The frequency of positive CT scan findings of pulmonary embolism according to the site within pulmonary arteries detected in 18 patients

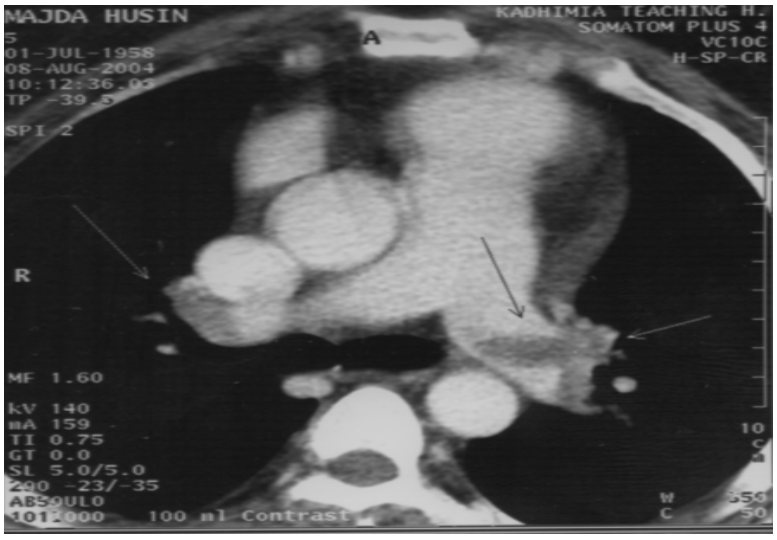

Figure 3: Filling defect in the main pulmonary arteries, the lobar arteries & the segmental arteries, which are totally occlusive on the RT. Producing" complete vessel cutoff sign".

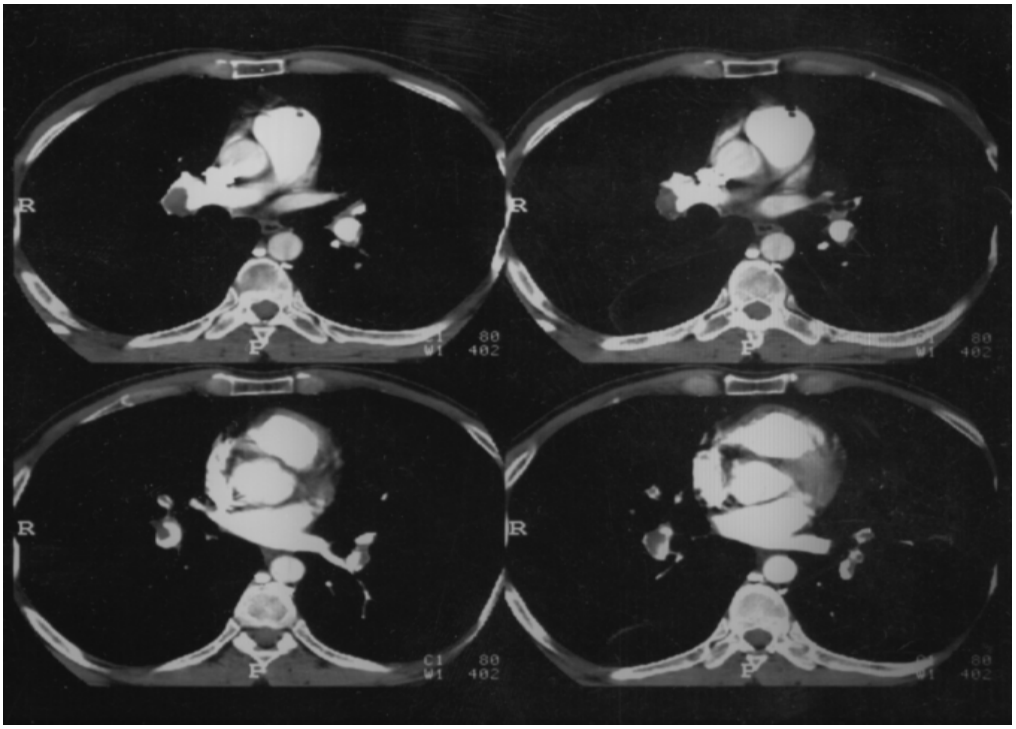

Figure 4: Multiple partially occlusive intra vascular filling defect in the pulmonary trunk & main pulmonary arteries, lobar & segmental arteries producing the" signet-ring, or polo mint, or rim sign" as viewed on axial CT section.

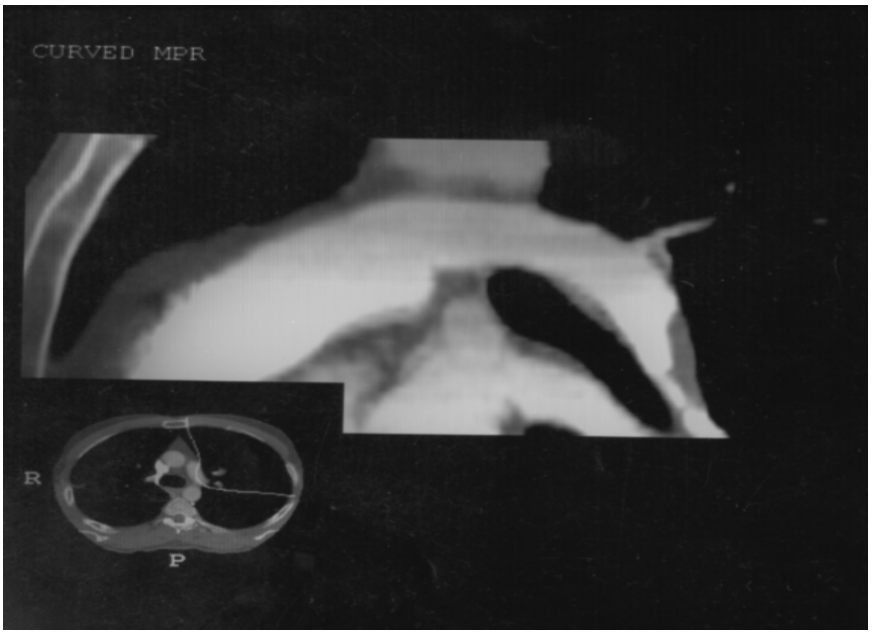

Figure 5: Reformatted sagittal oblique images showing the "railway track sign" of partially occlusive clot when viewed parallel to long axis.

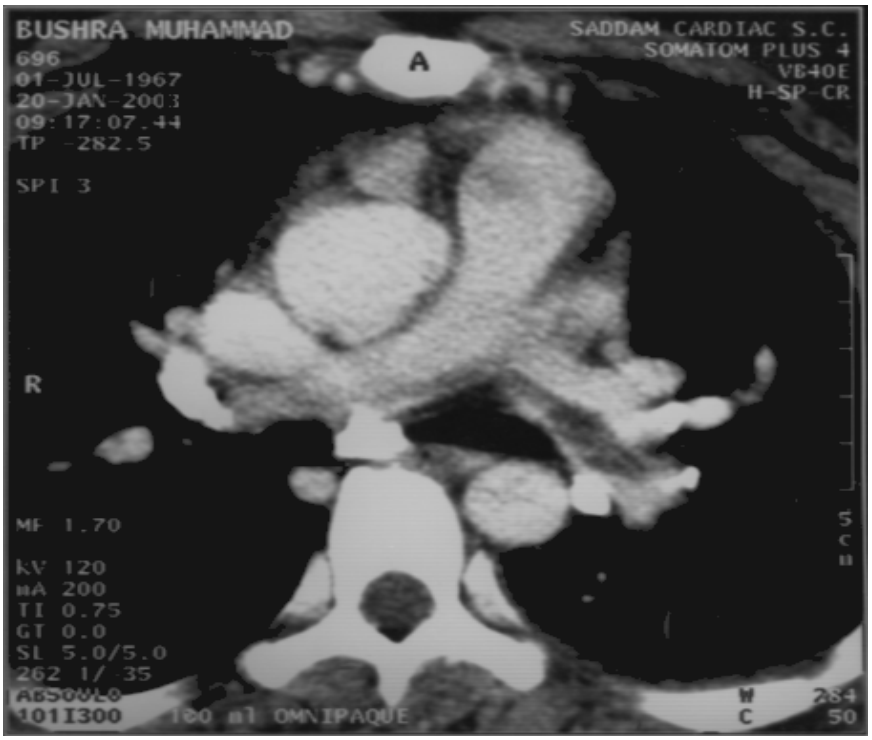

Figure 6 : single slice contrast enhanced spiral CT , shows saddle embolism

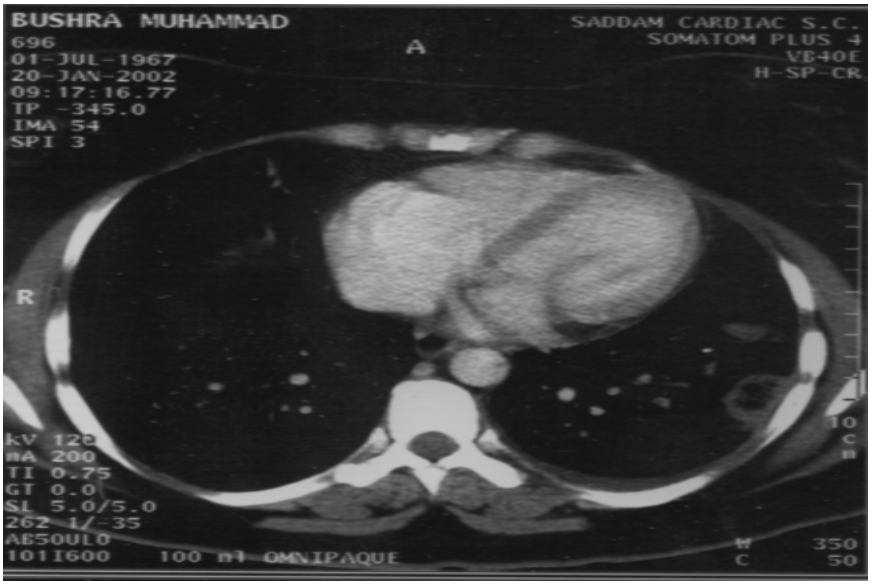

Figure 7: Single slice contrast enhanced spiral CT-scan show left lower lobe segmental artery emboli with the presence of peripheral wedge shaped pleural based consolidation "correspond to Hampton's hump" and non enhancing central necrosis with thickened vessel leading to the apex of consolidation "vascular sign" increase the specificity of infarction.

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The Prevalence of Silent Gall Stones And Its Relation To Some Risk Factors in Iraq

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ABSTRACT:

BACK GROUND :

Gallstone disease is a common condition all over the world as well as in Iraq. Symptomatic gallstones definitely need surgical or medical treatment . To remove or treat silent gallstones is still a debatable subject. Gallstones are about twice as common in women than in men and their incidence vary according to some physical fasters

METHODS :

1016 adult volunteers from both sexes and of different age groups were examined by ultrasound in Medical city teaching hospital between January 2004 and August 2005, for the estimation of the prevalence of silent gallstones among Iraqi people and it's relation to some physical and familial factors was studied .

RESULTS:

The incidence of silent gallstones in both sexes was 3.3%. It is more common in women 4.09% than in men 2.2% and it increases with age, parity, and the use of contraceptive pills, and high intake of black tea The size of most of the stones was less than 20 mm and they were less than three in number, the gall bladder was with a normal wall thickness, no associated mass , or gallbladder wall calcifications, and no association with specific blood group or obesity was found.

CONCLUSIONS:

Silent gallstones were found in 3.3% of healthy Iraqi individuals, and they are associated with the same risk factors of symptomatic gall stones such as age , parity , familial contraceptive except that obesity and blood groups are not a major risk factor.

KEY WORDS: Silent gallstones ultrasound , Risk factors.

INTRODUCTION:

Interest in the formation and clinical management of gallstone disease back to ancient times, as archeological evidence suggested that; members of the royal Egyptian families were affected by this disorder **(1)** .

Gallstones are about twice as common in women as in men. In elderly, the incidence rises to about 20% and sex incidence is roughly equal after the age of 80 years. about 5-15 % of gallstones are asymptomatic (2-3). Ultrasound is the technique of choice to detect gallbladder stones , its diagnostic accuracy is 90-95% , ultrasound may also show thickened gallbladder wall (> 3mm)as well as other features of gallbladder diseases such as tender gallbladder (sonographic Murphy sign) and pericholecystic fluid . Non visualized gallbladder also suggests a disease or non fasting patient **(4-5)**.

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Risk factors for gallstones:

1. Age and gender: Because gallstones are rarely dissolved spontaneously, the cumulative prevalence of gallstones increases with age.

In addition to that, cholesterol secretion into bile increases with age, where as bile acid formation may decrease **(6)** . Gender is the most prominent risk factor for gallstone formation, with most studies reporting a two to three – folds increase in females **(6)**

2. Obesity , weight , and Total Parenteral Nutrition: Obesity is also a well-known risk factor for cholelithiasis. A large prospective study of obese women reported a strong linear association between body mass index (expressed in kg/m^2) and the incidence of reported cholelithiasis. In the mentioned study, those with highest body mass index (>45 kg/m^2) had a yearly incidence of gallstone formation of approximately 2% per year. **(6)** **3. Pregnancy and parity:** Pregnancy is a greater risk factor for the development of gallstone, during pregnancy, bile become more lithogenic as a result of increased levels of estrogen, in addition

to that, the volume of gallbladder will be doubled, and stasis is developed **(6- 7)**.

4. Drugs and contraceptive pills: Estrogen is the most extensively studied drug or hormone that is associated with gallstone formation, also men taking estrogen have an increased incidence of symptomatic, gallstones.Other drugs include :
lipid lowering drugs (clofibrate) , somatostatin analog (octreotide) for acromegaly **(6)** .

5. Diet and dietary cholesterol : Diet seems to be a logical variable that could account for some of the discrepancies in gallstone prevalence reported from various countries. Unfortunately, this has not been the case. The results of studies have been conflicting, especially in regards to fat consumption. Studies are too numerous to elaborate on, but even in animal models, fat consumption may or may not cause an increase in gallstone formation depending upon which species are used for experiments. Increase dietary cholesterol increases biliary cholesterol but there are no epidemiological or dietary data to link dietary cholesterol with gall stones **(4)** .

NATURAL HISTORY OF ASYMPTOMATIC GALLSTONES 1-2 % of patients per year with asymptomatic gallstones develop biliary symptoms, once it is symptomatic patient have 50 % chance of having their next attack within a year, and they have 1-2% per year risk of developing acute cholecystitis or other complication. The patient with most frequent and prolonged attacks of colic over several months are at greatest risk for acute cholecystitis. **(7-8-9-10)** .

MANAGEMENT OF ASYMPTOMATIC GALLSTONES: A question is often raised about what to advice asymptomatic patients found to have gallstone during the course of unrelated studies. The presence of any of the following suggests a more serious course and should probably serve as a reason for prophylactic cholecystectomy : **1.** Diabetes mellitus , because the frequent and serious complications and high death rates (10-15%) in acute cholecystitis .
2. Large stones : greater than 2cm in diameter because they produce acute cholecystitis more often than smaller stones.
3. Calcified gallbladder wall , because it is so associated with carcinoma.
4. The association of gallstones with colo-rectal carcinoma indicates cholecystectomy for silent gallstone.
5. In laprotomy for other disease cholecystectomy is indicated when there is silent gallstone.
However, most asymptomatic patients have non of these special features.and need no interference **(9-10-11-12)**.

RESULTS:

The incidence of gallstones in our total 1016 sample is 3.3% and in 610 women , which constitute 63% of total participants is 4.09% , higher than in 406 men which constitute 37% of participants which is 2.2% The age distribution of silent gallstones in women and men is shown in table (1).

Table 1: Showing Age Distribution Of Silent Gallstones In Women and Men

Age group years	No. of examined women	No. of women with stones	Incidence of stones %	No. of examined men	No. of men with stones	Incidence of stones %
(18-29)	180	2	1.1%	81	Zero	Zero
(30-39)	119	5	4.25%	65	Zero	Zero
(40-49)	123	5	4.06%	96	2	2.08%
(50-59)	89	8	8.98%	50	2	4.0%
above 60	99	5	5.05%	114	5	4.3%
Total	610	25	4.09%	406	9	2.2%

For gender women showed a higher rate and are 1.8 times (A.R=44%) more than men with silent stones (25 cases out of 610 in women while only 9 out of 406 in men) (table 2).By measuring A.R (women 40 years and above) are 2.5 times more liable to have silent stones than who where(less than 40 years) (A.R =60%) (table 2) Among risk factors most obvious was multiparity 350/685 of woman were multiparity . Silent stones was found in 20 /350 cases of multiparous women while only five have stones,5/235 were non multiparous women and by measuring R.R multiparity associated with 2.7 times (A.R=63%) more than non multiparous for having silent stones (table 2) Silent stones where found in 10 /685 women with contraceptive pills intake out of 189 women with pills intake and do not have silent stones . Contraceptive pills associated with 1.4 times (A.R=28%) risk of having silent stones than those who never take it (table 2). There was 267 /1016 obese patients , 7 of them have silent gallstones , while 27 out of 715 of healthy or over weight have gallstones , R.R=0.7 , so obesity is not a risk factors for silent gallstones , p= 0.35 (table 2). For

blood groups the highest number of positive cases found in blood group O , 17 out of 17 for all other blood groups (R.R=1), which means no associations between blood group s and silent gallstones. (p=0.44) (table2) For family history 30 patients have positive family history and silent gallstones out of 4 have negative family history and silent stones , 148 have negative family history and no stones , 834 have positive family history and no stones (R.R=1.3) , so positive family history associated with 1.3 times risk for gallstones (A.R=23%) P= 0.33, (table4) Most of our volunteers drink 3 or more 60 ml cups of tea each day (615 against 401) silent gallstones where found in 24 of them while in only 10 of those who drinks less than 3 (60 ml) cups each day (R.R=1.6 and A.R=37%) , P=0.39 (table 2) As regard the number and size of stones in our study : 7 men and 18 women have less than three stones , while only 2 men and 7 women have three and more silent gallstones (table 3) In 31 patients the size of the largest stone is less than 20 mm , while only in three of the subjects the size of largest stone was more than 20 mm(table 3) Non had gallbladder mass or calcifications and , all had normal gallbladder size and wall thickness.

Table 2: Showing The Effect Of Risk Factors

Risk factors	No. of cases with stones	No. of cases without stones	R.R (relative risk)	A.R % (Attributed risk percentage)
1- Gender				
Female	25	585	1.8	44 %
Male	9	397		
2- Age/ years				
Female 40 & above	18	293	2.5	60%
Female less than 40	7	292		
3- pills intake				
used	10	189	1.4	28.5%
never used	15	396		
4- Parity				
Multiparous	20	350	2.7	63 %
Non-multiparous	5	235		
5- Obesity				
Obese	7	267	0.7	
Non- obese	27	715		
6- Blood groups				
O positive /Others	17 / 1 7	491 /491	1	no association
A positive /Others	8 / 26	225/ 757	0.9	
B positive /Others	5 / 29	156 /156	0.88	
AB positive / Others	2 / 32	156/826	0.9	
7- Family history				
Positive	30	834	1.3	23%
Negative	4	148		
8- No. of 60 cc cups of tea / day				
three or more	24	592	1.5	33%
less than three	10	390		

Note a relative risk of greater than 1.0 indicate a positive association.

Table 3: Ultrasound Findings In Patients With Silent Gallstones

Number of stones	men	women
Less than three	7	18
Three and more	2	7
Size of largest stone		

(5-10 mm)	5	7
(11-20mm)	4	15
(21-30mm)	zero	zero
more than 30 mm	zero	3

DISCUSSION:

To the best of our knowledge, this is the first population based survey conducted in Iraq to establish the prevalence of silent gallstones and its association with some risk factors.

The prevalence of gall stones in different countries compared to Iraq (our study) is shown in (table 4) Asymptomatic gallstones is more common in women than in men at all age groups, the same results were found in a old shown in (table 4) as will as in our study in Iraq .

(14,15,16,17,18,19,20). The prevalence increases with parity and the use of contraceptive pills, the same results were found in a study conducted for Peruvian population (16).

There was no association with any of blood groups, the same result was found in a study conducted in India (21). Most positive cases were found in a healthy or over weight individuals this goes with a study in U.S.A (14) , showing that obese people with gallstones are more likely to develop severe events (complications) than those who where thinner , so probably referred to the out-patient with gallstone by most of primary physicians than are the others. In a study conducted in India in 1999 , showed no correlation of asymptomatic gallstone with obesity , diet or socioeconomic state (19). Positive family history is associated with increase risk for silent gallstones, the same results were found in a study conducted in India in 1995, showed that there is a strong familial tendency for gallstone formation in relatives of gallstone disease patients (22), also in a study done in New Zealand in 2000 (23) . We found that most of the patients with silent gallstones drink three or more of 60 ml cups of black tea each day this might be incidental finding ,so we mentioned it as an observational finding in which a further study is recommended to support or abolish this finding Only three cases of silent gallstone, the size of largest stone was more than 20 mm , non have calcified gallbladder wall , this probably because most of asymptomatic gallstone have non of these specific features (11). Most patients with silent stones have less than three stones in number, this probably because the obstruction of cystic duct as reflected by the development of pain which occurs more frequently when there are multiple gall stones (10) . We did not discover any incidental associated gallbladder mass or tumor suggesting gallbladder carcinoma , this goes with a study done by Comfort and associates , they found no carcinoma among 112 patients with asymptomatic cholelithiasis , while the incidence of cancer in gallbladder of patients with symptomatic gallstones ranged from 1-15% with mean 4.5% (1,10,24,25).

CONCLUSIONS:

1. Silent gallstone more common in women than in men in all age groups and it is more common in women 40 years and above than in less than 40 years of age.

2. The prevalence increase with age , parity, contraceptive pills intake and familiar factor.

3. Obesity and blood groups have no important association with silent gallstones

4. most silent gallstones are less than three in number and smaller than 20 mm in diameter , with normal wall thickness and no associated gallbladder mass or calcifications.

Table 4 : showing the prevalence of gallstone in different countries.

Country	in Men	in Women
U.S.A (14)	5.5 %	8.6%
High Altitude Peruvian Population (15)	4.10 %	18.20 %
Peruvian Costal Native and High Land (16)	10.7 %	16.1%
Danish Population (17)	12.9 %	22.4 %
Okinawa, Japan (18)	2.5 %	4 %
India (19)	0.4 %	2.6 %
Iraq (our study)	2.2 %	4.09 %

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Evaluation of Amino acid Homocysteine in Hypertensive Patients

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ABSTRACT :

BACKGROUND :

Hypertension is a world wide disease and in 90% of cases the cause is unknown . Its early detection and treatment can prevent serious complications such as ischemic heart disease (IHD) . Furthermore the association of lipids with IHD is a well-known fact . However abnormally high levels of homocysteine were found to be strongly linked to an increase risk of coronary artery disease .

OBJECTIVES:

To evaluate the total plasma homocysteine concentration in hypertensive patients.

METHODS :

Total plasma homocystein concentrations were measured using High Performance Liquid Chromatography(HPLC) with Ultraviolet(UV) detector in 60 hypertensive patients (27 male and 33 female) aged 35 years and more . Cholesterol , triglyceride , HDL-cholesterol , LDL-cholesterol , VLDL-cholesterol were determined .The prevalence of high total homocysteine values were determined by comparison with normal reference population.

RESULTS :

Total plasma homocysteine levels were significantly higher in patients than in normal population. Total serum cholesterol and triglyceride concentrations were also significantly higher in patients than in normal population with no association to the level of homocysteine which is regarded as a special independent vascular risk factor.

CONCLUSION :

The study involves the evaluation of homocysteine in hypertensive patients plasma homocysteine levels were significantly higher in patients than in control groups. There were no significant differences between male and female patients.

KEY WORDS : Atherosclerosis , Homocysteine , Hypertension , Ischemic heart disease .

INTRODUCTION :

The diagnosis of hypertension in adult is made when the average of two or more diastolic and systolic blood pressure measurements on at least two subsequent visits are more than 90 and 140 mmHg respectively (1). Its early detection and treatment can prevent a lot of serious complications such as heart attack, stroke , kidney diseases and heart failure(2). Among the most important available risk factors for hypertention is serum lipids particularly serum cholesterol which is a solid alcohol of high molecular weight present in diet and is mainly synthesized in the liver and small intestine and excreted unchanged in bile or converted to bile acids to be excreted (3). It was also found that abnormally high blood level of homocysteine is strongly linked to an increased risk of coronary heart diseases as it may harm the lining of the arteries and contribute to blood clotting (4) by causing endothelial injury followed by platelet activation and thrombus formation (5).

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Homocysteine is a sulfur-containing aminoacid (M.Wt.268) formed during the metabolism of methionine (6)which can be found in meats and dairy products ,therefore high dietary consumption of such products can result in the overproduction of homocysteine. Elevation in plasma homocycteine is either caused by genetic defects in the enzyme involved in its metabolism or due to nutritional deficiencies of vit.B6 , B12 or folic acid because these vitamins are essential co-factors for enzymes involved in the metabolism of homocysteine like methionine synthase and 5,10 methylene tetrahydrofolic acid reductase(7).

Hyperhomocysteinemia may be associated with several disease states and medications. It may increase in chronic renal failure often approaching concentrations that are up to four times the normal value which may explain the observed acceleration of atherosclerosis in end stage renal disease(8).

There is also some association between hyperhomocysteinemia and patients with hypothyroidism and rheumatoid arthritis which suggest a potential mechanism for the high incidence of vascular disease observed in those

patients. Several drugs like methotrexate and phenytoin interferes with folate metabolism and may cause mild hyperhomocysteinemia(9,10).

METHODS :-

The study was conducted on 60 hypertensive patients (27 male , 33 female) attending Kadimya Teaching Hospital as an out patient over 6 months period with an average age of 35- 85 years. Another 20 healthy subjects were studied as a control group . A full personal and family history was taken from each patient. Seven ml of blood was drown into a sterile disposable syringe.

Five ml from the sample were allowed to clot after 30 minutes in a plain tube and serum was separated by centrifugation at 3000 rpm under room temperature for 5 minutes. The serum was immediately analyzed for lipid profile .

The remaining 2-ml were put in a K3 EDTA tube and immediately put in a crashed ice . The plasma was separated from RBC by cooling centrifugation at 3000 rpm under 4C for 5 minutes then stored under – 20C until analyzed. Total cholesterol was determined by Cholesterol Enzymatique PAP Kits (BIOMERIUX). Triglyceride was determined using enzymatic method supplied by BIOMERIUX Lab. HDL-Cholesterol serum level was determined by precipitation of VLDL-Cholesterol and LDL-Cholesterol by the addition of phosphotungastic acid in the presence of magnesium ions.

The supernatant obtained after centrifugation contains only HDL-Cholesterol on which the cholesterol fraction was done by the same enzymatic method mentioned in cholesterol. Serum level of LDL cholesterol was calculated by Friedwald formula. Determination of the biologically active homocysteine (THCY)was done by using HPLC with Schimodzu SPD-6AV UV-visible detector within a wavelength of 195 – 700nm(11).

RESULTS :

The study showed a significant higher total plasma homocysteine concentration in our hypertensive patients (56 ±40.15 µmol/l) than in control group (14±3.9 µmol/l) with P< 0.05but the study showed no significant differences in homocysteine levels between male and female as shown in table-1. The severity of homocysteinemia is shown in table –2 where intermediate hyperhomocysteinemia was representing 55% of all cases compared to moderate and sever degrees which were representing 30% and 15% of all cases respectively(11). Table –3 shows that controlled hypertensive patients representing 73.33% of all cases with a mean homocysteine level of 50.7±29.2 µmol/l , while 26.66% were uncontrolled hypertensive patients with a mean homocysteine level of 62.6 ±27.4 µmol/l (12).

The study also demonstrated that total serum cholesterol concentrations in patient group were significantly higher than the concentration in control group. Mean serum triglyceride concentrations in patient group were also higher than in control group as shown in table– 4.

The table also shows that there were significant differences between patient and control groups in serum concentrations of HDL-Cholesterol , LDL-Cholesterol and VLDL-Cholesterol.

It is also clear from the study that 32 patients who had hypercholesterolemia more than 220 mg/dl and 21 patients who had hypertriglyceridemia more than 160 mg/dl had no relation to the level of homocysteine which is regarded as a specific risk factor independent from other risk factors as it is demonstrated in table-5.

Table 1 :Total plasma homocysteine concentration in patients and control groups .P< 0.05 .

	[Homocysteine µmol/l		
	N	Mean	SD
Patients	Male 27	58.15	3.66
	Female 33	55.45	4.36
	Total 60	56.8	4.014
Control	20	14	3.9

Table 2 :Classification of plasma homocysteine according to concentrated .

level in male and female patients.

		Male			Female		
		N	Mean±SD	%	N	Mean±SD	%
Moderate	15-30 µmol/l	8	22.9±7.8	29.6	10	20.3±5.2	30.3
Intermediate	30-100 µmol/l	15	55±20.6	55.6	18	45.7±15.8	54.5
Sever	>100µmol/l	4	102.8±40.1	14.8	5	117.4±50.5	15.2

Table 3 : Plasma homocysteine in controlled and uncontrolled hypertension

	Homocysteine µmol/l			
	N	Mean	SD	**%**
Controlled hypertension	44	50.7	29.2	73.33
Uncontrolled hypertension	16	62.6	27.4	26.66

Table 4 :Lipid profile in patients and control groups

	Patient		Control		P<0.05
	Mean	SD	Mean	SD	
Cholesterol (mg\dl)	221.94	45.82	173.20	18.57	Significant
Triglyceride (mg\dl)	182.09	111.04	117.89	43.22	Significant
HDL-chol. (mg\dl)	41.95	14.31	52.72	12.41	Significant
VLDL-chol. (mg\dl)	36.13	22.41	19.11	8.81	Significant
-LDL	138.19	49.70	83.55	18.31	Significant

Table 5 :Plasma homocysteine with hypercholestrolemia and hypertriglyceridemia

	Homocysteine µmol/l			
	N	Mean	SD	%
Hypercholestrolemia >220 mg/dl	32	53.99	33.74	53.3
Hypertriglyceridemia >160 mg/dl	21	56.47	31.9	35

DISCUSSION :

The study had demonstrated a significant increase in plasma homocystein concentration in hypertensive patients and an essential linear relationship between homocystein level and vascular risk which was also found by Graeme et al.(13). There was no significant difference in plasma total homocystein concentration between male and female patients with moderate and intermediate hyperhomocystein level.

This difference increased in patients with sever hyperhomocystinemia which is similar to the finding of Stamler ,et al (14) . Hyperhomocystinemia was found in 73.33% of all patients which may be due to along interval (without control of hypertension)of disease which was more than 10 years . The high concentrations of homocystein in uncontrolled hypertensive patients might be due to sever hypertension as a result of neglection of treatment by antihypertensive drugs(15).

Total serum cholesterol and triglyceride concentrations was significantly higher than the concentration in control group and this result agree with Neaton and coworkers who have reported increases in cardiovascular outcome events with increasing cholesterol levels (16).This also agree with a previous finding in another study which found a significant triglyceride – coronary disease association (17) . HDL-Cholesterol in patients and control groups were significantly different and a more recent study showed a basic association of high HDL and low risk of coronary diseases .

A similar finding for both LDL and VLDL were obtained . The results showed that increase in cholesterol and triglyceride levels have no relation to the levels of homocystein which is regarded as a special independent risk factor(18) .

These findings agree with Konecky et al. who showed that hyperhomocysteinemia is an independent risk factor for aortic diseases (19).

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Role of Color Doppler Ultra Sound Versus Histopathology in Differentiating Malignant From Benign Breast Masses

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ABSTRACT:

BACKGROUND:

Cancer of breast is by far one of the most important clinical problems that concerns the surgeon. It is the commonest form of cancer in females, when detected early and given the proper treatment can be cured. Color Doppler imaging is modern technique that can be used in preoperative assessment of breast lumps.

OBJECTIVE :

To asses the role of color doppler imaging in differentiating malignant from benign breast lumps in comparison with the histopathology in AL- Najaf teaching hospital.

METHODS:

Total number of patients was 80 female of different age groups were assessed during the period from first of February 2004 to the first of February 2006 , presented with variable palpable breast masses. After clinical assessment, all our patients underwent , color doppler imaging and then subjected to the excisional biopsy and histopathology for confirmation of the diagnosis.

RESULTS:

The results of color doppler imaging for all our patients were found as follow, benign lumps in 54 cases (67.5%),malignant lumps in 26 cases (32.5%),two cases diagnosed as benign by color doppler imaging proved to be malignant by histopathology
giving (2.5%),false negative rate. All obtained results of sensitivity, specificity and over all accuracy of color doppler imaging respectively as follow (92.85%),(100%),and(97.5%).

CONCLUSION:

Color doppler imaging is highly sensitive and specific method in evaluating malignant breast masses and is painless, non-invasive and time saving procedure, so we can reduce the rate of unnecessary surgery for histopathology.

KEY WORDS : Colored Doppler Imaging(CDI),Breast Mass, Breast Cancer.

INTRODUCTION:

Differentiation between benign and malignant solid breast lumps by means of (CDI) has gained increased interest. In (CDI), the doppler signals received from flowing blood are processed and color-encoded .[1,2] (CDI) could be thought to increase diagnostic confidence in two ways either by showing vascularity in morphologically benign lump(even in cancers with a negative B-mode nature of lumps that are benign) or indeterminate at B-mode ultrasound. [1] It has been shown that (CDI) can reduce the number of open breast biopsies. [3] Tumor angiogenesis is a well known phenomenon for tumor growth, increasing numbers of new vessels are associated with an increased risk for malignancy . Doppler analysis proved to be successful on it's own for distinguishing benign from malignant lumps. [4] Neoangiogenesis demonstrated as:

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- Feeding signal, entering the tumor through it's border with adjacent structure.
- Spotting signal, vascular spots within the tumor.
- Draining signal, coming out of the tumor.
- Penetrating signal, passing through the tumor zone.
- Feeding and draining vessels mostly present in primary tumor.

Neo angiogenesis is established in (80-85%) of primary malignant tumor and in (5-10%) of benign tumor concentrating on this phenomenon we can differentiate between benign and malignant tumors.[5]

PATIENTS AND METHODS:

80 patients presented with variable breast lumps were selectively chosen in this prospective study that was done in period from the first of February 2004 to the first of February 2006 in AL-Najaf teaching hospital. All cases were subjected to clinical evaluation (including history and physical examination), CDI and histopathological examination.

Every patient was submitted to examination by U/S using Siemens-versa Pro U/S machine and/or Kretz Voluson by 7.5 Mega Hertz(MH_2)linear probe (B-mode and color Doppler imaging) to evaluate structure and vascularity of breast masses and all patients were examined by the same operator.

Complete examination of the breast by 7.5 MH_2 probe with evaluation of mass vascularity, compared this with contra lateral area in the normal breast, the examination included the axilla of the both sides. After open surgical biopsy which is either incisional, excisional or mastectomy specimen, all specimens were fixed in 10% formaline solution and later stained by haematoxyline and eosins stain either in Al Najaf teaching hospital or in private laboratory.

RESULTS:

Total number of cases was 80, the ages range between 16-65 Years with an average of 40.5 Years. The peak age incidence was between 30-39 Years as shown in table (1). Regarding the marital status of our patients with benign and malignant lumps 66 cases were married (82.5%).

The first common presenting complaint of patients was Breast lump and the second presenting complaint was pain. The distribution of masses between both breasts. malignant breast lumps are mainly found in right breast(69.3%), while benign lumps are mainly found in left breast (63%). Among the malignant cases , 4 cases presented with Axillary lymphadenopathy.

The incidence of lumps in relation to the quadrants of breast were different and it is more common in upper outer quadrant in both benign and malignant lumps.

Results of CDI.

1- Malignant (positive) = 26 patients diagnosed as malignant by CDI and confirmed by histopathological examination, so there is no false positive diagnosis table(2).

2- Bengin (negative) = 56 cases the result of CDI were benign, but the histopathological examination confirmed that in 52 cases and only 2 cases were found to be malignant, so there were two false negative diagnosis, table(2). Results of Histopathological Examination(HPE). 52 cases were benign, 28 cases were malignant, table(3).

Table (1):The distribution of benign and malignant lumps of breast diagnosed by CD study according to the age group.

Age of the patient (years)	Benign lesion		Malignant lesion	
	No. of patient	%	No. of patient	%
10-19	4	7	-	-
20-29	14	25.9	-	-
30-39	30	55.5	4	15.4
40-49	6	11.6	12	46.2
50-59	-	-	6	23
60-69	-	-	4	15.4
Total	54	100	26	100

Table (2):Results of CD-study of 80 patients with breast masses.

Results of CD-study	No. of patients	%
Benign	54	67.5
Malignant	26	32.5
Total	80	100

Table (3):Result of histopathology of 80 patients with breast masses.

Results of Histopathology	No. of patients	%
Benign	52	65
Malignant	28	35
Total	80	100

Table(4):Correlation between CD-study and histopathological results.

CD-study	No. of patients	%	Histopathology		
-	-	-	Benign	Malignant	%
Benign	54	67.5	52	2	65
Malignant	26	32.5	-	26	35

Table(5):Validity of CDI in diagnosing malignant breast masses according to histopathological examination.

	+ve malignancy	-ve malignancy	Total
+ve malignancy	26/TP**	0/FP	26
-ve malignancy	2/ FN	52/TN	54
Total	28	52	80

* HPE (validating test) CDI

P<0.001

Sensitivity =TP/all malignant x100

26/28x100=(92.85%)

Specificity =TN/ all non malignant x100

52/52x100=(100%)

Accuracy rate=TP+TN/ totalx100

78/80 x100=(97.5%)

**TP=True positive, FP=false positive, FN=false negative , TN=True negative,
*HPE=Histopathological examination.

Tables (6&7) show the CD-findings versus histopathological findings of both benign and malignant lumps in 80 patient

Table(6):CD-study findings versus histopathological diagnosis of benign lumps in 54 patients.

Nature of mass	No. of patients	Findings of CDI	Ri	Size	Histopathological results
Cystic	4	Avascular	-	3.5-4 cm	Calactocoele
Solid	14	Avascular	-	0.5-1 cm	Fibroadenoma
Solid	26	Mild increment in the peripheral vascularity	0.40-0.55	1.5-2.5 cm.	Fibroadenoma(22 cases) Ductectasia (4 cases)
Solid	6	Mild increment in the central vascularity	0.55-0.60	1.5-2 cm	Fibroadenoma
Solid	2	Mild increment in the central vascularity	0.64	0.88-0.75 cm	Intraductal carcinoma
Solid	2	Mild increment in the central vascularity	0.66	2 cm	Fibroadenoma

Table(7):CD study findings versus histopathological diagnosis of malignant lumps in 26 patients.

No. of patients	Findings of CDI	RI	Size	Histopathological results
16	Increment of central and peripheral vascularity	0.69-0.82	1.5-2 cm	Intraductal carcinoma
6	Increment of internal vascularity	0.75-0.80	2-3 cm	Intraductal carcinoma
4	Increment of central and peripheral vascularity and presence of penetrating vessels	0.70-0.71	1.5-3 cm	Invasive lobular carcinoma

RI (Resistance Index) = S-D/S
(S) =Systole, (D) =Diastole .

Table (8): Statistical results of CD-study.

Statistical parameters	CD-study results
True positive	26
False positive	-
True negative	52
False negative	2
Sensitivity	92.85 %
Specificity	100%
Accuracy	97.5%
Positive predictive value	100%
Negative predictive value	96%

DISCUSSION :

The value of any diagnostic test lies in it's ability to detect the presence of disease when it's present (sensitivity)and verify the absence of disease when it is not present (specificity). [5] In this study CDI was used in the diagnosis of palpable breast masses in 80 female patients, all those patients were subjected to HPE. The number of patients was low due to different causes, the most important one is that many patients refused breast biopsies due to wrong believe that HPE may change benign lesion into a malignant one, other cause contributed in the reduction of number of visitors to our breast clinic was the last war's circumstances in Al- Najaf city which lead to close the teaching hospital for about 8 months. The breast lumps in our study occur in patients predominantly between 30-39 years old, the peak incidence of benign lumps was between 30-39 years old and of malignant lumps was 40-49 years old which is similar to that reported by Benedetto mentioned that carcinoma of the breast is extremely rare below the age of 20, but thereafter it is increasing. [6] Regarding the relationship of malignant and benign lumps to the quadrants of breast , the upper outer quadrant was involved in (75%) of malignant lumps and in (54.63%) of benign lumps, this is similar to other study done by Steinberg et al, 1996 and Meterissian et al, 1995. [7,8] In this study the use of contraceptive pills was in 24 patients(44.4%) with benign lumps and 15 patients (57.69%) with malignant lumps, in the contrast to other studies in which the place of oral contraceptive pills and hormonal replacement therapy as a low risk factor which remains controversial, because in our society the large usage of pills among females in contrast to other methods of contraception could be a matter of religion and this study is similar to the study was done by Powles et al (1991).[6] Lump and pain were the commonest associations in our patients presentation all patients presented with lumps, while in 24 (44.4%)of benign cases and in 4 (15.3%)of malignant cases were suffering from pain and this is consistent with other study by Arbor ; (2000), which reported that most breast cancers present as a hard lumps, while in benign the common symptoms are pain (47%)and lump in (35%). [7,8] The findings of CDI in malignant lumps revealed that all malignant lumps show increased central and/or peripheral vascularity where RI ranging 0.69-0.82 ,while in benign lump only 8 cases show minimal or mild internal vascularity where RI ranging 0.5-0.6, and the remainder lumps were a vascular and this is similar to that reported by Al-Gul et al, (2003) that neo angiogenesis was established in (80-85%)of primary malignant tumors and in (5-10%) of benign tumors ,[9]and to other study done by Reston (2001).[10] In our study the sensitivity, specificity and accuracy of CDI were ; Sensitivity =(92.85%), Specificity = (100%), Accuracy = (97.5%) these results are approximately similar to the study was done by Cosgrove et al. who used CDI to evaluate breast masses in 60 patients, they found that 20 of 21 breast cancers demonstrated the accuracy was (98.3%), the sensitivity was (95%) and the specificity was (97%), [11] other study by Raza et al (2003) with specificity of (95%).[12] In other studies, Jackson obtained a sensitivity of (96%) in the evaluation of 35 solid breast lumps in 1992, [13] Taylor et al . have identified abnormal Doppler signals obtained by Doppler ultrasound in a variety of malignant tumors, with accuracy of (86%) of 44 cases . [14] That difference between our study and other studies, because other studies were done in 1992 which was the near beginning use of CDI in breast lumps and this reflect the low experience and less developed machines.

CONCLUSIONS AND RECOMMENDATIONS:

1.CD-study is sensitive and highly specific method of evaluating breast mass for malignancy.

2.This type of procedure is essentially painless and non-invasive and can be done as outpatient procedure ,and can be repeated safely at the same or further session, but it is an operator dependant.

3.In this study I hope that CDI in addition to other diagnostic modalities such as ultrasound and fine needle aspiration cytology may replace histopathology in detecting breast cancer in future.

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The Pattern Of Indications, Complications Of Splenectomy Personal Experience

Nooraddin Ismail Allaqolli

ABSTRACT:

BACKGROUND:

A prospective study of Splenectomy personal experience,with changing pattern of indications.

METHOD:

During the study period (Feb, 1990-Jan, 2006), a total of 411 splenectomies performed in erbil governate for different indications.

RESULTS:

The study included 411 patients, the age rang were 4– 65 years. Out of 411 cases 201 cases were due to trauma (48.9%).

CONCLUSION:

Trauma of different types remains the most common indication for splenic surgery & complications like gastric or pancreatic fistula is historical complications.

KEY WORD: Spleenectomy.

INTRODUCTION:

Galen is credited with the phrase (The spleen is an organ full of mystery). Fetal splenic tissue develops from condensation of mesoderm in the dorsal mesogastrium. The weight of the normal adult spleen is 75-250 gm; it consists of white & red pulp that is surrounded by serosa & capsule that contain muscle.

The spleen has following functions:-

1. Immune function.
2. Filtration.
3. Pitting.
4. Reservoir function.
5. Cytopoiesis.

Prior to 1970 the treatment for traumatic ruptured spleen was splenectomy, the recognition that patient without spleen have an increased risk of death from overwhelming infection lead the surgeons to consider the method of splenic preservation & significant changes have occurred in the management of splenic injuries in the last two decades [1].

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PATIENTS & METHODS:

A personal prospective review of 411 patients' scheduled for splenectomy done in Erbil Governate in three hospitals (Rizgary teaching hospital, casuality & Hawler private hospital) from Feb, 1990-Jan, 2006. The diagnosis was on clinical bases & blood examination, augmented by ultrasound examination & confirmed by operation in traumatic cases.

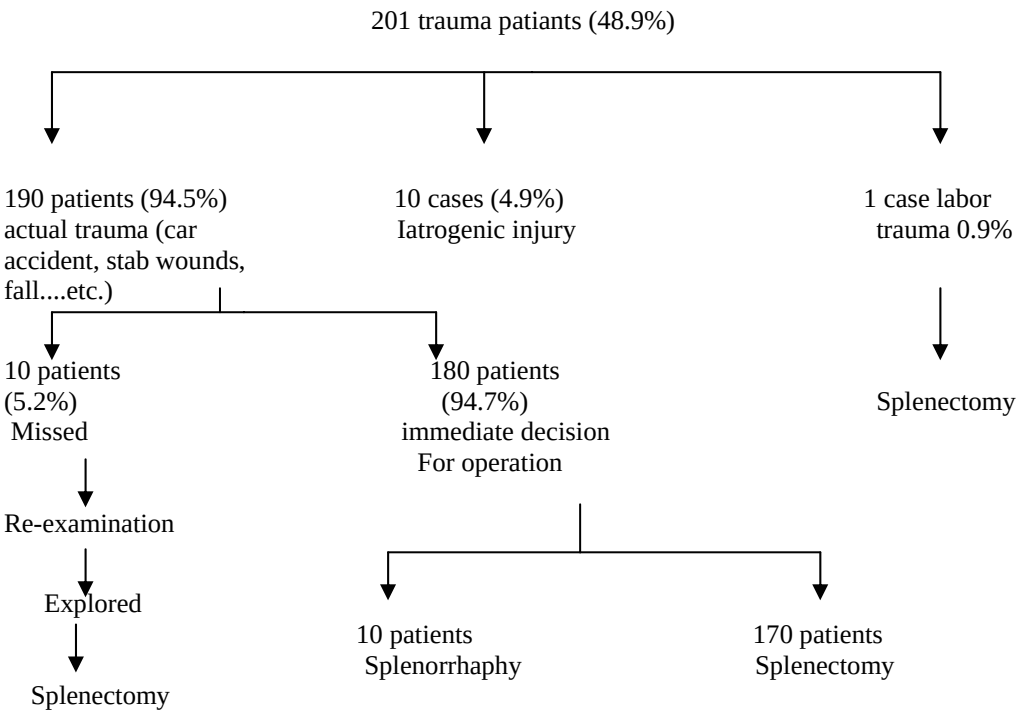
The ages of the patients were in between 4-65 years (mean age 57.4); males predominate on females (250 male to 151 female).

Investigations for hematological cases were done in medical side & splenectomy was performed on their advice.

RESULTS:

The study included 411 patients, their ages ranged between 4-65 years with mean age 57.5, there were 260 males (63%) & 151 females (37%).

Out of 411 patients, 201 cases were due to trauma as follows:



180 patients (43.7%) were splenectomised for blood diseases, all diagnosed by physicians & operated upon on their decision.
6 patients (1.4%) splenectomised for hydatid cyst.
2 patients (0.4%) splenectomised for infectious diseases (malaria).
1 patient (0.2%) splenectomised for abscess.
1 patient (0.2%) splenectomised for torsion of the splenic pedicle.

COMPLICATIONS

It occurred in 36 cases as follow:

- Atelectasis in 20 patients (2.4%).
- Wound infection in 10 patients (1.2%).
- Bleeding in 2 patients (0.4%).
- OPSI in 2 patients (0.4%).(overwhelming post splenectomy infection)
- Acute gastric dilatation in 2 patients (0.4%).
- Haematemesis (0%).
- Gastric fistula (0%).
- Pancreatic fistula (0%).

DISCUSSION:

While non operative management has been effectively practiced in developed countries, it is virtually absent in our region for the following reasons:

1. Lack of suitable image.
2. No real monitoring requirement.
3. Lack of high level nursing care.
4. Lack of rapid mobilization of operating room.

Out of 411 cases, 190 cases were explored due to trauma (46.2%), in a form of car accidents, stab wounds & fall from height. All these 190 cases explored depending on clinical examination, ultrasound report & peritoneal tapping.
All patients were resuscitated & transfused blood which has minimized post operative complications, which are the same findings in other study [2].(Solomonv 2000)
Although CT scan may guide management decision [3], but it was not done in our series since it is not available at casuality hospital.
Ultrasound examination was done & was sensitive in almost 90% of cases, though there are studies showing low levels of sensitivity [4, 5 & 6].
Reliance on free imtraperitoneal fluid may be not accurate because not all of them have free imtraperitoneal fluid.
Out of 190 patients, 180 patients explored immediately after resuscitation & 10 patients kept on conservative treatment & explored after 24 hours (mis diagnosis).
So out of these 190 cases, 180 patients (94.7%) were splenectomised & only in 10 patients (5.2%)

splenorrhaphy were performed, while this was done in other study in 12% [8].(Clancy 1997)

Although non-operative management was not practiced in our region, there were studies that practiced it only in 5% of cases [9] in the form of artery embolisation [10 &11] & they showed no age contraindications for non-operative management [12], but there are studies showing failure rate of 17% - 40% [13, 14, 15 & 16].

So it seems that appropriate selection is the most single important point in the decision [17].

In our series, 10 patients were missed (5.2%), while in other studies it is 2% [18]. the reasons for missing were:

1. Radiological misinterpretation.
2. Incomplete exploration.
3. Surgical inexperience.
4. Severe peritoneal adhesions.

Most of these cases occurred in our early life in surgery (1990-1995) due to lack of experience & unavoidable circumstances in our region. We & others [18] concluded that careful history, complete diagnostic procedures & good exploration are important factors.

IATROGENIC INJURIES:

Out of 201 patients with trauma, 10 cases (4.9%) were due to iatrogenic injuries {any injury which is unintentional damage caused by the surgeon or the assistant during operation}, all were splenectomised. It is a recognized complication, but usually underestimated.

The risk of injury was higher with previous operations, in obese patients, excessive traction & injudicious use of retractors & this injury increased operative time & blood loss as in other series [18].

As mentioned above, the rate in our series is 4.9%, while iatrogenic injuries in other series are between 9% - 40% [20 & 21].

The risk was higher during left hemi colectomy, Five cases (50%), while in other series it is 1.2% - 8% [22 & 23].

Iatrogenic injuries occurred in three cases (30%) during operations on stomach (vagotomy & drainage operation), this is the same finding as others [24].

In our series, we have one case of rupture spleen due to force pressure on the abdomen during labor by nurse. She was 40 years old gravida 8, para 7 & denied any history of malaria. Spontaneous rupture during pregnancy & labor is rare & usually occur during third trimesters [24].

Out of 411 cases splenectomised, 180 patients were for different hematological diseases (43.7%), while in other series was 25.5% [26], so it appears that the indications for splenectomy have changed & hematological diseases are emerging as second common indication.

The mean weights of the excised spleen in our series from hematological causes were 100–106gm, which is almost the same finding with others [26].

It is interesting to mention that we used to do total splenectomy for cases of hematological conditions, not only that we used to search for spleniculi in the peritoneum & remove it to prevent it's enlargement, but Stoehr used to do nearly total splenectomy claiming that total splenectomy is complicated usually by sever infection & thromboembolic events [27].

In our series, we have done 6 splenectomies for hydatid disease of the spleen (1.4%), while in other series it is (2.5%) [13].

Splenic cysts are rare & during the last two decades splenectomy can be done laparoscopically [29]. There are options nowadays to remove the cyst only to prevent complications of splenectomy [30].

In our series we have one case of splenic abscess (0.2%) of unclear aetiology. He was 15 years old male patient & has splenectomised. Splenic abscess are rare & are potentially serious surgical problem with high mortality rate [31].

One case of wondering spleen was found (0.2%) with torsion, while in other series it is 0.5% [7]. She was 18 years old female patient admitted with left loin pain & vomiting. Ultrasound revealed that spleen was not found in it's normal anatomical position, however a well defined homogenous mass of splenic texture with long pedicle was seen in the pelvis. She was explored & torsion of the pedicle found causing infarction of the spleen which was removed.

The presentation of wondering spleen is almost the same in all cases [32], which is a rare entity with only less than 500 cases reported so far [33]. It is usually due to congenital absence of intraperitoneal visceral attachment [34].

COMPLICATIONS :

In our series, comp lications occurred in 36 cases (8.7%) as follows:-

- Atelectasis in 20 patients (2.4%).
- Wound infection in 10 patients (1.2%).
- Bleeding in 2 patients (0.4%).
- OPSI in 2 patients (0.4%).
- Acute gastric dilatation in 2 patients (0.4%).
- Haematemesis (0%).
- Gastric fistula (0%).
- Pancreatic fistula (0%).

- Over all complications was 8.7%, while in other series it is 31% [32].

In our series there is no statistical difference between splenectomised patients & splenorrhaphy, this is the same finding in other series [33].

All patients received blood transfusion with no effect on rate of infection; this is in contrast to other's findings [35].

CONCLUSION:

Splenic operations {splenectomy & splenorrhaphy} are still among the common operations done by general surgeons. Trauma & hematological conditions remain the most two common indications for operation. Gastric & pancreatic fistulas are two historical complications in our series.

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Random Abdominal Flaps for Reconstruction of Upper Limb War Injuries: A Good Option for a Bad Time

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ABSTRACT:

BACKGROUND :

Soft tissue war injuries of the upper limb are usually extensive and multiple . Plastic surgeons face the challenge of reconstructing these injuries in patients who are multiply injured ,and in a time when facilities for free flap surgery are not available.

OBJECTIVE:

To demonstrate the efficacy of random abdominal flaps in reconstructing soft tissue defects of the upper limb.

METHODS:

From May 2003 to September 2005, 55 war injury patients with deep soft tissue loss in the upper limb were managed by random abdominal flaps of different shapes and directions, 13 of them had other soft tissue injuries affecting other areas in the body. The surgeries were done in busy general hospitals during war where time, personnel and facilities are limited.

RESULTS:

In 53 patients, the flaps had completely survived without complications, 2 flaps developed partial tip necrosis and healed later by secondary intention. Donor areas were covered by split thickness skin grafts in 52 cases, and directly closed in 3 cases. The largest flap dimension was 18 cm length and 15 cm width. The main disadvantages of the flap are donor site scar, bulk of the flap, and the need for a second stage for flap separation.

CONCLUSION:

Random abdominal flaps are easy, safe, versatile and operative time saving option for coverage of upper limb injuries. It can be designed in any direction to cover different soft tissue defects. These flaps still continue to be an excellent alternative for free tissue transfers during war time.

KEY WORDS: Random abdominal flap, Upper limb .

INTRODUCTION:

War injuries of the upper limb are usually penetrating and extensive , the special structural considerations of the hand make its reconstruction after a missile injury more difficult, and the closure of the resulting wounds cannot be simply done by direct closure or skin grafting. Extensive penetrating wounds may need complex flap coverage or free tissue transfers to obtain one-stage repair of the defect and reconstruction of the motor function of the muscle [1,2,3] . In a busy surgical ward ,when the surgical teams are overwhelmed with cases of war injuries, it may be impossible to perform complex surgeries like free tissue transfers .Moreover, the victims commonly have multiple injuries in different areas of the body ,and some of these injuries are life threatening and need to be surgically managed first [1,4] . Many flaps have been described for the coverage of soft tissue loss in the hand and forearm .

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Distant pedicled flaps like abdominal flap can provide an alternative method to free tissue transfer and performed as a salvage procedure in the management of war victims [5-9]. In this study missile injuries in the hand and forearm were reconstructed by random –pattern flaps based on different sites of the abdominal wall (Figure 1).

PATIENTS AND METHODS:

Between May 2003 and February 2006, 55 war injury patients (41 males and 14 females) were admitted to the plastic and reconstructive surgery departments in two hospitals in Baghdad. The patients age ranged between 13 and 76 years old. All of them have severe soft tissue loss and exposure of underlying bones, nerves and tendons. The injuries were caused by bullets or explosive missiles and are located in the hand, forearm , elbow or distal arm(Table1). In 13 patients , the upper limb trauma was associated with other injuries in the head , neck, lower limb , intra-abdominal organs ,as well as thermal burns necessitating emergency operations in some of them . The patients general conditions were stabilized first. Wound excisions and external

fixation of the forearm fractures were done in 21 cases before referral to the plastic surgery ward. (Table 2). Abdominal flaps were planned over the abdominal wall according to the requirements of the upper limb defect (Figure 2) . The flaps were located longitudinally, horizontally or obliquely , their pedicles were superiorly , inferiorly ,medially or laterally based (Figure 3) . In two cases the extremity had 2 penetrating wounds (volar entry and dorsal exits), two flaps were elevated (one is based superiorly and the other inferiorly) and the extremity was sandwiched in between (Figure 4). The operations were done under general anesthesia , elevation of the flap started at its distal end ,including the skin and the entire thickness of the subcutaneous tissue .The recipient wound edges were undermined for one centimeter and then sutured to the flap edges by silk sutures. Donor sites were closed directly in 3 cases or by split thickness skin grafting and the hand and forearm were kept in place by adhesive plaster wrapped as slings around the shoulders and trunk.

Flap separation was done after 21 days and the remaining stump of the flap was set again in the abdominal wall. Further reconstructive procedures (bone, tendon or nerve grafting) were deferred until a stable soft tissue cover was provided.

Table 1 : Sites of injury and their numbers

Site	Number of cases	Percent
Fingers	12	21.8
Hand	17	30.9
Forearm	23	41.8
Elbow	2	3.6
Distal arm	1	1.8
Total	55	100

Table 2: Surgical interventions before referral to the plastic surgery ward

Operations	Number of cases
Lapratomies	6
Lower limb amputations	2
Forearm fracture fixation	21
Lower limb fracture management	5
Tracheostomy	4
Wound debridment in different areas	27

RESULTS:

In 53 patients the flaps completely survived and the wounds were adequately covered , 2 patients developed partial flap loss one of them is a diabetic and hypertensive elderly man, the necrotic areas were left to demarcate and separate spontaneously ,after that the areas healed by secondary intention (Figure 5). The largest flap dimensions were 18 cm length and 15 cm width and the largest Length-to-Width ratio was 1:3 . The flaps were based on different directions and planned in different shapes to fit the needs of the reconstructed wounds (Table3). The site of the flap was also variable, and its selection was dependant on the local condition of the abdominal wall and the location of the recipient site (Table4). All the complications were correctable and most of them were due to skin maceration at the palmar aspect of the hand due to continuous moisture (Table5).

In 3 patients ,the donor sites were closed directly , in the remaining 52 donor sites partial thickness skin grafts were needed because the flap dimensions were large.

Table 3: Orientation of the flaps

Flap pattern	Number of cases
Superiorly based	22
Inferiorly based	10
Medially based	3
Laterally based	3
Obliquely oriented(superomedially based)	15
Double flap coverage	2

Table4: Locations of the flaps.

Location	Number of cases
Flaps elevated from the ipsilateral side of abdomen	19
Flaps elevated from the contralateral side of abdomen	23
Flaps crossing the midline of the abdomen	10
Flaps elevated from the abdominal wall and based on the chest wall	3

Table 5: Complications.

Complications	Number of cases
Partial flap loss	2
Infection	1
Skin maceration	5
Partial donor site graft loss	2

DISCUSSION:

The horrible events of war cause so much human pain , suffering and loss of lives. Surgeons face the challenge of managing such overwhelming number of war victims .In this study ,we managed many cases of war injuries in a busy surgical ward with a remarkable shortage of equipments and facilities . All the wound were deep with exposure of underlying bones, tendons, nerves and vessels. Surgical options for such cases are local flaps, distant pedicled flaps, or free flaps.

The wide zone of the injury made the use of local flaps impossible ,the same reason made distant flaps like the groin or the superficial inferior epigastric flaps are inadequate. Other pedicled flaps like tensor fascia lata flap cannot cover more proximal wounds in the forearm. The advent of microvascular free flaps has revolutionized the approach to the hand injuries with extensive soft tissue loss. It provides the best option in one surgical stage .However ,in our circumstances, microsurgical expertise and equipments are not available , and the patients do not have the ability to tolerate a multi-hour surgical procedure.

For these reasons we reconstructed the defects by random abdominal flaps which proved to be simple and easy to excute ,time saving, provide adequate donor tissue for coverage of large defects, versatile, and can be performed without the need for special facilities or equipments. Because of the safety of the flap ,some authors elevated the flap from scarred abdominal skin successfully [10] ,others performed thinning of the flap to have the best appearance [8,9,11] . In this study we did not perform thinning of the flap at the primary operation since secondary surgical debulking is more safe.

We found that the vascularity of the flap is excellent when the flap is planned in any direction and wherever the base is made .Ylmaz and colleagues based the flap on the paraumbilical perforators to improve the vascularity which enables narrowing of the pedicle [12] ,although we based the flap on the paraumbilical perforators in a number of cases, we did not narrow the pedicle as this may increase the tension on the base and increase the adverse effects of any minor kinking in it. Making the flap in the upper part of the abdominal wall have the advantage of decreasing the edema in the hand but it increases the risk of elbow stiffness. On the other hand, if the flap is elevated from the contra lateral side, the positioning and turning of the patient in the bed are more easily performed , while using a flap from the ipsilateral side will decrease the downward pull on the flap base if the weight of the extremity (and probably the external fixator) is not properly supported by plaster straps. However, the main criterion which decides the side of the donor area is the site of the defect on the extremity, wounds in the digits, hand, or the distal forearm are adequately positioned if the flaps are located in the contra lateral side, and the opposite is true for more proximal wounds. Making the flap length longer than the actual length needed is very important in preventing kinking at the base, decreasing the tension at the base, and facilitates donor area care. But making it too long will jeopardize the vascularity, in this study we made the length: width ratio as much as 1:3 in some cases without flap loss, except in 2 cases when partial loss was observed, one of them was a diabetic elderly man. In both cases the resulted wounds closed spontaneously by wound contraction and epithelialization. Stabilizing the hand to the abdominal wall at the end of the operation is a critical step in supporting the extremity weight. Long adhesive plaster straps are more effective in forming slings around the shoulder and trunk than elastic bandages. An opening in the plaster acting as a window is also vital for monitoring and detecting any kink in the flap base.

The main disadvantage of the flap is the donor site deformity , since in most of the cases the flap

dimensions are large, donor areas were surfaced by split thickness skin grafts. The resulting area will be a conspicuous contracted and pigmented graft, a disadvantage which is commonly mentioned in literatures [12, 13].

We think that this shape can be improved by tissue expansion of adjacent skin in later stages. Some authors performed abdominoplasty and the donor scars were converted to standard abdominoplasty scar [12], a solution which is only suitable for special situations when the area is small and sited at the lower abdomen in a patient who has lax lower abdominal skin. The other disadvantage of the abdominal flap is the flap bulk. The transplanted skin retains the fat-cell characteristics of abdominal skin, increase in body weight will result in thickening of the transposed flap [13].

In many patients, flap bulk was accepted when the flaps were used for the forearm, elbow or distal arm. Some patients asked for flap debulking which is done as a secondary operation with excellent results. Liposuction has been mentioned as another way of decreasing the flap bulk [13] . On the contrary , flap bulk was a major concern for patients with digital reconstruction by abdominal flaps .

They showed inadequate improvement even after three stages of surgical debulking. For this reason we think that this flap is mainly suitable for reconstruction of the hand ,forearm and elbow.

Its use in localized injuries of the digits can be replaced by other types of flaps that provide thinner skin and subcutaneous bulk and are suitable for digital reconstruction. Other disadvantages include staged reconstruction , and positioning of the hand for 21 days. Although positioning of the hand at the abdomen is better than in the case of groin flap because it provides elevation of the affected limb and a decrease in edema .

we can conclude that although microvascular free flaps are the first choice for extensive upper limb reconstruction, it is not always possible ,at least for so many cases of war injuries, to have the experience and facilities for such complicated surgeries. In these circumstances, random abdominal flaps provide an excellent and safe alternative .

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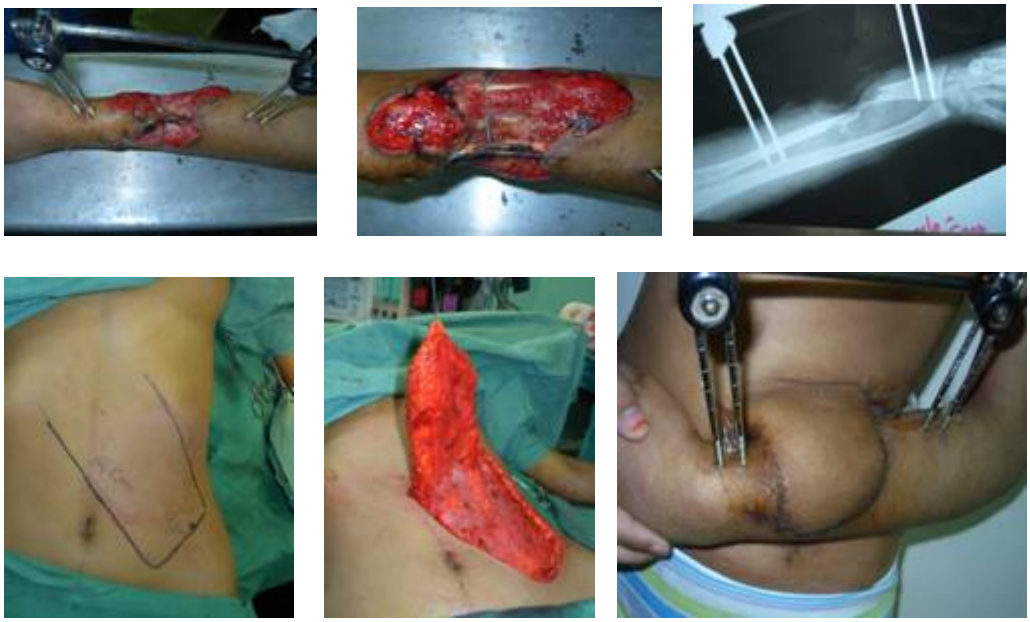
Figure1: Multiply injured patient , inferiorly based abdominal flap was done for his forearm wound



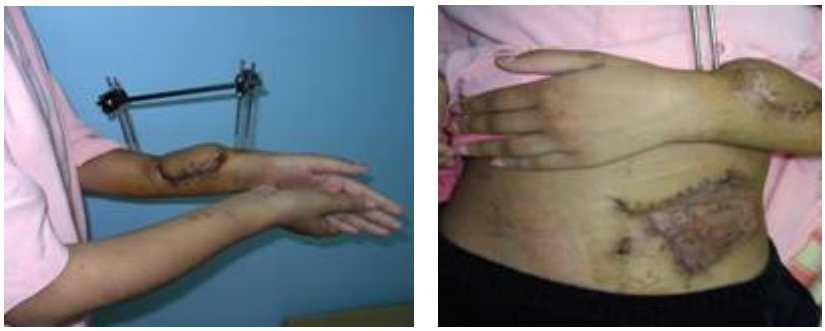


Figure 2: 21 year old female with bullet injury in the forearm, Large obliquely oriented abdominal flap was elevated to reconstruct the area, donor area was covered by skin graft and the flap separated after 21 days .

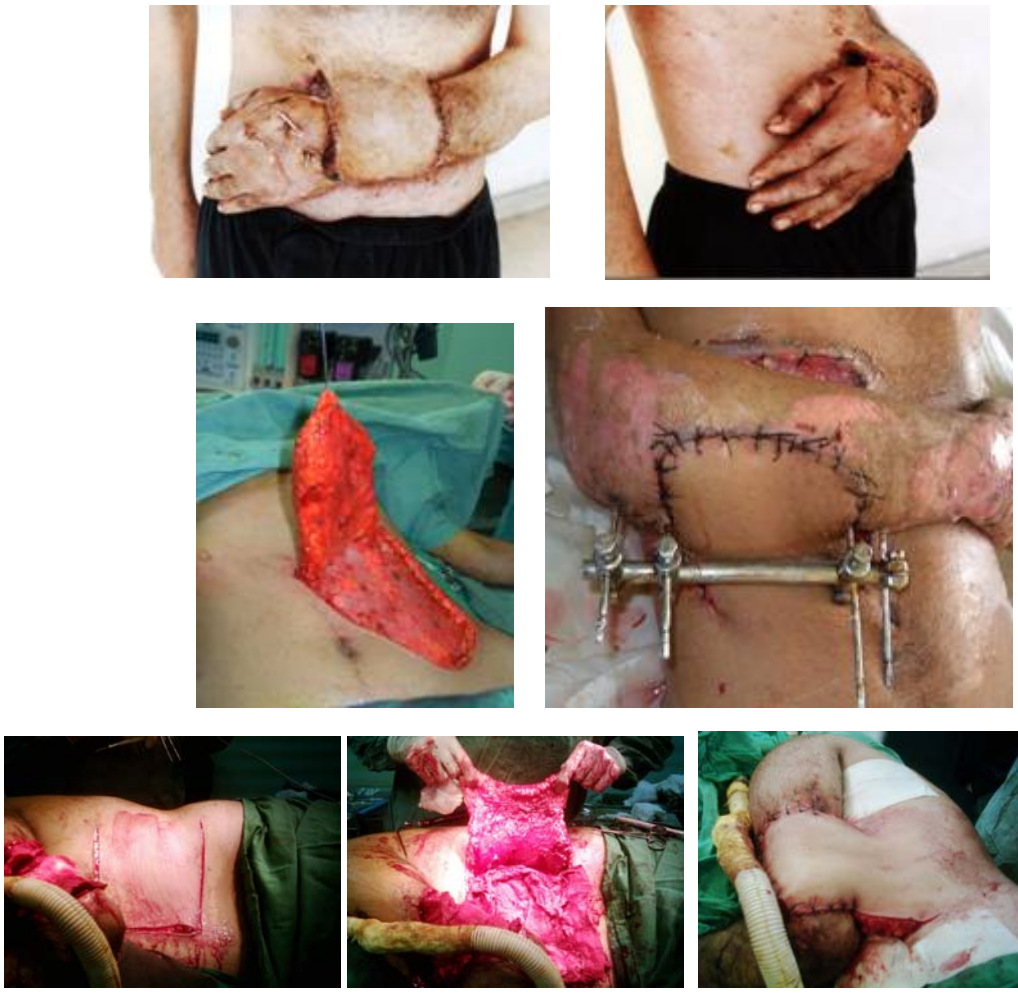


Figure 3: The flaps were based in any direction according to fit the requirements of the recipient site .

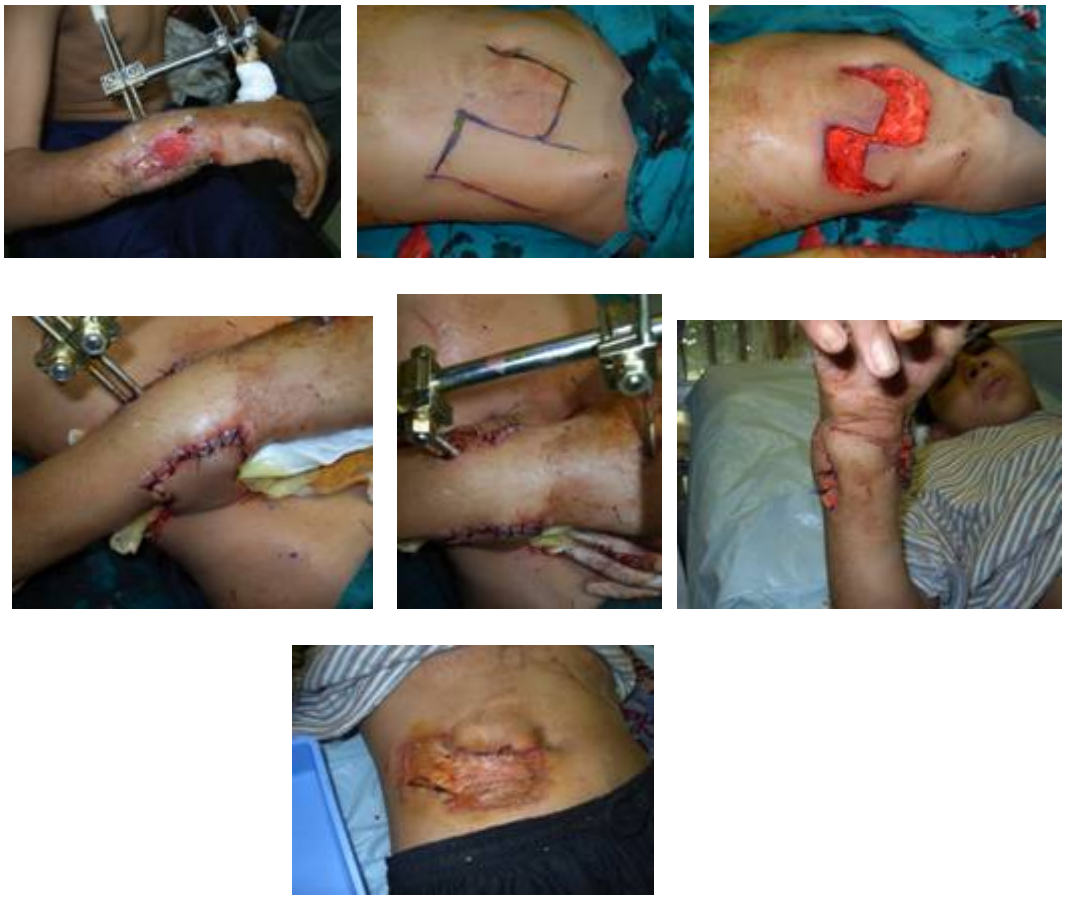


Figure 4: Young boy with penetrating wounds at the volar and dorsal aspects of the forearm. S-shaped incision was done to form two flaps which sandwiched the forearm .

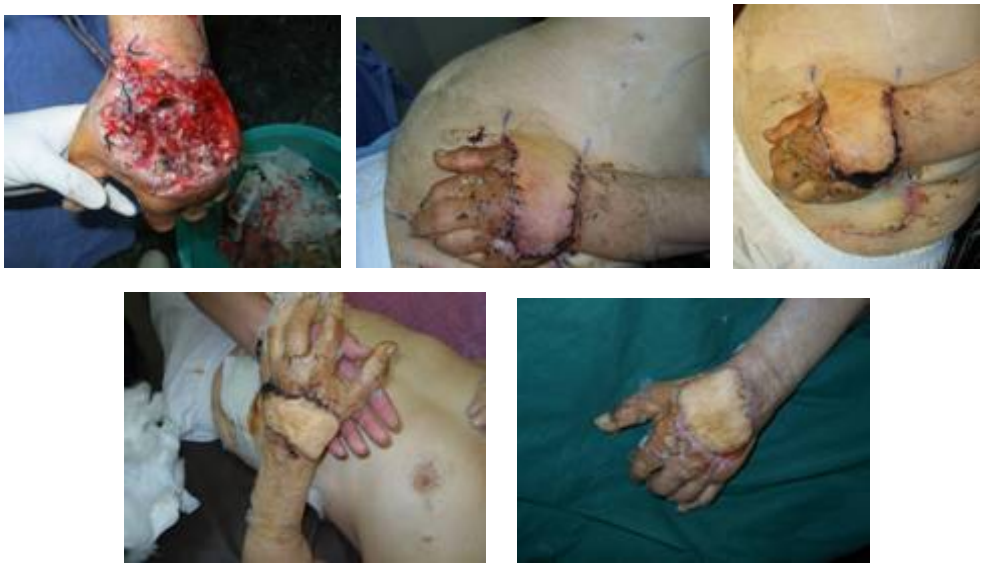


Figure 5: Elderly man with bullet injury in the dorsal aspect of the hand, abdominal flap showed partial loss at the tip, after separation of the necrotic tissue the flap healed by secondary intention .

Post Stroke Shoulder Pain Problem

Zeki Nooh Hassan

ABSTRACT:

BACKGROUND:

Shoulder pain is one of the complications that happened in patient suffered of hemiplagia our aim is to study this problem in hemiplegics patients due to stroke

METHODS:

56 patients affected by different types of stroke were enrolled in this study , each patient was examined by neurologist , CT scan then done and referred to a consultant rheumatologist at alkindi hospital for assessment of his shoulder area , the patient then investigated thoroughly for his or her shoulder pain

RESULTS AND CONCLUSION :

The study showed high correlation between shoulder pain and older age patients, aphasia, cortical sensory defects

The study showed that the frozen shoulder is the commonest cause of post stroke shoulder pain

KEY WARD: shoulder pain, stroke .

INTRODUCTION:

Stroke is sudden neurological dysfunction, resulting from sudden vascular insult involving cerebral vessels(1); it is either ischemic due to cessation of blood supply to the brain (2), or hemorrhagic stroke; due to different types of intracranial hemorrhage (2) (3). Shoulder pain is very common problem facing the neurologist when managing patients with stroke (4); 20 – 70% of patient with stroke develop hemiplegic shoulder pain (5) (6). Shoulder pain affects stroke outcome in a negative way. It can cause considerable distress , reduced activity and can markedly hinder rehabilitation resulting in negative interference with recovery after stroke (7.8). The cause of hemiplegic shoulder pain is the subject of considerable controversy (8). There are many pathological processes have been postulated as causes of painful hemiplegic shoulder after stroke. One of the causes is Rotator cuff syndrome, which results from impinging of rotator cuff tendon between acromion and humeral head, resulting in acute pain at the lateral surface of shoulder (9) (10). Other causes of shoulder pain are biceptal tendonitis; which affect large head of biceps resulting in pain at the anterior surface of the shoulder joint (10). Subacromial and sub deltoid Bursitis, which causes pain at lateral aspect of shoulder. Adhesive capsulitis (frozen shoulder) is a common painful condition associated with loss of active movement in the direction of external rotation and abduction (10).Other causes include glerohumeral sub luxation (11) soft tissue trauma (12) Brachial plexus traction Neuropathy (13) and central cortical post stroke mechanism may play a role (7). The aim of this study is to analyze this complaint and its correlation with the different clinical aspects of cerebral dysfunction .

Department of Neurology / Al-kindi College of Medicine

PATIENTS AND METHODS :

56 patients; aged from 29 – 73 years; suffered from stroke with hemiplagia during the first(6) months post stroke were studied at Alkindi teaching hospital, between January 2004 – June 2005 , all the patients had CT scan of the brain at the onset of the stroke .; the patient was classified into ischemic or hemorrhagic stroke according to CT scan results. Patients with Ischemic heart disease ,with diabetes mellitus and with any joint problems were excluded from the study; the neurologist took Full history in details and examined the patient neurologically to diagnose and localize his neurological deficit. Speech was assessed in all patients according to the steps of speech examination published in Bickerstaff *Neurological examination in clinical practice* (14) which includes:

1- spontaneous speech assessment
2-comprehension
3-naming objects
4-repetition
5-reading
6- writing) the patients with left sided weakness were examined for cortical sensory loss ; astreognosis was examined by asking ability to identify 2

centimeter cube object and 2 centimeter circumference ball we use this a little pit large sizes because of paralysis of the hand and poor hand grip (14).graphesthesia was assessed by drawing the Arabic shape of number 9 and 2 on the patient palm using pencil . sensory inattention assessed by ability to recognize simultaneous stimuli on both sides of the body the abnormal response is when the patient can recognize only the sound right side of the body at simultaneous stimulation and normal response of both side when examined separately ;the patients with aphasia did not involved in cortical sensory examination because of barrier of difficult communication . Muscle power grading was assessed according to medical research counsel scale (MRC)of great British which recorded the power in 6 grades(15) the patients then referred to rheumatologist who examined the patient. The patient had full blood count, blood sugar, ECG, Chest X-ray, Cervical X-ray and Shoulder X-ray, electromyography /nerve conduction study were done by the examining neurologist ; the rheumatologist at last diagnosed the cause of the shoulder pain P value < 0.05 was considered significant and was used whenever applied.

RESULTS :

56 patients aged from 29 – 73 years; with stroke suffering from shoulder pain were studied; 29 patients had ischemic stroke and 27 had hemorrhagic stroke (intra cerebral hemorrhage). 17 of those with ischemic stroke were female (58.6%) and 12 out of 29 were male (41.4%). (tab. 1) .10 out of 27 patients with intracerebral hemorrhage were female (37%) and 17 were males (63%). (Tab. 1) .23 patients out of 56 aged above 60 years; 18 patients aged between 51-60 years; 10 patients ages were between 41-50 years; 3 patients' ages were between 31-40 years and 1 patient was 29 years old age. (Tab. 2) .In patients with Ischemic stroke No patients had shoulder pain in the first month after stroke, 17 out of the 29 developed the shoulder pain in the second month after ischemic stroke, and 10 patient with ischemic stroke had the shoulder pain in the third month post stroke, one ischemic stroke patient in the 4th and one patient in the fifth month. No ischemic stroke patient in the study developed the shoulder pain in the 6th month post stroke. (See tab. 3) In hemorrhagic stroke; one patient had the shoulder pain in the first month, 10 patients in the second month, 10 patients in the third month, 4 patients in the fourth month, one patient in the fifth month and one patient in the sixth month after hemorrhagic stroke. (See tab. 3) .Right-sided weakness was seen in 13 out of 29 patients with ischemic stroke (44.8%) the right-sided weakness was seen in 12 out of 27 (44.4%) patients with intra cerebral hemorrhage. (Tab. 4) Left-sided weakness was seen in 16 out of 29 patient with ischemic stroke (45.6%) and seen in 15 out of 27 who had intracerebral hemorrhage (55.6%) (Tab. 5). 15 of the patients with ischemic stroke have grade 0-1 shoulder muscle power 12 patients have grade 2-3 and 2 patients have grade 4 shoulder muscle weakness. Those with hemorrhagic stroke, 17 patients having grade 0-1, 5 patients having grade 2-3 and 5 patients having grade 4 shoulder muscle power. (Tab. 6) . Aphasia was found in 21 out of 25 patients those with right sided weakness, Abnormalities of cortical sensory functions (astreognosis , sensory inattention and graphesthesia) was seen in 27 patients out of 31 patients with left sided weakness. (Tab. 7). 23 patients were diagnosed as frozen shoulder; 20 patients were diagnosed as refereed pain from other sites (neck, elbow); 5 patients had direct trauma to the shoulder by fall on ground; 4 patients had shoulder subluxation and 4 patients had rotator cuff syndrome. (Tab. 8)

Tab (1) male/female ratio having shoulder pain in ischemic and hemorrhagic stroke.

	Ischemic stroke.	Hemorrhagic stroke.	Total
male	12	17	29
female	17	10	27
total	29	27	56

P= 0.17 no significant

Tab (2) classification of the patients according to age groups

≤ 30 years	31- 40 years	41 – 50 years	51 – 61 years	> 60 years
1	3	10	19	23
1.7%	5.3	17.8%	33.8%	41.1%

P= 0.0001 significant

Tab (3) the time of presentation per month

	1st month	2nd month	3rd month	4th month	5th month	6th month
Hemorrhagic stroke	1	10	10	4	1	1
Ischemic stroke	0	17	10	1	1	0
	1	27	20	5	2	1

Tab (4) relation of the shoulder pain to the right side of weakness

	Ischemic	hemorrhagic	Total
Right. Side weakness	13	12	25
No	16	15	31
Total	29	27	56

P = 0.81 non significant

Tab (5) relation of the shoulder pain to the left sided weakness to shoulder pain

	Ischemic.	Hemorrhagic.	Total
Left. Side weak.	16	15	31
No	13	12	25
total	29	27	56

P= 0.81 non significant

Tab – (6) relation of shoulder muscle power grade to shoulder pain

	Grad 0 - 1	Grad 2 – 3	Grad – 4	Total
Ischemic .	15	12	2	29
Hemorrhagic.	17	5	5	27
Total	32	17	7	56

P= 0.12 non significant

Tab – (7) relation of shoulder pain to aphasia and other cortical sensory loss.

	Yes	No	Total	
Aphasia	21	4	25	P <0.005 significant
Cortical Sensory signs	27	4	31	P <0.005 significant

Tab (8) causes of shoulder pain

diagnosis	Frozen shoulder	Referred. pain	Direct trauma	Sub luxation	Rotator cuff
Number of patients	23	20	5	4	4
percentage	41.1%	35.7%	8.9%	7.1%	7.1%

P=0.0001 significant

DISCUSSION:

The shoulder pain is very common problem facing the neurologist in the management of patients with stroke (1 – 6). This problem occurs in both types of stroke whether hemorrhagic stroke or Ischemic stroke. The present study showed no significant difference between ischemic stroke and intracerebral hemorrhage in the development of shoulder pain, this agreed with. Other studies Hanukah, Sashama, and Ohkawa etal (5) Anderson (7). The present study showed an equal male – female occurrence of the shoulder pain in both types of strokes, and this result is in agreement with the results of other studies;Hanukah, Sashmi, Ohkawa etal(5)Anderson study(7), Jeperson .Jogenson (8)and Walsh study(12). The nearly equal male/ female occurrence of shoulder pain with no significance difference between both stroke type in occurrence of shoulder pain; support the opinion that the shoulder pain is

related to the hemiplagia, whatever of its cause and not related to the type of stroke or to the gender difference and this is in agreement with Roy,Sand,Hill study(4) Hanukah, Sashama,Ohkawa etal(5) Anderson (7) Jeperson – Jogenson (8) Chaca, Wolf study(11)andWalsh study (12). In the present study the shoulder pain happened more frequently in elderly than in younger aged group; this correlation with older age, group is related to already diseased joint, as well as less active life style in elderly patients and this agreed with Walsh study (12). The present study showed no significant correlation between weakness side (whether right or left sided weakness) with occurrence of shoulder pain and this is in contrast to Roy,Sand,Hill etal (4) whom found a significant relationship with non-dominant left sided weakness And in agreement with Walsh who reports no correlation with side of weakness(12).the present study analyze the relation of higher cerebral function, concentrating on aphasia. whatever its type, cortical sensory dysfunction(graphesthesia, astreognosis. and sensory inattention.); We found significant relation between shoulder pain with higher cerebral dysfunction and prove that the patients with cortical involvements are at higher risk for shoulder pain development .this result is agreed with Roy,Sand,Hill etal (4) The study showed that the shoulder pain occurs mostly in the second and third months post stroke in both types of stroke. The present study showed no significant correlation between muscle power grading and the development of the shoulder pain. And this result is contrasting to Roy,Sand,and Hill study(4) Hanukah, Sashama, Ohkawa etal (5) Anderson (7) Jeperson – Jogenson (8) Chaca, Wolf study (11)and Walsh study (12). The study showed that frozen shoulder is the most common cause of the hemiplegics shoulder pain; other causes like refereed pain form relatively high percentage (35.7%) from the causes of shoulder pain. Other causes like direct trauma (8.9%) shoulder joint sub luxation (7.1%) and rotator cuff syndrome (7.1%). We did not found Brachial plexus traction neuropathy in our patients.

Those finding is in contrast to Walsh (12), Braus (13) studies which showed high incidence of shoulder subluxation more than other causes, small sized sample in comparison to those studies may explain the last difference in causes of shoulder pain between the present study and other studies.

CONCLUSION:

1) The shoulder pain is very common problem in stroke.2) The shoulder pain development is more common in older age group.3) Patients with cortical involvement are at high risk to develop shoulder pain4) The shoulder pain development is not related to the side of hemiplagia, sex and grading of muscle weakness.5) Rheumatologist should examine every patient and plan for early exercise should be encouraged.

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Distally Based Fasciocutaneous Flaps In the Management of Soft Tissue War Injuries of the Lower Half of the Leg

Tariq Abdul Qadir* , Harith Abdul Jabbar Al Ani ** .

ABSTRACT:

BACKGROUND :

Soft tissue war injuries affecting the lower half of the leg are a major challenge facing plastic surgeons .Distally based fasciocutaneous flaps have an important role in the management of these defects.

OBJECTIVE :

To demonstrate the role of distally based fasciocutaneous flaps as an alternative to free tissue transfer in the management of lower leg soft tissue defects.

METHODS :

Thirty seven war injury patients with soft tissue loss in the lower half of the leg were managed by distally based septocutaneous flaps (19 cases),and distally based superficial sural artery flap (18 cases) , donor areas were closed by partial thickness skin grafts in all of the cases.

RESULTS :

All of the 37 flaps survived and healed uneventfully except in 4 cases in whom partial necrosis of the flap occurred at the tip. Two cases of distally based superficial sural artery island flaps developed venous congestion and edema .

CONCLUSION :

Distally based fasciocutaneous flaps are an important alternative to microvascular tissue transfer in treating lower leg war injuries. The versatility and arc of rotation of the distally based superficial sural artery flap were better than in distally based septocutaneous flaps.

KEYWORDS:

INTRODUCTION:

Lower extremity war injuries are usually extensive causing wide spread soft tissue and bone loss. Usually other areas of the body are also involved and the patient is in a critical situation if it is not fatal. After stabilization of the patient general condition and management of any life threatening injury, plastic surgeons face the challenge of soft tissue reconstruction . Starting from the simplest surgical techniques in the ladder of reconstruction ,namely direct suturing or skin grafting, till reaching the most sophisticated techniques like microvascular tissue transfer. Coverage of soft tissue defects in distal areas of the leg and foot is always a difficult yet common problem because of the tightness, poor circulation , and the limited local tissue available for reconstruction [1-5].

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Various methods of reconstruction to cover such defects with exposure of underlying bones and tendons include local cutaneous ,fascial or fasciocutaneous flaps based either proximally or distally, cross-leg flaps, distally based muscle flaps, and free flaps and each has its merits and drawbacks [6,7,8,9,10]. Generally , microvascular techniques have revolutionized the treatment modalities ,free flaps are superior to other methods because they allow reconstruction of large defects with well vascularized tissues that have exact physical properties needed [9,11,12]. In a busy surgical ward with a remarkable shortage of equipment and facilities ,microsurgical tissue transfers are impossible as they require time and facilities in addition of being technically demanding necessitating the availability of a trained microsurgical team . In these situations ,surgeons usually try to find suitable alternatives to free transfers. Fasciocutaneous flaps of the lower extremity are a relatively a new concept largely attributable to the clinical work of Ponten who described 23 of these flaps in 1981 [3,13] .

The early designs extending along the vertical axis of the leg with a proximal pedicle have been modified by the use of the transverse flaps with a lateral or medial pedicles , and lastly, with distal pedicle [12] . Distally based Fasciocutaneous flaps are commonly vascularized by the medial septocutaneous perforators arising from the tibial artery, the lateral septocutaneous perforators arising from the peroneal artery, or can be based on both [1,3] .Over the past 13 years some authors have focused on the concept of neorocutaneous flaps [14] . Arteries that are accompanying superficial sensory nerves give nutrient vessels to the skin ,and some flaps can be designed over these areas [10,14] .

In 1992, Masquelet and colleagues first described the distally based superficial sural artery island flap based on the vascular axis of the sural nerve and its anastomosis with the lowermost septocutaneous perforator from the peroneal artery [4,10].

Many surgeons demonstrated the versatility of the flap and its advantages in the reconstruction of the lower leg [1, 4, 10,15,16] .

In this study we managed 37 war injury patients with soft tissue loss in the lower half of the leg by distally based fasciocutaneous flaps .

PATIENTS AND METHODS:

Thirty seven patients with soft tissue loss and exposure of tendons ,joints or bones in the lower half of the leg and foot were treated with distally based fasciocutaneous flaps over a period of 26 months from April 2003 to June 2005.

All of the wounds were war injuries resulting from either shell or bullet injuries. Their ages range from 10 to 73 years.Fourteen of them were females and 23 were males. Life saving surgeries were done for 5 of them (Lapratomy and thoracotomy) and the patients were admitted to the plastic surgery unit when the general condition of the patient is stable ,and any bony injury was fixed externally and necrotic soft tissues excised .

In 18 patients there was a bone gap necessitating bone grafting after 6 to 12 weeks. The soft tissue defects were closed by the following distally based fasciocutaneous flaps :

1. Distally based superficial sural artery island flap (9 cases).
2. Distally based superficial sural artery interpolation flap (9 cases).
3. Distally based septocutaneous flap based on the medial septal perforators (6 transposition flaps and 3 island flaps).
4. Distally based septocutaneous flap based on the medial and lateral septal perforators (8 transposition flaps and 2 island flaps).

The selection of the type of the flap depends on the site of trauma and the presence of intact donor area. All the flaps were done secondarily after wound excision ,under general anesthesia ,and with the aid of a tourniquet .

Distally based superficial sural artery flap:

The flap is based on the median superficial sural artery which originates from the popliteal artery and accompanies the sural nerve and descends between the two heads of the gastrocnemeous muscle . In 65% of cases this artery descends to the lateral maleollus ,in 35% of cases the artery fades distally to a vascular net at the distal third of the leg, in both cases the artery has a constant distal anastomosis with septocutaneous perforators from the peroneal artery which will supply a reverse – flow flap [9,10,17] . The axis of the flap is along the course of the sural nerve which is over a line joining the midpoint of the popliteal fossa and a point approximately 1 cm behind the lateral maleollus. The center of the flap is placed on the midline in the posterior surface of the leg and outlined according to the dimensions of the defect .The standard flap described is the island flap, we also made 9 interpolation flaps. The upper border of the flap is incised and the and the lesser saphenous vein ,medial sural nerve and accompanying arteries are divided and ligated.

The flap is then elevated with deep fascia making sure that the vessels and the nerve are included in the flap. In cases of island flaps, a longitudinal strip of fascia containing the sural nerve and lesser saphenous vein is taken with the pedicle.

And the skin island was tunneled subcutaneously to reach the defect . In all of the cases the dissection stops 5-7 cm proximal to the lateral maleollus and the donor areas were closed by a split thickness skin grafts.(Figure)

Distally based septocutaneous perforator flaps:

These flaps are fasciocutaneous flaps based on septocutaneous perforators passing in the medial intermuscular septum with or without septocutaneous perforators passing in the lateral intermuscular septum. The flap is either centered over the medial intermuscular septum to include the septocutaneous branches of the posterior tibial artery ,or based over the entire posterior area to include the septocutaneous perforators of the peroneal artery in the lateral septum also. They were elevated in the subfascial plane till reach 1 cm proximal to the lowest septocutaneous perforator if a Doppler ultrasound device is available. Unfortunately in most of the cases Doppler exams were unavailable, in these cases the dissection

stops 8 cm proximal to the medial maleollus, although most of the surgeons advocated the use of Dopller preoperatively ,some authors did not believe that its use is helpful [2]. Donor sites were closed by split thickness skin grafts.

Flap delay for one week was done in 3 cases when the planned flap was very long reaching few centimeters from the knee joint flexion crease and intraoperative bleeding points from the tip of the flap were scanty. The patients were followed up for a period of 5-23 months , donor and recipient sites were evaluated and early and late complications were recorded.

RESULTS:

All of the 37 flaps survived and healed uneventfully except in 4 cases in whom partial necrosis of the flap occurred at the tip. Two of them were flaps based on the medial septocutaneous perforators ,one was based on both medial and lateral septocutaneous perforators and one flap was distally based superficial sural artery flap. The necrotic tissue was either excised or sloughed spontaneously and a split thickness skin grafts were applied to close the resulted wound in one case, the other 3 wounds healed by secondary contraction. The sural nerve and the short saphenous vein were included in all of the reverse superficial sural artery flaps .The nerve was included in only 5 septocutaneous flaps and the short saphenous vein was included in 10 septocutaneous flaps. The largest dimensions of the flap were 11 cm in width and 19 cm in length and the donor areas extended to the middle or proximal thirds of the posterior leg . Donor areas were closed by partial thickness skin grafts in all of the cases. During the follow up period 11 patients developed complications .All of them showed improvement in a period of 1-5 months and became ambulatory after the completion of wound healing if the bones were not involved. The complications were:

- Partial flap loss (4 cases).
- Venous congestion or prolonged edema (2 cases).
- Infection (One case).
- Donor site complications (3 cases).

Donor site complications included scar hypertrophy (one case),partial skin graft loss(one case), and painful neuroma (one case). Parasthesia in the foot after nerve division is considered a sequel rather than a complication.

The distally based superficial sural artery island flap showed excellent versatility and its reach was better than the septocutaneous flaps . The flap can cover defects on the medial ,posterior or anterior aspects of the leg and foot.

DISCUSSION :

Distally based fasciocutaneous flaps in the leg were described as a safe, easy , and time saving option for soft tissue reconstruction in the distal half of the leg .In Iraq were plastic surgeons face a large number of war injuries among civilians of different ages , and were there is a great shortage of medical supplies and lack of facilities , the use of conventional pedicled flaps remains a good alternative for microvascular free tissue transfer and reduces the number of amputations. The blood supply of the septocutaneous flaps is via the septocutaneous perforators arising from the tibial artery (medially) or the peroneal artery (laterally) .The flap should be based on the lower perforators to make the flap pedicle as long as possible , the exact location of the perforators is variable.

Peroneal artery perforators travel in the septum between the peroneus longus and the flexor halusis longus muscles in the proximal two thirds of the leg and between the peroneus longus and the soleus muscles in the distal one third of the leg . the average number of perforators is 4.8 per leg with a range of 1 to 7.They are concentrated at the seventh and eighth tenths the lateral longitudinal distance (7.4 cm above the lateral maleollus) .In the distal leg perforators may course medially to connect to the posterior tibial system [13] . El Khatib showed that these lateral septocutaneous perforators are located 7-10 cm from the tip of the lateral maleollus [16] ,other studies demonstrated that they are 5 perforators with a 3-5 cm intervals between them and the lowest one is 5-10 cm above the maleollus [3] . The posterior tibial artery perforators pass in the septum between the soleus and the flexor digitorum longus muscles .The average number of perforators is 3.1 ,with a range of 0 to 6 [13] .They are located 3.5-7 cm from the tip of the medial maleollus [16] . Other studies showed that the lowest perforators are located 9-12 cm from the medial maleollus. It is obvious that there is some variation in the exact locations of the lowest septocutaneous perforators of both systems .That makes preoperative Doppler ultrasound identification of the perforators sites is of great importance since surgical dissection of the perforators and intraoperative identification are risky .In most of our cases Doppler identification was unavailable .we made the pivot point for the reverse superficial sural artery flap rotation 7 cm from the lateral maleollus for safety. The site ranges in the literatures from 3 cm to 7 cm [2,4,5,6,7,9,10,16] . For the same reason , we made the pivot point for the septocutaneous flaps based on the medial septocutaneous perforators 8 cm above the medial maleollus .

The distally based sural artery flap was used to cover large defects in the anterior ,posterior and medial aspects of the distal half of the leg ,the flap was elevated from the middle third of the posterior leg ,this coincides with other studies done previously [2,7,9] , in 4 of our cases the flaps were elongated to include skin from the proximal third of the leg ,in one of them the bleeding points from the tip of the flap was scanty ,we did a delay of 7 days to increase the vascularity of the flap and it healed later uneventfully .Husamettin and co workers elevated the flap from the proximal third leaving just 1-2 cm of skin from the popliteal crease and used the term (super sural neurofasciocutaneous) to identify these flaps [6] . Partial skin necrosis occurred in 4 cases , all of them affected the distal 1-3.5 cm of the flaps , in 3 cases the wounds healed by wound contraction and in one case split thickness skin graft was used to cover the wound. Partial necrosis secondary to venous congestion is one of the disadvantages of the distally based sural artery island flap. Unfortunately ,the distal tip is usually the portion that fails which is commonly the part of the flap that one needs the most [18] .

Two cases developed prolonged congestion and edema both of them are distally based sural artery island flaps .the edema decreased with time but it did not disappear .

A tight subcutaneous tunnel through which the flap pedicle is passed leading to vascular compression (especially the venous drainage) may explain this edema. Venous drainage of the flap occurs via superficial venous network, which is formed by small concomitant veins ,lesser saphenous vein and septocutaneous veins of the peroneal venous system [5,6].After flap elevation ,direct reflux through the valves does not occur, small concomitant veins were present along both sides of the lesser saphenous vein and were considered to be venae comitantes of accompanying arteries of the vein .These small veins receive blood from the lesser saphenous vein and play a role in bypassing the valves [6]. Many surgeons reported the possibility of pedicle compression in the tight subcutaneous tunnel affecting the venous drainage system when using the standard distally based sural island flap .Technical modifications have been described to overcome this problem.

The flap can be planned in a tear drop configuration so that there is cutaneous extension over the neurovascular axis [6,10] ,other workers considered the tear drop configuration inadequate in decreasing tunnel tightness and prefer division of the skin bridge of the tunnel and skin grafting over the pedicle [5,19].

Ogun et al did not experience any circulatory impairment and considered the reported tightness as a result of improper tunneling [2] . In this study when the distally based sural island flap was used 5 cases developed venous congestion early after the operation, they showed improvement with time and the edema disappeared later except in 2 cases one of them developed partial skin loss later .

In both cases the skin over the subcutaneous tunnel was either edematous or scarred. When we used the distally based superficial sural artery flap as a peninsular interpolation flap, we did not encounter any venous congestion postoperatively, but this type of flaps necessitate the need of a second operation for flap separation .The same approach is described by Maffi and his co workers [18] .

Although flap edema was seen in a number of septocutaneous island flaps ,we did not find congestion leading to tissue loss or long lasting edema for more than 3 months even when tunneled under adjacent skin. The full width fascial pedicle may have better venous drainage ,in addition to that, the small angle of pedicle rotation and the vicinity of the recipient site made the possibility of pedicle compression inside the tunnel less .

The sural nerve should be included in the distally based superficial sural artery flap making numbness at the lateral aspect of the foot unavoidable , trials of preserving the nerve have led to partial flap loss [2,9,10] .On the other hand, the nerve can be preserved when distally based septocutaneous flaps are pedicled on the medial septocutaneous perforators making it an advantage when using these flaps. Most of our patients showed slow improvement and a decrease in the level of numbness one year after the operation, but the symptoms did not disappear during the period of follow up, Ogun showed that these sensory changes improves with time and does not constitute a major problem [2] . A similar result was reported by Singh and Naasan [7], and by Costa Ferreira et al [9]. Despite the risk of flap congestion in the subcutaneous tunnel and donor site numbness ,we found that the distally based sural artery flap is more versatile and reliable than the distally based septocutaneous flaps based on the medial septocutaneous perforators (or perforators of both sides) .It provides a large skin island which can be mobilized to cover soft tissue defects in different locations in the lower half of the leg and foot. This coincides with the results of other studies which showed that the flap meets almost all criteria for an ideal flap,

making it an excellent choice for covering soft tissue defects of the lower leg [2] .

CONCLUSION:

The technique of the distally based fasciocutaneous flaps in general was simple and safe .The operation can be done in a short time without interfering with the blood flow of one of the major vessels of the leg . In this study ,most of the patients were multiply injured ,lapratomies and thoracotomies were done for some of them prior to their referral to the plastic surgery ward. The facilities ,time, personnel, and the expertise for microvascular surgery were unavailable. The flaps were performed in busy general hospitals under suboptimal environment and shortage of medical supplies ,which are expected during wars. They obviated the need for microvascular tissue transfers in most of the cases saving the limb from amputation ,except when the degree of soft tissue loss and bone exposure are very extensive. The distally based superficial sural artery flap is an excellent option when the tissue loss involves the distal part of the leg and foot. Its versatility and (reach) made it superior to other distally based fasciocutaneous flaps . Careful technique and awareness about the possible complications that may arise can save the lower limbs of many war victims.

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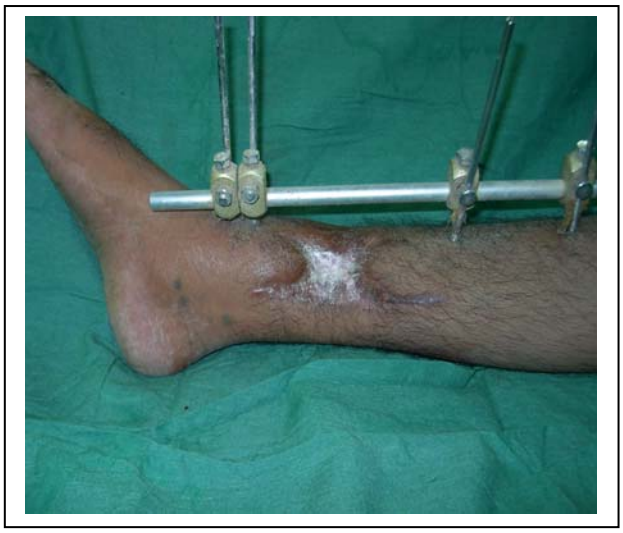

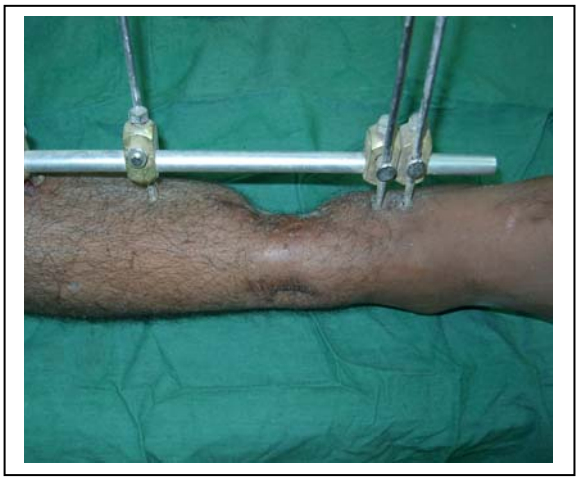

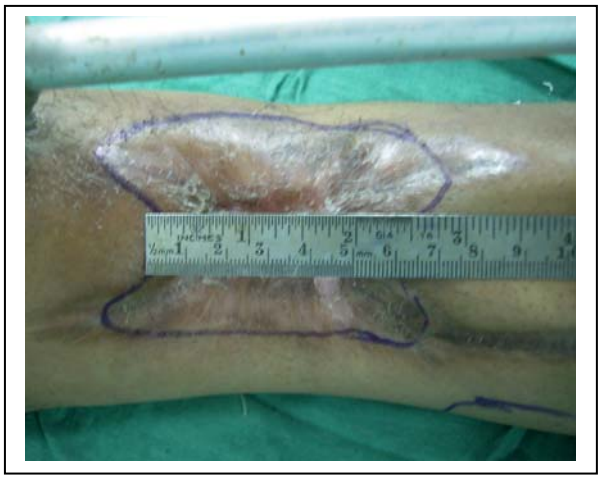

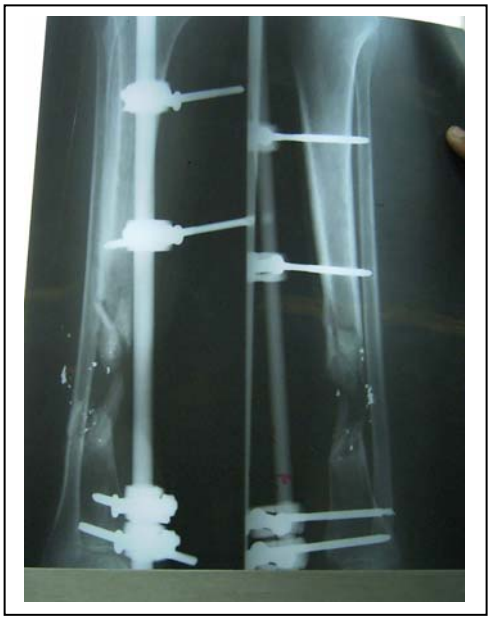

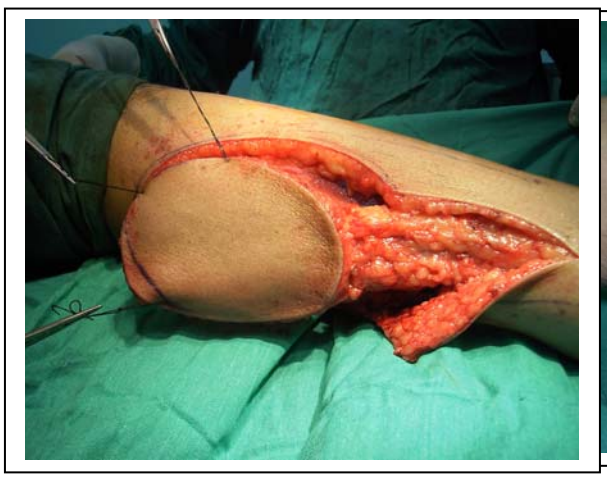

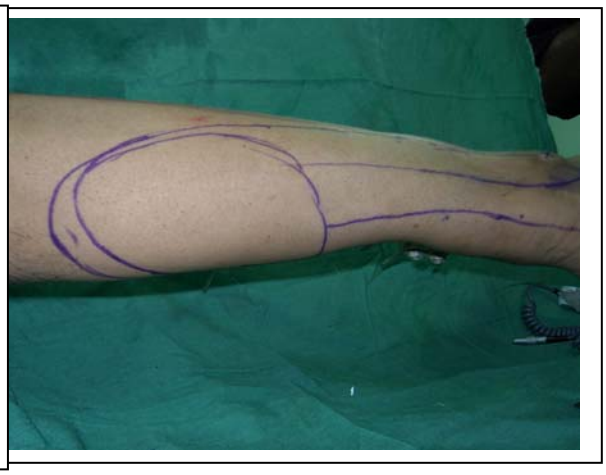

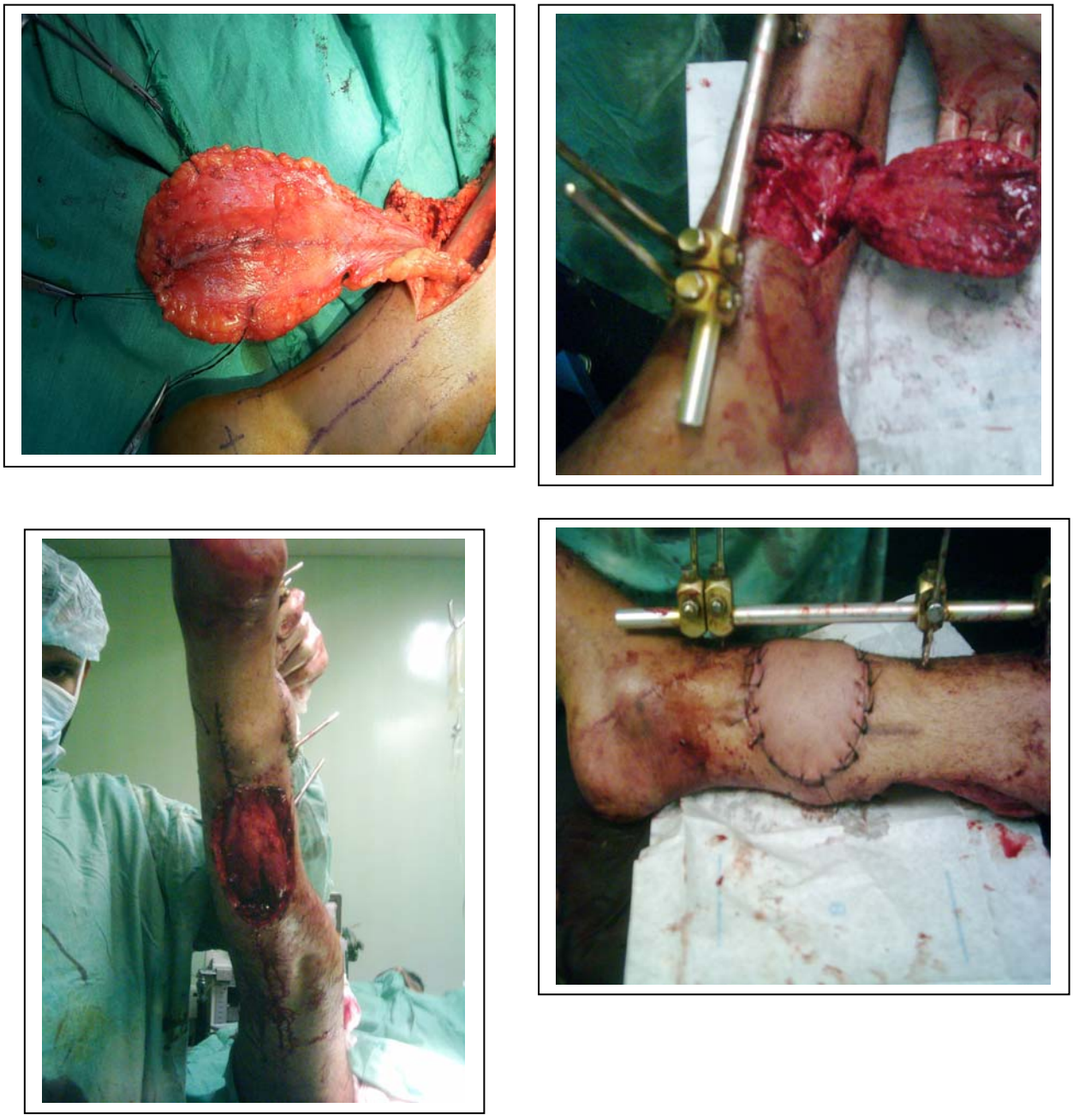

Figure : Distally based superficial sural artery flap for reconstruction of 8 cm by 6 cm post traumatic defect in the lower third of the leg.

MATERIAL AND METHODS :

Materials :

Preparation of extract : Aqueous extract of harmala seeds prepared as described by Adday et.al.1989 [11].

Parasites and their maintenance : A stock of L. donovani (MHOM/IQ/BRC1(AA3)) was obtained from Department of Biology , College of science , University of Baghdad .

Animals : Males of Balblc mice , 6-8 weeks of age and 18-20 gm body weight , these animal were obtained from Al-Nahrain medical college .

Experimental protocol :

Note : each group contain 6 animals .

1- control group .

1-1- group injected intra peritoneally with (125µg / 20 gm b.wt of P. harmala).

1-2- Group injected intraperiotoneally with 250µg of 20gm b.wt of p. harmala . 1-3- Group injected intraperitoneally with 500 µg /20gm b.wt of p. harmala .

1-4- Group injected intraperitoneally with $5x10^5$ promastigotes of L. donavoni .

1-5- Group injected with 0.2ml sterile PBS and serves as negative control .

2- Experiment groups :

2.1.1. Group was treated with 125µg/20 gm b.wt. I.P. of *P. harmala* (I.P.)and infected with $5x10^6$ promastigotes after (5) days .

2.1.2. Group was treated with 250µ/20gm b. Wt. Of *P. harmala* (I.P.) and infected with $5x10^6$ promastigotes afer (5) days .

2.1.3. Group was treated with 500µg/20gm b.wt. of P. harmala I.P. and infected with 5x106 promastigotes after (5) days .

2.1.4. Group was treated with 125µg/20gm b.wt. P. harmala I.P. and infected with 5x106 promastigotes after (10) days .

2.1.5. Group was treated with 250µg/20 gm b.wt. of P. harmala I.P. and infected with 5x106 promastigotes after (10) days .

2.1.6. Groups was treated with 500µg/20gm b.wt. of P. harmala I.P. infected with 5x106 promastigotes after (10)days .

2.1.7 Groups was treated with 125µg /20gm b.wt. of P. harmala I.P. for successive six days then infected with 5x106 promastigotes .

2.1.8 Groups was treated with 250 µg / 20 gm b.wt. of P. harmala I.P. for successive six days and infected with 5x106 promastigotes .

2.1.9 Groups was treated with 500 µg / 20 gm b.wt. of P. harmala extract for successive six days and infected with 5x106 promastigotes .

All these groups of mice are sarcified after (8) week and concern on the following parameter .

1.Total of leukocytes and differential count .
2.Percent of leukocyte forming formazan .
3.Percent of infected cells and phagocytic index .
4.The changes of spleen and liver weight and Organ index .
5.Total parasite burden (TPB) and prophylactic index .Calculate : infected cells percent, leishmancidal index, macrophage forming formazan .

A. Preparation of peritoneal macrophages: five ml of HBSS contain 50 I.U/heparin were injected into the peritoneal cavity a septically, after gentle massage of the abdomen then the mouse was sacrificed .The skin from the ventral body was removed peritoneal fluid was pooled with pasteur pipette from small pores on the peritoneal membrane into the poethylene tube . 0.5 ml of peritoneal fluid was placed at tissue culture plate for 1 hour at 37°C after incubation three times washed with PBS , fixed with methanol for 3-5min , washed with PBS , stained for 20min with Geimsa stain , washed with PBS , examined under oil immersion , then leishmanial index was Calculated according to Al-Jorany et.al., 1992(6) .

Leishmanial index =

$$\frac{\text{Infected cells – infected and treated cell}}{\text{Infected cells}} \times 100$$

B. Calculate of Macrophages number which forming formazan .

This parameter depends on superoxide production by macrophages , then the superoxide reduced the undissolved yellow stain into dark blue indissoluble formazan salt which sediment in macrophage cytoplasm casper et. al., 1992 [12] .

Methods :

1.Peritoned macrophage prepared as in A .
2.0.5ml of NBT was added to macrophages and incubated at 3°C for 25min.
3.Wash macrophages by PBS, then calculate the macrophage which forming formazan among (200) macrophages .

Total parasite burden in spleen and liver :

A cut section of liver and spleen was plotted on filter paper and impression was made on glass slides , air dried smear were fixed in methanal for 3-5 min and stained with Geimsa for 20min . Slides were examined under oil immersion and the ratio of amastigotes to organ cells nuclei was determined . Total parasite burden were quantified according to stauber – 1956 [13] .

Leishmain donavani = liver or spleen wt mg x ratio of amastigoter x 200.000

Unit per liver or spleen While the prophylactic index was calculated according to Riffat et.al.,

1989 [14].Counting of monuclear leukocyte was carried out by using a haemocytometer .

Prophylactic index :

$$\frac{\text{No. Of amastigotes in spleen or liver in infected and treated animal}}{\text{No. Of amastigotes in spleen or liver in infected and non treated animal}} \times 100$$

Statistical analysis : ANOVA testy was used to compare the result .

RESULTS :

1- Quantified of Total white blood cells and differential count . Table (1) shows the changes in W.B.C. number in mice treated with (125, 250, 500) µg/20gm for (5,10) days and successive six days before the infection . The number of WBC for all treated group were ($P<0.05$) it reaching (8725, 9575, 10283) cell / cumm respectively comparing with the untreated control which was (7853.3) cell / Cumm in contrast with group infected with parasite alone which reach (1791-6) cell / Cumm . Also , the number of W.B.C. decreasing in groups treated with P. harmala seed extract and infected with L. donovani promastigates comparing with control ($P>0.05$) . In addition to that the increasing number was in the number of lymphocytes . Calculation of infected cells percent , lishmanial index in peritoneal macrophages and number of macrophages forming formazan . Table (2) shows the changes of leishmanial index and infected cells percent in peritoneal macrophages for treated mice with (125, 250 , 500)µg/20 gm b.w.t. On (5, 10 daily for six days) before infection with L. donovani promastigotes , also the same change for the infected mice . The result shows a decrease in infected cells percent for all treated groups . It reach to (32.4) cells in mice treated with 125µg for ten days , which were significant ($P<0.05$), while the leishmanial index increase for treated group , the higher level for it were obtained in the group activated with 250µg for successive six days it was (85.27%) .Table (3) shows the changes in mean of macrophage forming formazan , they reach (60.6) macrophage in group treated with 250µg which was significant($P<0.05$) in contrast to infected alone , the mean number of macrophages forming formazan in infected mice reached (8.83) .

3- Quantified of Total parasites burden and prophylactic index in spleen and liver. Table (4) shows the changes in amastigotes loads , and prophylactic index in spleen and liver of mice treated with (125, 250 , 500) µg of P. harmala extract on days (5, 10 daily for six days) before infection and the same change in mice infected alone . The number of Total parasite burden in spleen and liver for all treated groups were quite low , caused significant suppression of parasite burden ($P<0.05$) . Higher level of prophylactic index were obtained in the group treated with (500) µg for successive six days . The total parasite burden was $0.471x10^6$ amastigotes in spleen and $7.60x10^6$ in liver, while the prophylactic index reach (99%) in spleen and (97.8) in liver .

4- Estimation of spleen and liver weight and organ index .Table (5) shows the changes in spleen and liver weight and organ index of mice treated with (125 , 250 , 500) µg of P. harmala seed extract on day (5, 10 successive six days) before infection and same change of infected mice alone . The weight of spleen and liver for all treated group were quite low ($P<0.05$) comparing with the weight of infected mice which reach (0.22 , 1.78) gm for spleen and liver respectively . Higher level of organ index were obtained in the group of mice infected alone which reach (9.64 , 78.8) for spleen and liver respectively .

DISCUSSION :

The activation of macrophages by P. harmala leads to changes in macrophages surface receptors which used by leishmanial promastigotes for invading macrophages , among these receptors the mannose – fucose receptors (16) . The result were consistent with Mahmoud and Tuwajri (1991) (17) . When demonstrated reduction the proliferation of L. donovani amastigotes in both spleen and liver of glucan pre-treatment mice . Also , the reduce of parasites burden in spleen and liver were observed when treating infected mice with Esculetin (6,7 dihydroxy coumarin) (7) and polysacchorides of Rhizobium meliloti (6) . Murray, 1988 reported the same result when treated visceral leishmaniasis with interferon - γ . Also Rachamin and Jaffe (1993) (19) demonstrated that immumized mice with a protein purified from L. donovani promastigotes dp 72 before infected I.V. with L. donovani showed 78% reduction in liver parasites burden compared with control . Also , the result of this study demonstrated that the immunopotentiation with P. harmala induced significant prophylactic index in mice against infection with L. donovani *in vivo*. The mechanism(s) that mediated this effect are unknown at present time . The possible mechanism(s) responsible for this effect might involved an increasing the released of reactive oxygen , so this lead to augment that the capacity of macrophages to eliminate the infections especially the parasites very sensitive to hydrogen peroxide , generally , macrophages could be activated for engulfed and killing leishmanial parasites by Esculetin (7) . hydroxyethylstarch (20) and thymic ectract (14) . All treated groups showed

an increased number of macrophages forming formazan, this agreed with Gasper *et al.*, (1992) (12) study which measured the estimation of respiratory burst in non-infected macrophages by nitroblue Tetrazolium (NBT) because activated macrophages by non-particles of polyalkey anoaerylate (PACA) . While the decrease in number of macrophage forming formazan in infected mice were consistent with Mallinson and Coombs (1984) (21) when improved that the infected macrophages with leishmania amastigotes didn't reduce NBT , become the macrophages couldn't produce superoxide , due to ability of parasites to enhance lipoxygenation and cycloxygenation then enhance prostoglandins, leukotreins and thromboxanes which suppress the production of super oxide (22) .

Table (1) Total leukocyte and differential count in groups of mice treated with peganum harmala seeds extract and infected with L. donovani .

Differential count								Total W.B.C. count		Activation periods	Concentr-ation µg
Eosinophil		Neutrophil		Monocyte		Lymphocyte					
Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD		
	-	5.2717	± 0.1746	*4.1517	±0.4951	*90.576	* 0.435	*5100	±126.49	5	125
		6.3467	±0.333	*2.761	±3.0720	*92.208	±0.291	*6491.6	±66.45	5	250
		7.5050	±1.9265	*1.669	±0.582	*90.873	±1.531	*6708.3	±120.0	5	500
	-	13.3467	±0.1527	*4.223	±1.3495	*81.930	±0.2248	*5991.6	±149.72	daily six days	125
		12.1850	±0.3364	*0.2717	±0.099	*87.543	±0.2835	*7658.3	±73.59	daily six days	250
		10.7717	±0.9036	*1.6233	±0.9010	*88.2050	±0.149	*7783.3	±103.27	daily six days	500
	-	10.446	±0.221	*6.1417	±0.516	*83.4117	±0.510	*4350	±89.44	10	125
		9.825	±0.194	*3.2517	±0.4225	*87.690	±0.584	*5433	±136.62	10	250
		9.883	±0.488	*2.8417	±0.585	*87.275	±0.137	*5791	±97.032	10	500
	-	18.3650	±0.0909	*1.4317	±0.1059	*80.203	±0.033	*8725	±121.44	Treated alone	125
		15.4917	±0.8717	*1.295	±0.477	*83.213	±0.585	*9575	±154.11	Treated alone	250
		14.1	±0.1302	*1.4717	±0.2081	*84.928	±1.243	*10283	±112.54	Treated alone	500
0.360	± 0.2925	11.25	±0.6546	15.90	±0.634	*72.480	±0.4671	*1791.6	±58.45	-	C+
		14.3467	±0.257	0.3117	±0.1125	85.3417	±0.295	7858	±257.71	-	C-

* P< 0.005 .
Infected control : C+
Un infected : C-

Table (2) Percent of infected periotoneal macrophages and leishmanicidal index in groups of mice treated with P. harmala seeds extracts and infected with L. donovani .

Leishmanicidal index	Percent of infected cells Mean ± SD	Activation periods	Concentration µg
72.52	* 25.11 ± 0.072	5	125
75.1	* 21.77± 0.314	5	250
75.21	* 21.66 ± 0.268	5	500
79.98	* 17.051 ± 0.097	Daily six days	126
85.27	* 12.87 ± 0.185	Daily six days	250
85.07	* 12.13 ± 0.115	Daily six days	500
62.25	* 32.42 ± 0.301	10	125
70.22	* 27.68 ± 0.326	10	250
70.31	* 27.198 ± 50	10	500
-	91.6 ± 0.458	-	C+

C+ : infected control .
C- : un infected control .
* P < 0.05 .

Table (3) Total of isolated periotoneal macrophages forming formazan from groups of mice treated with P. harmala seed extract and infected with L. donovani .

Macrophage forming formazan		Activation periods	Concentration (µg)
Mean	±SD		
* 25.66	± 0.516	5	125
* 30.63	± 0.983	5	250
* 32.0	± 0.0	5	500
* 40.44	± 0.516	Daily six days	125
* 52.166	± 0.408	Daily six days	250
* 53.0	±0.0	Daily six days	500
* 22.0	± 0.0	10	125
*28.33	± 0.516	10	250
* 29.0	± 0.0	10	500
* 52.5	± 0.516	Treated alone	125
* 60.6	± 0.408	Treated alone	250
* 61.5	± 0.0	Treated alone	500
25.166	± 0.4082	-	C-
8.833	± 0.4082	-	C+

Infected control : C+
Un infected control : C –
* P <0.05 .

Table (4) Total parasites burden in spleen and liver in groups of mice treated with P. harmala seeds extracts and infected with L. donovani

Prophylaction index	Total parasite in liver		Prophylactic Index	Total parasite in spleen		Activation periods	Concentr-ation µg
	Mean x 10^6	±SD		Mean x 10^6	±SD		
93.1	*24.45	±0.413	93.1	*3.326	±0.150	5	125
95.8	*14.75	±0.187	94.8	*2.528	±0.040	5	250
96.0	*14.0	±0.1414	94.9	*2.475	±0.028	5	500
95.7	*15.250	±0.1871	95.3	*2.27	±0.054	Daily six day	125
97.6	*8.33	±0.307	98.9	*0.496	±0.021	Daily six day	250
97.8	*7.60	±0.322	99.0	*0.471	±0.024	Daily six day	500
93.6	*22.71	±2.155	93.0	*3.40	±0.0216	10	125
95.01	*17.67	±0.402	94.1	*2.88	±0.080	10	250
95.21	*16.96	±0.103	94.1	*2.855	±0.0394	10	500
-	358.6	±2.155	-	49.27	±0.255	-	C+

C+ : infected control .
* p<0.05 .

Table (5) The changes in spleen and liver weight / (gm) in mice treated P. harmal seeds extract and with L. donovani.

Differential count

Liver index		Spleen index		Liver weight		Spleen weight		Activation periods	Concentr-ation µg
Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD		
*51.01	±	*5.82	±1.85	*1.1581	±0.029	*0.132	±0.035	5	125
*49.67	±	*5.66	±1.33	*1.27	±0.035	*0.128	±0.027	5	250
*47.93	±	*4.97	±1.2	*1.08	±0.043	*0.115	±0.010	5	500
*50.19	±	*5.39	±1.76	*1.155	±0.024	*0.124	±0.002	daily six days	125
*44.4	±	*4.77	±0.33	*1.019	±0.006	*0.106	±0.007	daily six days	250
*44.03	±	*4.44	±0.31	*1.012	±0.001	*0.102	±0.002	daily six days	500
*50.56	±	*6.08	±1.23	*1.163	±0.027	*0.140	±0.022	10	125
*49.65	±	*5.85	±1.6	*1.1421	±0.018	*0.134	±0.031	10	250
*49.98	±	*5.57	±1.7	*1.150	±0.020	*0.128	±0.0194	10	500
*55.0	±9.46	*6.57	±0.63	*1.154	±0.011	*0.144	±0.017	Treated alone	125
*56.11	±14.74	*7.16	±1.2	*1.793	±0.02	*0.145	±0.001	Treated alone	250
*13.1	±56.1	*7.19	±1.2	*1.795	±0.01	*0.146	±0.003	Treated alone	500
*78.8	±	*9.64	±1.21	*1.786	±0.087	*0.22	±0.046	-	C+
*43.78	±	4.3	±1.14	1.0	±0.007	*0.098	0.015	-	C-

* P< 0.005 .
Infected control : C+
Un infected : C-

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The Effect of the Aqueous Extract of Peganum harmala Seeds on the Mammary Glands of Virgin, Pregnant, and Lactating Rats

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ABSTRACT:

BACKGROUND:

Penganum harmala is a well known herb that is used by different societies. It is used as a medical herb in treating various diseases and disorders .There was no earlier published work on the effects of the aqueous extract of Peganum harmala . Therefore, we designed this study to investigate the effect of the aqueous extract of Peganum harmala seeds on the mammary gland at the various physiological states (virgin, pregnancy and lactation) making use of the available histological, histochemical, and immunohistochemical means.

METHODS:

Aqueous extract of Peganum harmala was given for two weeks by an orogastric tube on single regular daily dosage to Norway albino female rats. Animals were subdivided into subgroups according to their physiological states. Mammary glands of these animals were routinely processed for histological, immunohistochemical and histochemical studies using formalin fixative ,paraffin embedded sections in the first two studies and formal calcium, frozen sections in the third study. Experimental specimens were compared with that of control subgroups.

RESULTS :

Harmal induced mammogenesis in the mammary glands of virgin rats. It's aqueous extract was able to initiate lactogenesis in a well-prepared mammary gland (i. e. during pregnancy) and finally this aqueous extract promotes lactogenesis when administrated during lactation.

CONCLUSION :

Peganum harmala is a mmammogenic herb .

KEYWORDS: Peganum harmala, mammary glands, alkaline phosphatase, estrogen and progesterone receptor's.

INTRODUCTION:

Peganum harmala is a well known herb that is used by different societies. It is used in treating various disease and disorders (1). It is also used as a galactagogoue for lactating women and has long been described as an abortifacient-emmenagouge. (1, 2). A number of herbs, known to be galactagouges have been investigated on a sound clinical pharmacological principle to determine their effect in promoting milk secretion. These herbs were fenugreek (3), fennel, cumin and garden cress (4). Based on the earlier mentioned reports and as there was no earlier published work on the effects of the aqueous extract of Peganum harmala seeds on the rat mammary glands (Medline and Extramed search 1965-2005); we designed this study to investigate the effect of the aqueous extract of Peganum harmala seeds on the mammary gland at the various physiological states (virgin, pregnancy and lactation) making use of the available histological, histochemical and immunohistochemical means .

MATERIAL AND METHODS:

Sixty, female, albino, Norway rats (Rattus norvegicus) were employed in this study. Animals were grouped according to their physiological states into three groups (table 1). The experimental group was treated daily with the aqueous extract of Peganum harmala seeds at a concentration of 125µg/gm body weight/day (5, 6). The aqueous extract was given through orogastric tubes and the duration of treatment was 2 weeks. The control group received 1 ml of distilled water as a placebo under similar conditions. From each ether-anesthetized rat, three pieces of the mammary glands were excised together with one piece of the liver. Two pieces of the mammary glands were immediately fixed in 10% formalin for 24 h.

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Those specimens were processed for routine haematoxylin and eosin and for immunohistochemistry. (7). The 3rd specimen of the mammary gland and the small piece of the liver were immediately fixed in formal calcium at 4C° for 18h., rinsed in tap water and finally placed in gum sucrose at 4C° for 18h. (7). The tissues were quenched (in liquid nitrogen) and sectioned to 6microns thickness (at-22, using SLEE cryostat). These sections were processed for demonstration of alkaline phosphatase activity (7) .

Via cardiac puncture, blood samples were obtained from each ether anesthetized rat to measure the level of estrogen, progesterone and prolactin in their serum. Morphometrical study was done using an eye piece micrometer fitted to a light microscope at 10×40 magnification making use of mammary gland sections stained with haematoxylin and eosin.

The diameter of the alveoli and the number of nuclei per one alveolus were studied morphometrically.

Table 1 Showing the animal groups

Groups	Number of Rats		Description
	Control	Experimental	
Adults	10	10	Normal, two months old virgin.
Pregnant	10	10	Seven days pregnant
Lactators	10	10	1st day of lactation

RESULTS:

1.Histological study: Mammary tissue from virgin rats treated with harmal demonstrated an increase in the size of the lobuler, alveoli and ducts when compared with their controls. Furthermore some alveoli revealed a pink homogenous material in their lumen. (Fig. 1 A & B). In pregnant rats treated with harmal, the mammary glands exhibited a remarkable increase in the size of their lobules when compared with the control group. Each lobule was packed by dilated alveoli which were lined by a single layer of cuboidal epithelium circumscribed by another layer of myoepithelial celles (Fig. 2 A & B). Alveoli and ducts, in the mammary tissue of lactating rats treated with harmal, were seen filled with milk secretion. Epithelial loss in the interlobular ducts were identified with a probable pouring of milk from the adjacent alveoli (Fig. 3 A & B).

2.Histochemical Study: Mammary tissue of virgin rats treated with harmal exhibited positive alkaline phosphatase activity (black rings) around the basal part of the secretory epithelium of the alveoli. However, no such black rings were observed in control virgin rats (Fig. 4 A & B). Positive alkaline phosphatase activity (black rings) were observed around the basal part of the secretory epithelium in control pregnant rats. Similar black rings were reported in the mammary tissue of pregnant rats treated with harmal but they were thinner (Fig. 5 A & B). Mammary tissue of control lactators showed thin, discontinuous black rings around the basal part of the secretory epithelium. The discontinuous black rings were more thinner in the mammary tissue of harmal treated lactators (Fig. 6 A & B).

3.Immunohistochemical study: Mammary tissue of virgin rats showed strong (+++) expression of both estrogen (nuclear staining) and progesterone (cytoplasmic staining) receptors. Virgin rats treated with harmal exhibited moderate (++) expression of both receptors. (Fig. 7 A & B). Moderate expression (++) of both estrogen and progesterone receptors were observed in the mammary tissue of control pregnant rats These receptors were stained weakly (+) in the mammary tissue of pregnant rats treated with harmal. (Fig. 8 A & B). Mammary tissue of control lactators showed weak expression (+) of both receptors. Nearly similar expressions of these receptors were observed in the lactators treated with harmal (fig. 9 A & B).

4.Morphometrical study : Diameters of the alveoli were significantly ($p<0.05$) increased in virgin, pregnant and lactating rats treated with harmal than their controls. (Table-2). The number of nuclei per one alveolus was significantly increased in virgin, pregnant and lactating rats treated with harmal than their controls. (Table-2).

5.Hormonal Study: Radio immunoassay for estradiol, progesterone and prolactin were observed using mean + SD. Progesterone and prolactin were significantly increased in virgin rats treated with harmal when compared with their controls (Table - 3). Estradiol, progesterone and prolactin were significantly increased in pregnant rats treated with harmal than their controls (Table-3). In lactators treated with harmal, estrdiol, progesterone and prolactin were significantly increased when compared with their controls (Table-3).

DISCUSSION :

The results of all parameters indicated that harmal is a mammogenic herb since it induced mammogenesis in the mammary glands of virgin rats. It's seeds were able to initiate lactogenesis in a well-prepared mammary gland (i. e. during pregnancy) and finally this herb promotes lactogenesis when administered during lactation. Alkaline phosphatase study showed the presence of black rings around the basal part of the secretory epithelium of alveoli in virgin, pregnant and lactators treated with harmal and these black rings were thinnest in lactators. It has been found by Al-Yawer and other workers (9,10) that alkaline phosphatase can be taken as a marker for the activity of both basement membrane and myoepithelial cells in the mammary gland and other structures. From this we can conclude that the basement membrane and myoepithelial cells were more developed in virgin, pregnant and lactating rats treated with harmal than their controls. Expressions of both estrogen and progesterone receptors were detected more in the mammary tissue of control virgin rats than that in harmal treated virgin group. Expression of these receptors decreases when pregnant rats treated with harmal and decreases more when lactating rats treated with harmal. These results indicate that a reduction in the expressions of progesterone and estrogen receptors coincident with functional differentiation and harmal may induce more differentiation in the mammary glands when administered.

It has been found by Shyamala (11), that progesterone receptor present in the mammary gland of non-pregnant female mice is reduced during pregnancy and is virtually undetectable during established lactation. Saji, et al. (12) had found that female mammary gland undergoes a surge of cell division during puberty and throughout adult life. In pregnancy, estrogen receptors expression is low and this percentage will become lower during lactation. 13, 14, 15. Morphometrical study showed that the diameters of alveoli and the number of nuclei per one alveolus were significantly increased in virgin, pregnant and lactating rats treated with harmal.

This may be due to the increase in the number and activity of the alveolar cells and these cells synthesize and secrete more milk when harmal was administered. Similar findings were observed when fennel, cumin and garden cress were given to virgin rats (4). Estradiol was significantly increased in pregnant and lactating rats treated with harmal but there is no such significant increase of estradiol in virgin rats treated with harmal.

On the other hand, progesterone and prolactin were significantly increased in virgin, pregnant and lactating rats treated with harmal. Taken together, these findings may indicate that progesterone and prolactin are necessary for the development of the mammary glands and estradiol may be necessary for the early but not the late development of the mammary glands. It has been demonstrated by Shyamala (11) that progesterone is essential for lobuloalveolar development and not for ductal morphogenesis. On the other hand, Bole, Feysot et al. agreed that prolactin is the hormone primarily responsible for the synthesis of milk proteins, lactose and lipids and the terminal stage of the mammary gland development, lobulo alveolar growth is directly regulated by prolactin.

Histological Study :

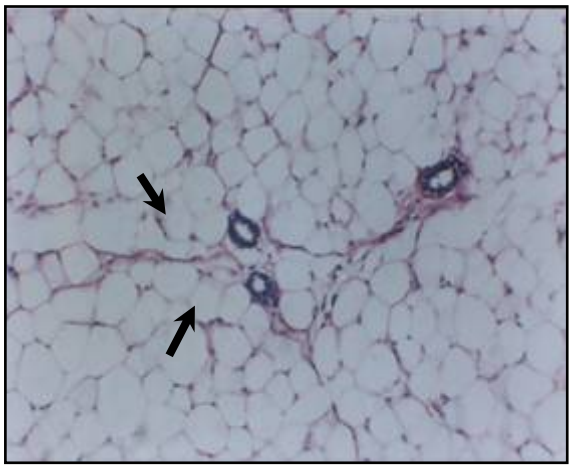
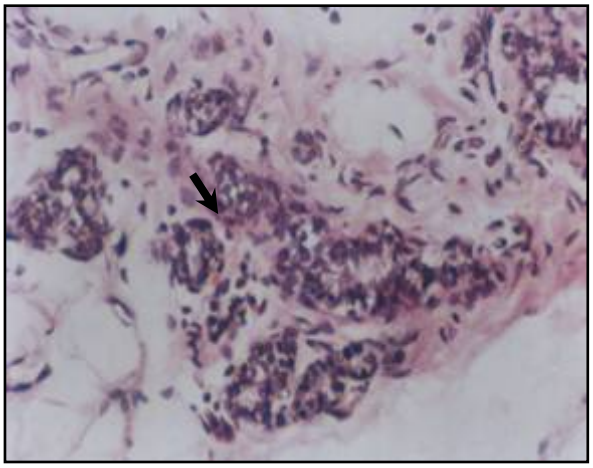


Fig. 1 (A) Control Virgin



(B) Virgin treated with Harmal

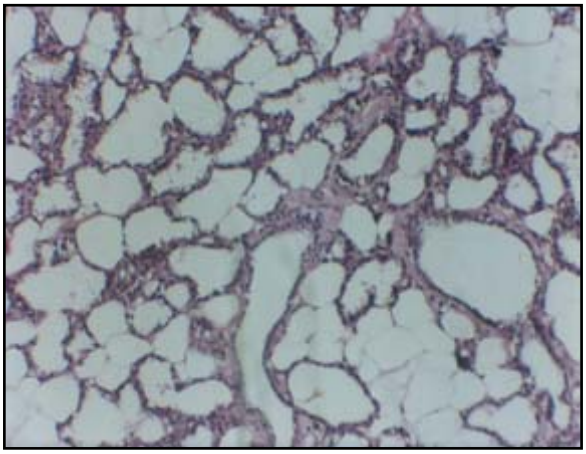
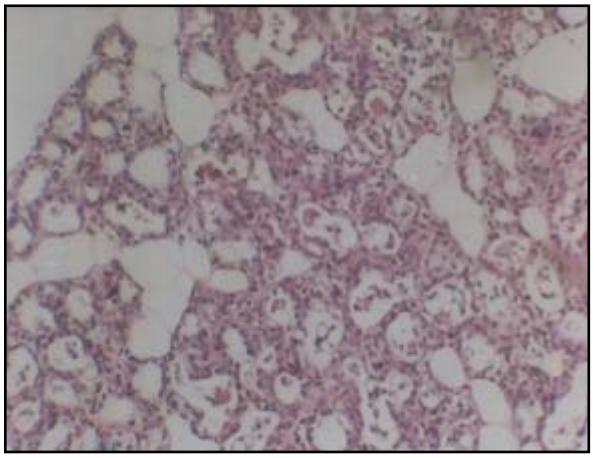


Fig. 2 (A) Control Pregnant



(B) Pregnant treated with Harmal Borage

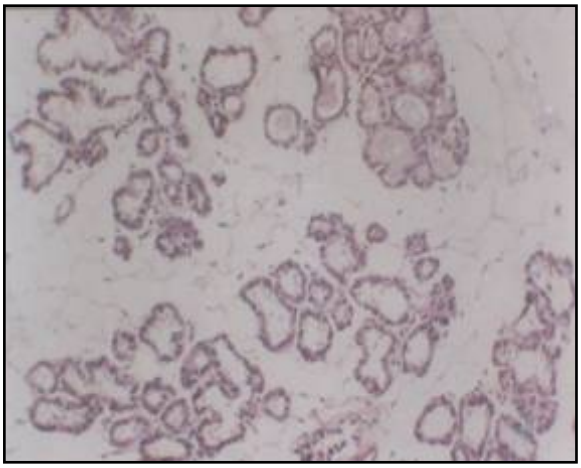
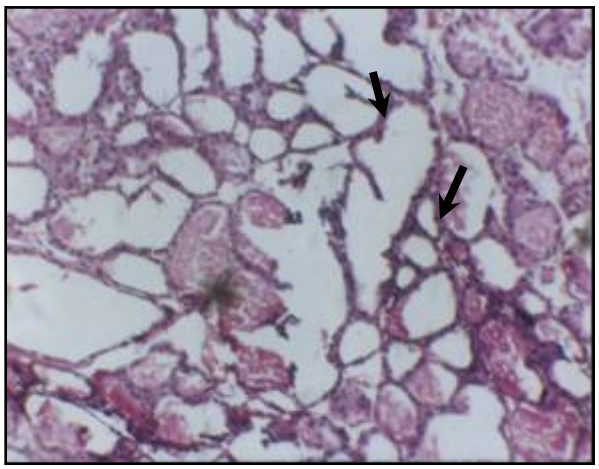
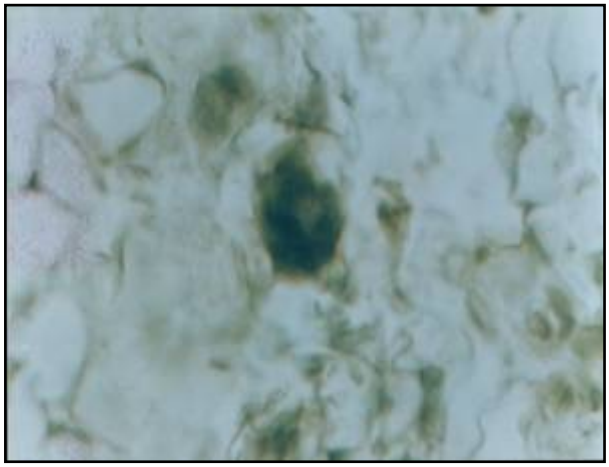


Fig. 3 (A) Control Lactating



(B) Lactating treated with Harmal

Histochemical Study :



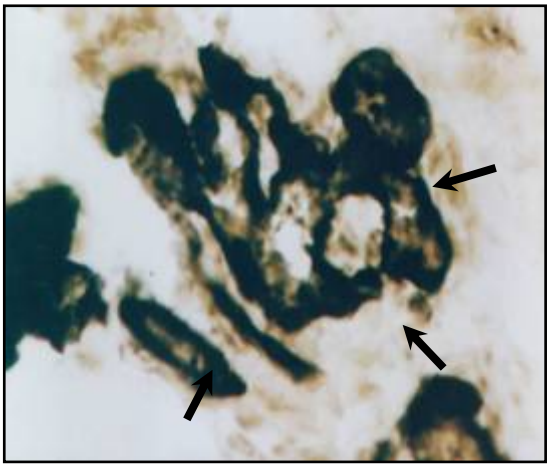
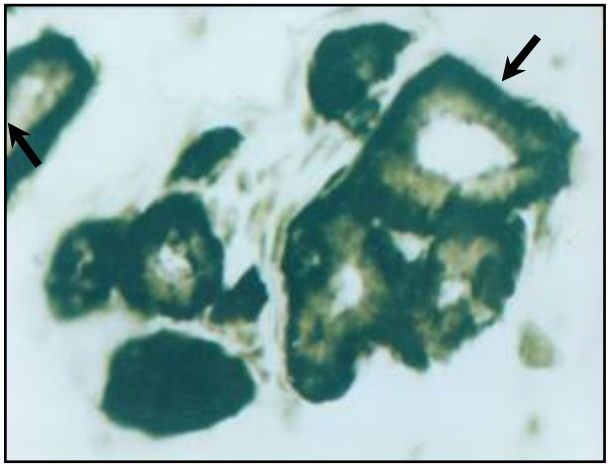


Fig. 4 **(A) Control Virgin** **(B) Virgin treated with Harmal**



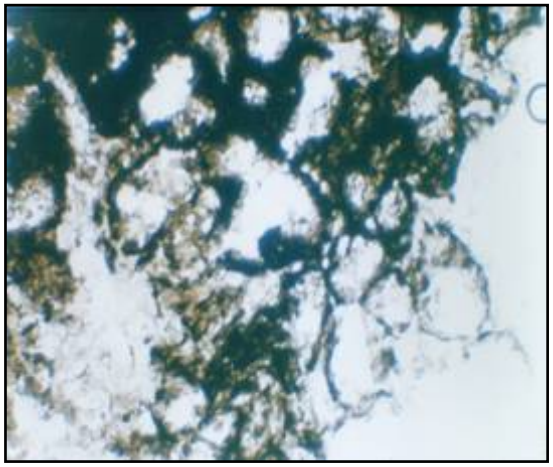
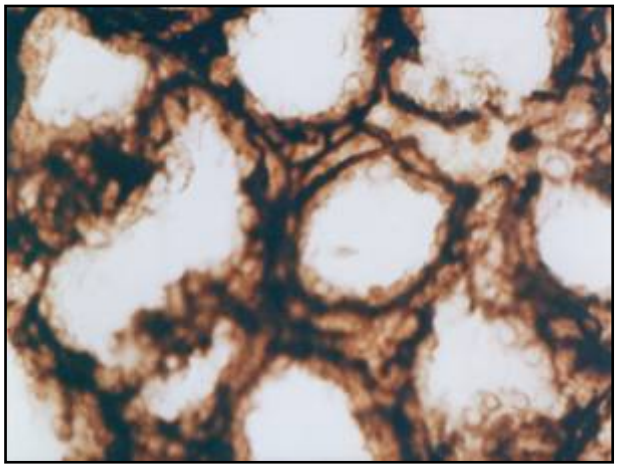


Fig. 5 **(A) Control Pregnant** **(B) Pregnant treated with Harmal**



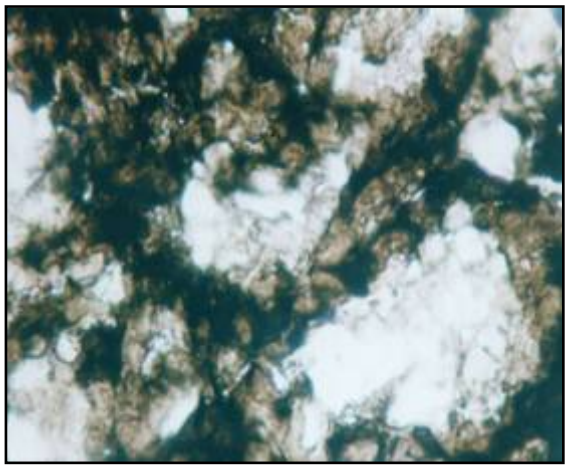
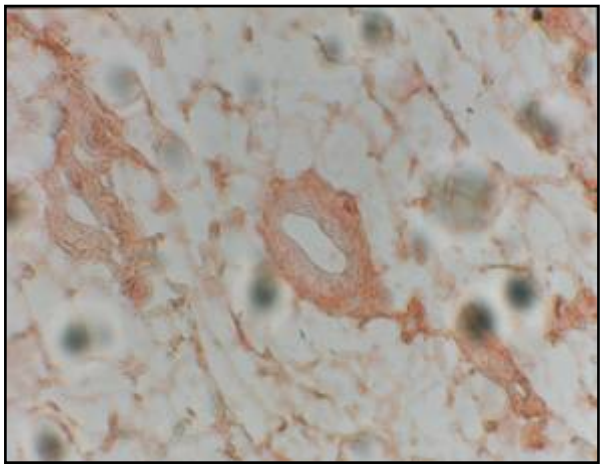


Fig. 6 **(A) Control Lactating** **(B) Lactating treated with Harmal**

Immunohistochemical Study:



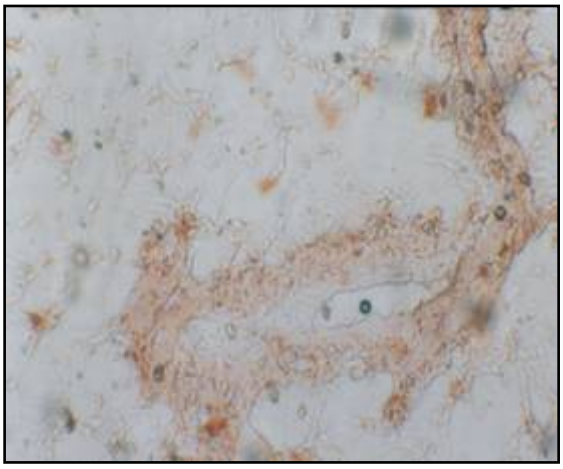
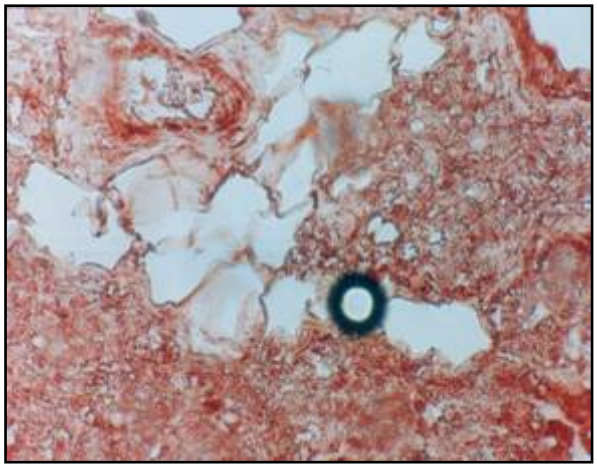


Fig. 7 **(A) Control Virgin** **(B) Virgin treated with Harmal**



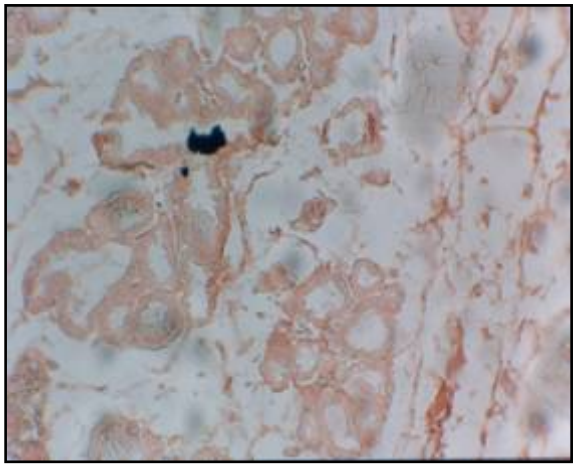
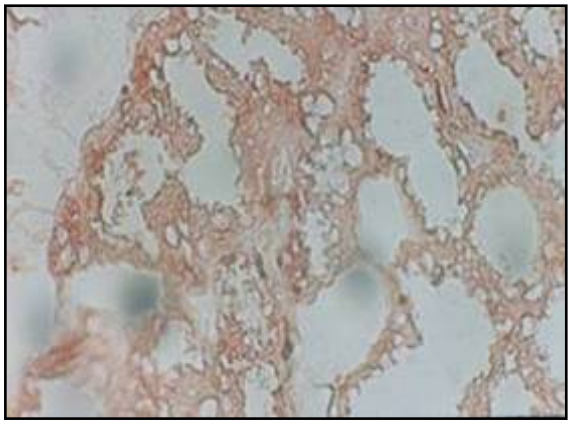


Fig. 8 **(A) Control Pregnant** **(B) Pregnant treated with Harmal**



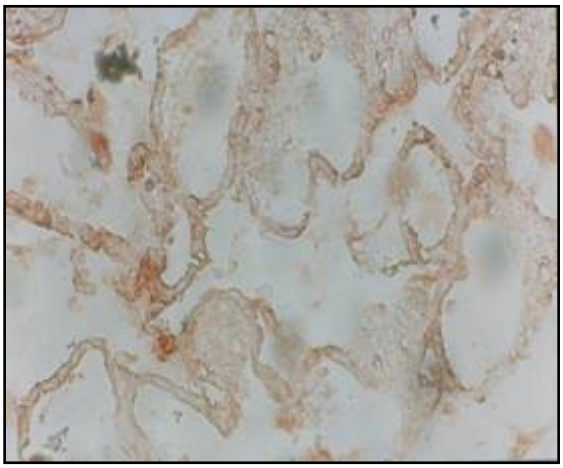


Fig. 9 **(A) Lactating Control** **(B) Lactating treated with Harmal**

Fig1: Mammary gland of virgin rat treated with aqueous extract of harmal (B) Dilated alveoli was seen. Each lined by a single layer of cuboidal cells (arrow) Control mammary gland of virgin rat (A) demonstrated few ill defined secretory tubules (arrow). (H&E) (X200).

Fig2: Mammary gland of pregnant rat treated with harmal exhibited a remarkable increase in the size of their lobules (B) when compared with their controls (A). (H&E)(X200).

Fig3: Mammary gland of lactating rat treated with harmal (B) demonstrated an increase in the size of their alveoli with pouring of milk from the adjacent alveoli (arrow). (A) Control lactating mammary gland (H&E)(X200).

Fig4: Mammary gland of virgin rat: (B) treated with harmal, positive alkaline phosphates activity (black rings) was noticed around the basal part of the secretory epithelium of the alveoli (arrows). No such black rings were demonstrated in control mammary gland (A). (Alkaline phosphates) (X200).

Fig5: Mammary gland of control pregnant rat (A) demonstrated thick black rings around the basal part of the secretory epithelium (arrows). Mammary gland of pregnant rats treated with harmal (B) demonstrated relatively thin black rings (Alkaline phosphates) (X200).

Fig6: Mammary glands of lactating rats of the experimental group (B) showed thinner, discontinuous black rings when compared with the control group (A) Alkaline phosphates (X400).

Fig7: Mammary glands of virgin rats of control group (A) showed strong (+++) expression of both estrogen and progesterone receptors, those treated with harmal (B) exhibited moderate (++) expression of both receptors (Estrogen and progesterone receptors) (X400).

Fig8: Mammary glands of control pregnant rats (A) showed moderate expression (++) of both estrogen and progesterone receptors. Weak expression (+) of these receptors were noticed in those treated with harmal (B) (X200).

Fig9: Mammary glands of lactating rats demonstrated weak expression (+) of both estrogen and progesterone receptors in both control (A) and experimental group (B) (Estrogen and progesterone receptors) (X200)

Table 2: Showing mean of alveoral diameter (µm) and number of nuclei of the epithelium lining the alveolus in control and experimental groups.

Groups	(Mm) mean of alveolar diameter			Number of nuclei of the epithelium lining the alveous		
	(virgin) mean (µm) ±SD	(pregnant) mean (µm) ±SD	lactating mean (µm) ±SD	(virgin) mean ±SD	(pregnant) mean ±SD	lactating mean ±SD
Control	23.98±2.026	32.87±2.1667	38.393±0.879	11±1.53	17±0.58	21.0±1.0
Harmal	*36.34±1.260	*42.17±1.0013	*50.58±1.478	14.0±1.0	17.0±1.0	23.3±1.53

P* < 0.05

Table 3: Serum progestrone, Estrogen and Prolactin in Control and Experimental Groups

Groups	Control Group			Groups treated with harmal		
	(virgin) mean ± SD	pregnant mean ±SD	lactating mean ±SD	Mean ±SD	mean ±SD	mean ±SD
Estradiol ng/ml	72.9±0.2	120.76±1.89	96.363±0.3	75.7±0.3	*15.5±4.3	*107.4±0.52
progesterone ng/ml	7.103±1002	11.36±0.37	15±0.2	*8.4±1.0	*14.4±0.4	*22.3±0.2
Prolactin ng/ml	4.5±1.0	9.4±0.4	10.4±0.4	*8.167±0.1	*10.3±0.2	*12.56±0.28

P* < 0.05

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Differentiation Between Two Isolates Of Entamoeba histolytica Isolated From Different Clinical cases After Intraceacal Inoculation In Hamsters

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ABSTRACT:

BACKGROUND:

The aim of this study is to differentiate between virulence of two isolates ,one was isolated from severe clinical symptoms of amebiasis isolate (A) the other was from asymptomatic carriers isolate (B).

METHODS :

12 male hamsters were employed in this study , they were divided into 3 groups . 1st group (5 hamsters), inoculated intracecally with isolate isolate (A) from human case of severe amebiasis . 2nd group (5 hamsters) inculated with isolate (B) from asymptomatic carriers .3rd group (2 hamsters)inoculated with saline solution as control group .

RESULTS :

1st group of hamsters showed a flask shaped ulcer with sever inflammation of cecum with liver abscesses , after four weeks of infection The 2nd group showed only mild inflammation of cecum without ulceration and liver abscess. While 3rd group were normal control group.

CONCLUTION:

Our study may be Considered as a step for Characterization of Entamoeba histolytica that cause the invasive intestinal and extraintestinal amebiasis and E.dispar an intestinal commensal parasit in Iraq.

KEY WORDS: Entamoeba histolytica , Virulence, Amebiasis **.**

INTRODUCTION :

Amoebiasis is due to invasion of the colon mucosa by E-histolytica, it is an important parastic disease in human , have a variety of affects ,[2] .

E-histolytica trophzoites first colonize the human of larg intestine in 80% to 90 % of cases E-histolytica remains in the lumen and there are either no clinical symptoms or patients complain of mild gastro intestinal disconfort [3] .

In 16% of cases the amoebae become invasive adhering to and digesting the wall of intestine forming flask shaped ulcer [4]. E.histolytica has recently been reclassified as two distinct but morphologically identical intestinal parasites, E.histolytica causative organism of invasive amebiasis and E.dispar non pathogenic intestinal commensal parasite [5] , this reclassification stemmed from a cumulative Clinical , Biochemical , Immunologic and genetic data [6].Infection with E.dispar is approximatly 10 times more common E.dispar be revivad for the beign specie. In 1997, the world health organization (WHO) formally accepted this redefinition [7].

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MATERIAL AND METHODS :

Two isolates of Entamoeba histolytica were used from stock cultures of morphologically identical E.histolytica cultivated and maintained on Cleveland and Collier medium at 37°C .The amoebae were chosen carfully from patients with symptomatic dysentry and asymptomatic carrier . 0.05 ml of culture suspention adjusted to contain about 500,000 trophozoites were inoculated interacecally in hamster under ether anaesthesia. 0.05 ml of saline solution were used for inoculation control group of hamster .12 male hamster mescaricetus auratus were used as experimental animals their wieght range between 100-120 gm .

Groups of animals :

Group I 5 animals inoculated with 0.05 ml suspention from positive amoebae culture isolated from acute case of amoebic dysentery (isolate A).

Group II 5 animals inoculated with 0.05 ml from positive amoebae culture isolated from asymptomatic cases (isolate B).

Group III 2 animals inoculated with 0.05 ml saline solution as control group . Hamster were killed with an overdose of ether at the end of experiment which was 4 weeks post inoculation . Direct wet smear of hamster stool specimens were

examined before and after inoculation . The caecum was removed and gently washed with saline , it was cut open with scissors, the mucosa was exposed , portions of caecum which were regarded as grossly damaged was taken , liver was removed and examined to absorve the number and severity of abscess . Specimens were taken from theses organs , fixed with 10% formalin embedded in paraffin sectioned and stained with eosin and hematoxyline for histophathological studies .

RESULTS :

Group I Cecum . Histopathological studies showed a flask shaped ulceration with infiltration of acute and chronic inflammatory cells Fig (1), inflammatory exudate is occupying the lumen mainly , trophozoite are seen within the lumenal exudate Fig (1,2). The amoebae pentrates the mucosal epithelium as a role of polymorphic leucocytes in the production of amoebic lesions .

Group I Hepatic Lesions: (4 of 5) infected hamsters showed lesions . The lesions were focal , well demarcated creamy white areas scattered irrgularly , lesions were mostly subcapsular .

Microscopic wet smear examination of stool specimens shows positive trophozoite of E.histolytica. The body weight loss was 15 gm table (1).

Group II Cecum .Histopathological studies showed mild inflammation , inflammatory cells include Lymphocytes plasma cells and neutrophil without ulceration .

Group II Hepatic Section Hepatic section was normal No Lesions were seen . Stool examination was positive for trophozoite with increase in body weight table (1). ***Group III*** Stool examination showed negative for E.histolytica . Body weight showed increased (10 gm) and normal grossly apperance of Cecum and liver .

DISCUSSION :

From above observations it seems that the experimental model showed variation in the symptoms of the infected animals due to the virulence of the strain [7]. The major Clinical syndromes that result from infection with isolate (A) include chronic invasive colitis with liver abscesses which suggest that the isolates was highly virulent and invasive (8,9) , in contrast isolate (B) which was isolated from asymtomatic carriers showed only mild inflammation of cecum without ulceration of the liver [10] . Our results are confirmative to other recent study done on the same isolates , when isolate (A) was highly virulent produced multible liver abscesses and acute inflammation while isolate (B) showed mild inflammation only (a virulent) when its inoculated interahepatically in hamster [11] . These results are also confirmative to other study done on the same isolates using electrophorysis analysis, was showed different electrophorysis patterns [12]. The presence of different isonenzyme varient groups revealed that there are certain isoenzyme patterns for each isolate [13,14] .

conculation is reached that there are two strains of morphologically identical E.histolytica that cause invasive intestinal and extraintestinal amebiasis and E.dispar that has never been shown to cause human disease [12,13] .

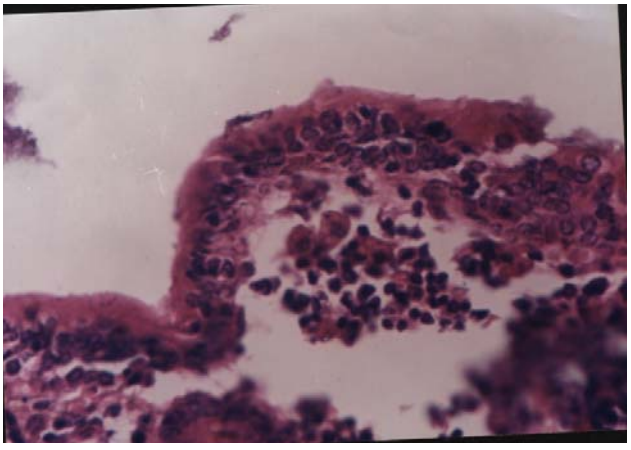


Figure 1 : Cecum section showed a flask shaped ulcer, trophazoites are seen after intraceacal inoculation of isolate (A) (x600)

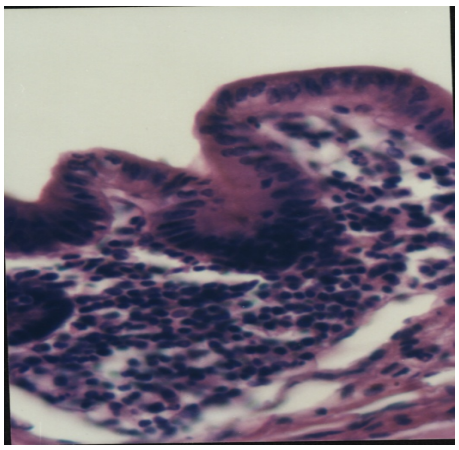


Figure 2 : Cecum section showed ulceration with acute and chronic inflammatory cells after intraceacal inoculation of isolate (A) (x600).

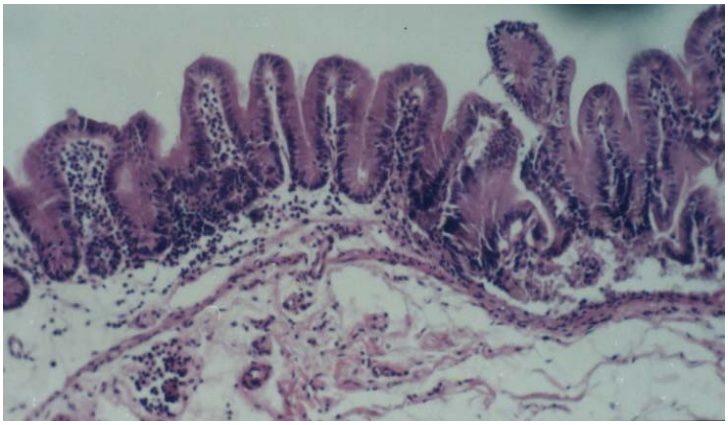


Figure 3: Cecum section showed mild inflammatory cell , lymphocyte plasma cell and neurtrophile after intraceacal inoculation with isolate (B) (x400)

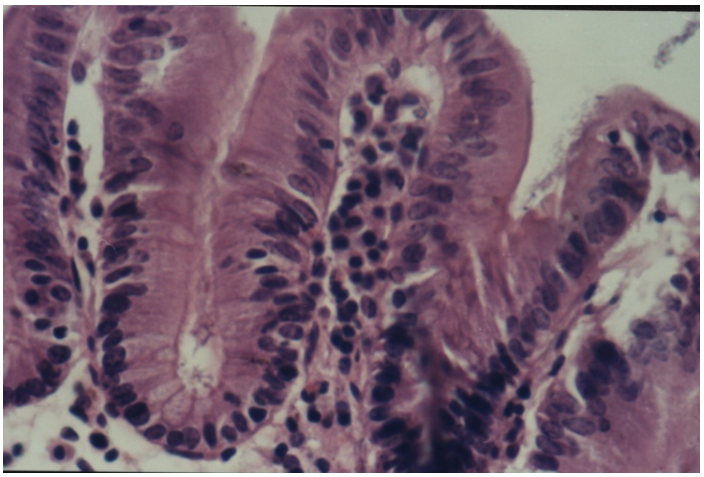


Figure 4 : Cecum section showed mild inflammatory cells after intraceacal inoculation with isolate (B) (x600)

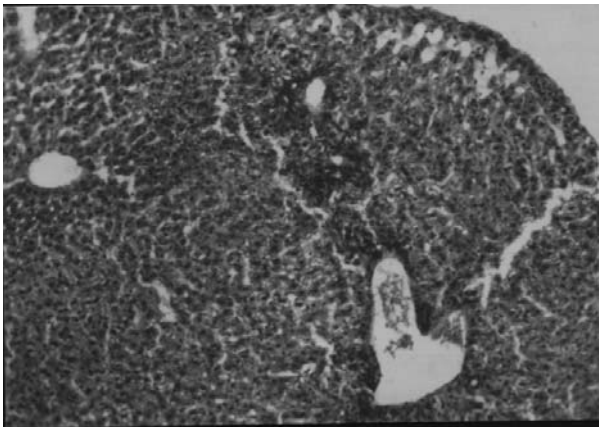
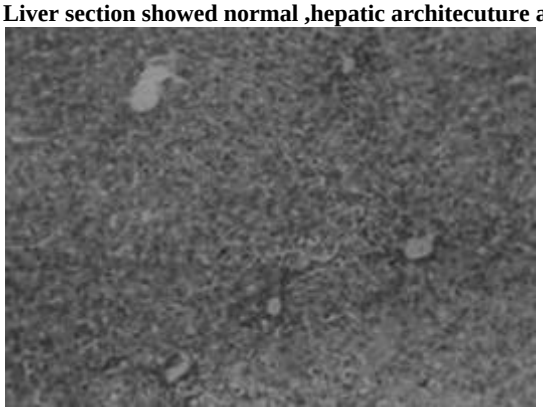


Figure 5 : Liver section showed Liver abscess after intraceacal inoculation with isolate (A) (x600)

Figure 6 : Liver section showed normal ,hepatic architecuture after



intraceacal inoculation with isolate (B) (x600)

Animal group	Stool examination	Change in the body weight	Cecum	Liver
Group I	Positive with trophozoite	-15 gm	Severe ulceration	Many abscesses
Group II	Positive with trophozoite	+5 gm	Mild inflammation	No abscesses
Group III	negative with trophozoite	+10 gm	No intestinal Lesions	No abscesses

Table 1: stool examination, body weight and grossly apperance of three group of animals included in this study

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Epstein- Barr Virus In Iraqi Patients With Nasopharangeal Carcinoma

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ABSTRACT:

BACKGROUND:

Epstein-Barr virus (EBV) was ubiquitous Herpes virus that had a role in the development of undifferentiated carcinoma of the nasopharynx, Burkett's lymphoma, acute infectious mononucleosis and other lymphoprolifrative disorders.

METHODS:

Thirty Iraqi patients with nasopharangeal carcinoma were referred to Oncology Unit in Al-Kadhemia Teaching Hospital from 1992-1994. Sera of those patients were tested for the presence of antibodies against Epstein-Barr virus nuclear and early antigens using indirect immunoflourescence test. Cellular immunity for those patients was tested for the CD4+, CD8+, CD4/CD8 ratio, T-cells % and B -cells %. Their results were compared with twenty-two normal apparently normal individuals.

RESULTS:

Antibodies to Epstein-Barr virus nuclear and early antigens were detected in nasopharangeal carcinoma Iraqi patients and not in the control group. There was significant difference between two groups in CD8+ cells, T- cells % and B- cells % and there was no significant differences between two groups in CD4+ cells, CD4/CD8 ratio.

DISCUSSION:

EBV infection was stopped by T- cells immune response that was capable of eliminating virus infected cells and virus neutralizing antibodies against nuclear and early antigens which prevent the spread of infection. Lymphocytes were predominantly CD8+ cytotoxic T lymphocytes, which recognize and destroy EBV infected cells.

RECOMMENDATIONS:

Other antibodies to viral capsid antigens (IgG, IgA and IgM). Other methods must be used other than indirect immunoflourescense test like western blot method and enzyme linked immune sorbent assay (ELISA).

KEY WORDS: Epstein-Barr virus, nasopharangeal carcinoma, antibodies.

INTRODUCTION:

Cancer can be induced by different causes, one of them viral infection. The best example was undifferentiated carcinoma of the nasopharynx that Epstein-Barr virus (EBV) had a role in the development of this tumor (2), this virus is a member of the Herpes group of viruses. By the age of three years 99% of children in developing countries had been subclinicaly infected with EBV while in developed countries, infection occurred between the 15-25 years (1).

The virus was excreted in oropharangeal secretions and responsible for person-to-person transmission. This virus was isolated from cells of an east African individual with Burkitt's lymphoma. In fact, Epstein and Barr discovered this in 1964.

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It was also associated with nasopharangeal carcinoma by replication of this virus in the epithelial cells of some nasopharangeal carcinoma, a tumor that occurred primarily in China, and with thymic carcinoma and B-cells lymphoma in the United States. However, cells from Burkitt's lymphoma patients in the United States show no evidence of EBV infection while cells isolated from East African individuals with Burkitt's lymphoma contain EBV DNA and nuclear antigens (6,7). Viral EBV antigens were divided into three classes based on the phase of the viral life cycle in which they were expressed: latent phase antigens, these included EB nuclear antigens and LMP1 antigens. Their expression revealed that an EBV genome was present and revealed past infection. Second antigens were early antigens, which included non-structural early antigens; their expressions indicated the onset of productive viral replication and were often found in nasopharangeal carcinoma.

Lastly, late antigens that included viral capsid and viral envelope antigens. They were produced abundantly in cells undergoing productive viral infection (7). Immunity to (EBV) infections elicited an intense immune responses consisting of antibodies against many virus specific antigens, a number of cell mediated responses and secretion of lymphokines (7). Primary EBV infection was stopped by two defenses: cellular immunity by T – cells immune responses capable of eliminating almost all virus infected cells with characteristic atypical lymphocytes were predominantly CD8+ cytotoxic T lymphocytes. The other arm of immune responses was humoral immunity by induction virus neutralizing antibodies which prevent the spread of infection from one target cells to other and the pattern of antibodies responses to different EBV antigens help in distinguishing acute or subclinical infection from past EBV infection (1,3,17).

EBV escaped from immune system and cause viral persistence by down regulating the expression of highly immunogenic antigens of the virus or by direct modulation of the host cytotoxic T lymphocytes responses by virus encoded proteins (3). Immunological and virus serological testing were frequently performed in order to determined the pattern of specific antibodies to different classes of EBV antigens and their diagnostic and prognostic significance.

The aim of this study was to detect whether those patients had a high level of antibodies against EBV, and to study the cellular immune responses in Iraqi patients with nasopharangeal carcinoma caused by EBV infection. So this revise mainly concerned EBV serology and related immunological parameters.

PATIENTS AND METHODS:

1. **Patients group:** consisted from thirty Iraqi patients with nasopharangeal carcinoma who were referred to Oncology Unit in Al-kadhemia Teaching Hospital from 1992-1994. Their age ranged from 25-65 years. Males were fifteens and the rest was females.

2. **Control group:** included twenty-two apparently healthy individuals. Their age ranged from 23-66 years. Males were thirteen and the rest was females.

METHODS:

1. Sera from both groups were separated from the blood and tested for the presence of antibodies directed against EBV nuclear antigens and early antigens using indirect immunofluorescence test (GULL LABORATORIES, USA).
2. Lymphocytes from the blood of both groups were separated using Ficol-Hypaque lymphocytes separation media and then enumeration of lymphocytes subsets (CD4+, CD8+, CD4+/CD8+ ratio) were done using indirect immunofluorescence test (16).
3. Counting of T-lymphocytes was done using sheep rossating method and enumerations of B-cells were done by direct immunoflourescence test (16).

Statistical analysis was done using student t-test.

RESULTS:

Serological test for detecting antibodies against EBV antigens (EBVNA) were detected in 93.33% of the patients with nasopharangeal carcinoma living in different parts of Iraq.

Antibodies against EBVEA were also detected in 93.33% of patients. There was a significant differences (P>0.05) between patients group and control group in these two antibodies as shown in table-1-. In case of testing lymphocytes subsets (CD4+, CD8+ and CD4+/CD8+), there was a significant difference (P>0.05) between two groups in cytotoxic T-cells CD8+ cells only. The mean of this cells was 27.56 while in the control group was 20.40 as demonstrated in table-2-.

The last table –3- showed the significant differences (P>0.05) in the percentages of T and B-cells between these groups. In the patients group the percentage of T-cells was 37.1% and B-cells was 3.33%.

Table1: Antibodies to EBV nuclear antigens and to EBV early antigens in Iraqi patients with nasopharangeal carcinoma compared with control group.

Serological tests	Patients group(♂ and♀) Positive reaction No.= 30		Control group Positive reaction No.=22	
	No.	%	No.	%
EBVNA	28	93.33 (1)	1	4.54
EBVEA	28	93.33 (1)	1	4.54

(1) P>0.05

Table 2: Lymphocytes subset in Iraqi patients with nasopharangeal carcinoma (mean +_ standard error mean)(X+_ SEM) and control group.

Lymphocytes subsets	Patients group (♂ and♀) No.=30	Control group No.=22
CD4+% X+- SEM	38.16 +_ 4.03 (N.S.)	41.22+_3.03
CD8+ % X +- SEM	27.56+_2.02 (1)	20.40+_1.03
CD4+/CD8+ ratio X+- SEM	1.53+_0.21 (N.S.)	1.95+_0.35

(1): P>0.05
(N.S.): Not significant.

Table3: Percentages of T and B-lymphocytes of Iraqi patients with nasopharangeal carcinoma in comparison with control group.

Tests	Patients group (♂ and♀) No. =30	Control group No.=22
T cells % X+_ SEM	37.10 +_ 1.76 (1)	50.90+_ 2.86
B cells % X+_ SEM	3.33 +_ 0.25 (1)	5.18+_ 0.36

(1): P>0.05

DISCUSSION:

Epstein –Barr virus was a member of human herpes virus family and like other herpes virus maintains a life long latent association with B-lymphocytes and a permission association with stratified epithelium in the oropharynx. Clinical manifestations of primary EBV infection range from acute infectious mononucleosis to symptomatic persistence infections. EBV was also associated with a number of malignancies in the human by induction B-cells proliferation and activation of a cellular oncogens (4,6,7).

The studying of immune responses in EBV infected patients was important because some patients with EBV infections had failure of immunity and induction of malignancy in patients receiving immune suppressive therapy (1). In the present study, several standard immunological and serological parameters were tested. We found that sera from Iraqi patients with nasopharangeal carcinoma contained elevated levels of antibodies to different viral specific antigens {EBV nuclear (EBNA) 93.33 % and EBV early antigens (EBEA) 93.33 %}, which was in acceptance with other reports (1,6,12,17) that the presence of EBNA was developed about four months after infection, and remain for life while EBEA appear during primary infection and considered an indicator of active infection and could be useful diagnostically.

We conclude that this cancer was present in Iraqi patients and EBV had involved in the development of carcinoma of the nasopharynx because those patients had a high levels of antibodies to EBV both EBVNA and EBVEA while control group did not had this antibodies. Other reports showed that antibodies were developed against viral capsid antigens of IgM type that appear early in the course of infection and of IgG type that was helpful in the diagnosis of infection (1,6,12). The presence of these antibodies in the serum of Iraqi patients suggested that EBV had involved in the development of this cancer in Iraqi patients. Other reports in other countries showed that EBV infection occur early in life, with immunity to EBV acquired primarily after four years (6,15). In addition to EBV specific antibodies, non-specific hetrophile antibodies were found that react with any components of EBV and disappear within six months after recovery (6). Studying cellular immunity in this revise, we found that CD8+ cytotoxic cells was significantly increased 27.56 +_ 2.02 and T lymphocytes (percentage of rosette-forming cells in the peripheral blood) and B lymphocytes percentages were decreased significantly (37.1+_ 1.76 and 3.33 +_ 0.25 respectively while CD4+ cells and CD4+/CD8+ ratio did not affected.

This may be due to EBV produced IL-10 which had IL-10 like activity and like IL-10 tends to suppress TH1 activity by cross-regulation and reduce the cell mediated response to EBV thus conferring a survival of the virus.

This reflect the role of TH1 / TH2 balance in determining the outcome of disease (17). This virus may also due to lymphopenia and suppression of cellular immunity cause it and certain degree of humeral responses detected by hyper gammaglobulinaemia in sera of some patients (5). All above disturbances in the results had no prognostic value in predicting the treatment response to chemotherapy (5).

In our study the increase in the CD8+ cells, this may be due to cytotoxic T cells recognize virally determined epitopes on infected cells make up the major effecter arm and control the infection.

This results was in agreement with other studies, which detected that there was a high number of peripheral activated CD8+ cells with low cytotoxicity (8), this was due to tumor cells were unable to process EBV antigens and presented to cytotoxic T cells presumably because of defect in antigen processing genes such as TAP1 and TAP2 genes (4). Other studies showed that an important regulatory mechanism for the maintenance of EBV latency in B-lymphocytes was T cells competition for growth factors produced and utilized by EBV immortalized B cells (14).

EBV encoded genes that ensure its persistence in human B-lymphocytes and encourage B-cell proliferation and evade immune recognition (9). Evasion from cytotoxic T lymphocytes surveillance might be an important step in the pathogenesis of Epstein-Barr virus by down regulation of all transformation associated viral antigens except EBNA-1and certain HLA class I alleles. EBNA-4 was the predominant target of HLA restricted cytotoxic T cells and EBNA-6 was the lesser target for these cells (10).

The decrease in the percentages of T and B cells were in agreement with Ware *etal* (11) who showed decrease in the number and increase in the expression of surface lymphotoxin and tumor necrosis factor on activated T, B (CD20+) and natural killer cells (CD56+) in peripheral blood. The long term T cell immunity to Epstein-Barr virus was considered to play an important role in suppressing proliferation of EBV infected B cells and out growth of EBV associated tumors (13).

So this reduction in the percentages of both T and B cells might be due to virus induced lymphopenia.

RECOMMENDATIONS:

Other antibodies to EBV antigens like IgA antibodies to EBV capsid antigens appear to be a useful screening test for early detection of nasopharangeal carcinoma using different new methods. Other cellular and immunological testing must be done to study the immune response in those patients. We recommend doing a control group with some patients with other forms of carcinoma of the head and neck.

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Evaluation of EBV serum and salivary IgA antibodies level in head and neck cancer patients

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ABSTRACT:

BACKGROUND:

Many previous studies revealed that salivary and serum IgA response to infectious agents such as EBV-coded antigens in patients with nasopharyngeal carcinoma (NPC) had a vital role in the combat against tumorigenesis. Moreover, it could be considered as a reliable index for the humoral limb of anti-cancer immunological action.

METHOD:

One hundred twenty two head and neck cancer (HNCA) patients were selected randomly from two main hospitals, Alkadhimya hospital and radiotherapy center in Baghdad. Also 100 apparently healthy control subjects (HC) underwent the same examinations and tests. Enzyme-linked immunosorbent assay (ELISA) test was applied on all HNCA patients sera and saliva, in order to measure anti-EBV IgA antibodies .

RESULTS:

Revealed that NPC patients were the only group that showed a sero-positive ELISA readings of anti-EBV serum IgA. No saliva EBV antibodies were detected in the studied groups .

CONCLUSIONS:

S.IgA level seems to be an applicable index for evaluating EBV burden originated from nasopharyngeal cancerous cells. Such index might help in diagnosing early and specifically the carcinogenesis of NPC, moreover, might help in evaluating the progress of the disease.

KEY WORDS: Head and neck cancer, HNCA, nasopharyngeal carcinoma, NPC, immunosuppression.

INTRODUCTION:

In context of humoral immunity role in HNCA, several early reports on the salivary IgA response locally to infectious agents such as viruses provided a basis for investigations of the specific IgA local immunological response to EBV-coded antigens in patients with NPC who has become a good patient sample for the humoral limb of anti-cancer action (1, 2). Serum IgA levels were elevated during pre- and post- therapy periods and serum immune complexes are elevated in the post therapy periods (3, 4). Some reports revealed that there was little correlation between the degree of elevation of IgA level and clinical course of disease in HNCA while in NPC, however levels of IgA specific to EBV have shown a reliable correlation with tumor load (5). Some reports revealed that the time of elevation of serum IgA and immune complexes is correlated with the concomitant drop in CMI activity (1, 6). While other study showed that IgA levels were elevated constantly in HNCA mainly in younger adults more than older ones, and this elevation is associated with an enhanced immunologic helper state (7). In NPC IgA, IgG, IgE serum levels against EBV antigens were elevated, indicating the virion antigens are triggering components of humoral immunity, and serve as a tumor-associated antigens , which could be external or internal, and such antibodies may clarify the HNCA etiology (1, 3). In such cases it is important to determine the external etiological agent of cancer that confirmed by serology, for example a virion is serving as a primary cause or a co factorial one, is reactivated from a latent state. Additional support for an immune response to HNCA is derived from observations of antibody-secreting plasma cells either in or bordering the tumor, especially observed in high number in leukoplekias with dysplasia and much fewer such cells are seen in more advanced tumor (8). The relationship between elevated serum antiviral antibodies and local infiltrating antibody-producing cells in the tumor site, focuses our attention on the possible existence of specific relationship between the tumor antigens and the humoral immune response in HNCA,

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whether this response is beneficial or not (9). Soluble immune complexes were found to be also elevated in about 75% of HNCA patients.

Basler demonstrated the principle of high levels of IgA immune complexes in NPC and HNCA due to the close relation of this tumor to mucosal tissues, and mucosal tissues associated lymphocytic tissue (MALT) which are the primary sites for IgA production (10).

One of the most important diagnostic tools in the diagnosis of EBV-related HNCA is the measurement of anti-EBV serum IgA (S. IgA). Serum level of anti-EBV IgA has been considered as an unique feature of NPC in many regions in the world (11). But in Iraq such lightening on the importance of S. IgA against EBV has not yet been achieved. So, we did a full comparison of anti- EBV S. IgA levels among HNCA patients and with HC subjects. In our study we intended to evaluate the relationship between the level of serum and saliva IgA EBV antibodies and the HNCA disease and NPC in particular, in attempt to find out a reliable diagnostic and/or prognostic tool for HNCA patients.

PATIENTS AND METHODS:

Patients were selected from those with HNCA and at different stages of the disease progression from two main centers; Alkadhimya hospital and radiotherapy center in Baghdad. 122 head and neck cancer (HNCA) patients were involved, composed of 66 patients of CA larynx, 42 patients of NPC, 14 patients of Hypopharyngeal CA. While other types of HNCA including 4 patients of tonsillar carcinoma, 2 patients of the rest which include post-pharyngea, tongue, epiglottic and retromalar carcinomas, were all neglected for statistical inconvenience. The age of HNCA patients was ranging between 16 to 74 years old (median,53 years) and (mean, 51.8 years).

Control group consisted of 100 apparently healthy people in good general health, ranging age from 21 years old to 66 years old and non of them was taking medicine regularly. Blood was drawn into glass tubes of 10 ml in size for serum separation. Serum stored at –20 ^{Co} until used in ELISA (12). Saliva samples were taken from patients and control group put in glass universals cup and directly were frozen at –20 ^{Co} till their use later on by ELISA, to detect the presence of secretory IgA in saliva, which is specific for EBV antigens in patients who proved sero-positive against EBV antigens and also in sero-positive control population.

ELISA assay **:** ELISA assay was designed to detect quantitatively the serum and salivary IgA against EBV antigens. We prepared our local ELISA kit by coating 96 flat bottom plates at concentration of 10 ug/ml of the crude EBV antigen (Wellcome, UK).

Duplicate wells were made for every patient and proceeded the assay as recommended by (13).

Calculation of cut-off value for S. gallolyticus seropositivity **:**

Cut off value is considered as the upper limit above which all readings were considered as positive. Therefore, ELISA readings of control subjects (n=100) were used to calculate the cut-off value according to (14): 99% Confidence interval (cut-off value) = mean + 2.626× Standard error mean. (2.626): taken from the table of student's t-test under the p=0.01 for the 99 degrees of freedom.

Statistical analysis**:**

Data analysis was performed by the following computer statistical programs:Microsoft EXCEL 97and Statistica.

RESULTS AND DISCUSSION:

Out of 122 patients of HNCA we can notice the predominance of squamous cell carcinoma (SCC) over all types of HNCA (74% in CA larynx and 68% in hypopharyngeal CA), except for NPC patients that elicited a different feature of having 93% of highly undifferentiated carcinoma, namely Lymphoepithelioma (LE).

Statistical analysis showed that only NPC group was significantly of higher S. IgA level (mean ELISA reading = 1.34) than other groups of HNCA (mean ELISA reading for CA larynx= 0.98; for hypopharyngeal CA =1.02) (p<0.01), while there was no real difference between Hypopharyngeal CA and CA larynx (p>0.05).

The cut-off point calculated was 1.22. Depending on this cut-off point, it was found that only 9 patients within NPC group were sero-negative for anti- EBV S. IgA , while in CA larynx only 6 patients were sero-positive and finally in Hypopharyngeal CA only 3 patients was sero-positive and the rest of patients were sero-negative (Table 1). This important serological feature reflects a fact that there is a strict relationship between EBV and etiology of NPC in Iraqi patients. Very rare those reports that revealed a positive detection of salivary anti-EBV IgA of HNCA, especially in NPC patients.

The majority of reports tell us that there is no positive results regarding this field (1, 3, 15).

We applied ELISA technique on saliva as well as on sera of HNCA patients. After completion of assay we did not detect any positive ELISA reading for the salivary IgA (mean ELISA reading = 0.099) when compared to HC group (mean ELISA reading = 0.089).

Therefore, we inferred that immunoassay on saliva anti-EBV IgA is not sufficiently sensitive for the diagnosis of EBV-related tumors.

The detection of anti-EBV S.IgA is still more specific than the detection of anti-EBV S.IgG, because anti-EBV S.IgA is only found in NPC patients, while anti-EBV S.IgG could be found in low levels in a large proportion of the population due to the childhood IM infection and in high levels in immune deficiency conditions, and few other cancers in addition to NPC (1, 3).

Because of the fact that 78.5 % of the sera of NPC patients in this study were positive to EBV S. IgA antibodies, thus, using serological kits for the detection of EBV antibodies seems to be a useful, cheap and non-invasive method to diagnose about 78.5% of NPC cases in Iraq, which is a very high percentage of cases that make the use of these serological tests seems really worthy.

In contrast to serum EBV antibodies, saliva antibodies against EBV were not detected at all. This has a very important impact on our imagination on what is happening inside the nasopharyngeal tissue.

According to many reports, it has been postulated that the real reservoir for EBV latent infection within the nasopharynx is the tissue infiltrating lymphocytes (TIL) which are in continuous recycling with PBL, and the transfer of EBV infection is Bi-directional between the source TIL and the recipient cells, the nasopharyngeal cells. But not all the nasopharyngeal tissue layers are susceptible to get EBV infection, it has found that only the deep layers have this criteria because they have CD21 receptors, the gate for EBV entry (16, 17). Therefore, S.IgA are triggered far more than salivary IgA which depends on the availability of EBV-encoded antigens on the superficial layers of nasopharyngeal tissue cells.

Hence, we conclude that S.IgA levels might be applicable index for evaluating the EBV burden that originated from nasopharyngeal cancerous cells. Such index might help in diagnosing early and specifically the carcinogenesis of NPC, moreover, might help in evaluating the progress of the disease and give a glimpse to the humeral limb of immunity among NPC patients.

Table 1 : Correlation of different HNCA types with each other depending on sero-positive / negative ELISA readings for anti-EBV S.IgA

NPC		Hypopharyngeal CA		*p* value
Sero +	Sero -	Sero +	Sero -	0.009chi
33	9	3	11	0.018 F.E
NPC		CA larynx		0.00001 chi
Sero +	Sero -	Sero +	Sero -	0.0001 F.E
33	9	6	60	
Hypopharyngeal CA		CA larynx		0.28 chi
Sero +	Sero -	Sero +	Sero -	0.52 F.E
3	11	6	60	

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Prevalence of Autoantibodies to Various Tissue Antigens Before and During S2 – Complex Immunotherapy in Head and Neck Cancer Patients

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ABSTRACT :

BACKGROUND:

A total of 63 terminal untreatable stage IV head and neck cancer patients were investigated for clinical responses and presence of autoantibodies to various tissue antigens before and during S2- complex immunotherapy.

METHODS:

S2 –complex is a new low molecular weight biological response modifier (BRM) with a potant immunostimulating and anti tumor activities.

RESULTS:

Autoantibodies detected at pretreatment period were those directed towards the following antigens : nuclear, thyroid microsomal, epithelial cells, gastric parietal cells, smooth muscles, peripheral leukocytes, T-lymphocytes, B-lymphocytes, monocytes, thymus reticular epithelial cells and Hassall's corpuscles. Beside, autoantibodies with specificities to glomerular basement membrane and vascular endothelial cells were present at low incidence. Short term use of S2- complex induced a transient increase of the following autoantibodies: nuclear, thyroid microsomal, epithelial, parietal cells, smooth muscle, thymus reticuloepithelial cells, Hassal's corpuscles, thymocytes, peripheral blood leukocytes, T, B-lymphocytes, monocytes, as well as kidney glomerular basement membrane and vasculr endothelial cells.

DISCUSSION:

In the later follow up period i.e. 2-6 months most of these autoantibodies responses returned to normal healthy control levels. Moreover, two exceptions were demonstrated which were the incidences of the antiglomerular basement membrane and vascular endothelial antibodies which remianed higher than the pretreatment frequencies. In addition, autoantibodies specific to mitochondria , thyroglobulin and red blood cells were only occasionally seen in our head and neck cancer patients both before and during S2-complex therapy.

KEY WARDS: Head and neck, Cancer, Autoantibodies.

INTRODUCTION:

Host immune response may play an important role in the origin and progression of head and neck cancer. Studies in the early 1970 revealed a depressed cell mediated immune response to cutaneaous antigenic stimulus of 2, 4 dinitrochlorobenzen (DNCB) skin testing (26). Various autoantibodies have been identified in the sera of patients with epithelial and hematological malignancies (1,2). Local recurrences and metastatic spread occurred more often among patients with autoantibodies (3). Another physiologic condition associated with increased titers of autoantibodies was aging (4,5).

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Cancers are more prevalent in the elderly than in the younger individuals.Treatment of head and neck cancer is surgery, radiotherapy, chemotherapy and immunotherapy. These methods either used alone or in combination depending on the type, site, stage of tumor, patients age and general health. Immune therapy is a new type of cancer treatment that can boost or restore the body's immune system and act as an adjuvant after and / or with other three modalities of treatment(27). This can be achiveed by using immune adjuvants like levamisole, IL-2 and interferons.

S2-complex (Synthetic-2)is one of a new immune therapy used in this study. It is a low molecular weight synthetic organometalic complex (Iraqi classification No.6, International classification No.61K). It is submitted to the insurance of innocence of invention No.2836 in 15/1/1992.

It is used in the treatment of advanced stages (stage III and stage IV) of HNC after surgery and / or radiotherapy or chemotherapy in the oncology unit. It had been found that immune therapy lead to production of auto antibodies in the patient serum like treatments with interferons lead to auto immune thyroiditis due to polyclonal activation so in this study, we try to demonstrate the incidence of various autoantibodies to different tissue antigens (Ags) in advanced head and neck cancer (HNC) patients stage IV before and during 6 months (ms.) of S2- complex immunotherapy to evaluate the effect of this immunotheraputic agent in exacerbation of auto immune responses.

PATIENTS & METHODS :

1. Patients group (treated with S2-complex):

Sixty three patients with advanced head and neck squamous cell carcinoma (HNSCC) , most of them failed the conventional forms of therapy (surgery, radiotherapy, chemotherapy) and refered to Oncology Unit in University Hospital of Iraqi Medical College o from year 1992 to 1994 for immunotherapy with S2-complex (0.1-0.5 mg/ kg body weight) for five consecutive days and then once at weekly interval for 6 months. Their aged ranged (13-79 ys.) Male to female ratio was 51/12. Most of them were heavy smokers. They had carcinoma of different sites in the head and neck area (larynx, hypopharaynx, parotid and maxillary sinus ..etc.).

2. Patients group without treatment:

70 patients with HNC. Sera were obtained from 30 patients with carcinoma of the larynx, 25 with carcinoma of hypopharaynx and nasopharaynx and the rest from other sites. Their age's ranged from 14-65ys., medianage was 50 ys.

3. Control group:

Consisted from twenty healthy individuals. Their age ranged (23-73ys.). All of them were in good general conditions, not smokers and did not take any medication. Sera of both groups were separated from the blood and used for detecting the presence of autoantibodies against different tissue antigens using indirect immunofluorescence method (6).

RESULTS:

The first autoantibody which had been detected in our study was antinuclear antibody (ANA) table -1- was detected in 65.07 % of head and neck cancer patients. S2- complex induced transient increase in this autoantibody then decrease to 40 %. The pattern of reaction was speckled in most of reactions. The predominant isotype was IgG and the titer was more than 1:20. the regression index increase early after treatment then decrease after 6ms. Of treatment. The same condition was noticed with other autoantibodies like antimicrosomal antibodies table-2-. There was a transient increase in the level of autoAbs then decrease after long period of follow up. The titer was also IgG type and the titer was 1:10. regarding antithyroglobulin Abs, It occurred only before treatment and after one mounth of treatment. Other autoAb was the antiepithelial antibodies (table-3-) also showed a transient increase in these autoantibodies and their titer mostly of 1:10 and also increase in minority of cases reaching 1:640. The isotype of these autoantibodies is of mixed types (IgM, IgG, IgA). In case of antiparietal cell antibodies (table-4-), there was a progress decrease in antipariatal cell Abs reaching 6.6% after 6ms. And decrease in the regression index in all periods of follow-up. The titer in most periods is 1:10 and of IgG type while antismooth Ms. AutoAbs, there was a decrease in a slower rate and increase in the regression index. The titer was 1:10 and of IgG type. Regarding anti basement membrane antibodies and antiendothelial cells as shown in table 5 and antireticular antibodies (table-6-) demonstraed a transient increase in these autoantibodies and then decrease after long period of follow-up . The titer was 1:10. In case of antierythrocytes antibodies and antimitochonderial antibody, they were not detected in both control and head and neck cancer patients (table -7-). Other Ab was Anti Hassale's corpusels antibody , completely disappeare after 6ms. And anti thymocytes Abs detected in very small percentages (table-6-). Lastly antileukocytes, anti T- cells and B- cells in table -8- showed slight increase in autoantibodies titers and then decrease after six months of follow - up .

Table 1: Antinuclear antibodies,immunoglobulin isotypes and antibody titers in head neck cancer patients before and during S2-complex treatment and in healthy controls.

Individual	Positive Reaction No. %	**Regression index over those of treatment responses %	Titer NO. Of Sera %	Isotype > 1:40 No. %	Pattern Of Reaction no. %
Control N=20	2 10	----------	1:10 2 100	---------	Speckled 2 100
Before N =63	41 65.07	----------	1:10 35 85.3 1:20 4 9.7 1:40 1 2.4 1:80 1 2.4	IgG 1 50 Mix 1 50	Speckled 8 19.5 Rim 9 21.9 Mixed 24 58.5
After 2 Weeks N=51	31 60.7	6.7 *	1:10 ▲ 27 87.09 1:20 2 6.2 1:40 ▼ 2 6.2	IgG 1 50 IgG 1 50	Speckled 21 65.6 Diffuse 1 3.1 Mixed 10 31.2
1 m. n=45	33 73.3	12.6 *	1:10 30 90.9 1:20 ▲ 3 9.09	----------	Speckled 24 72.7 Rim 2 6.06 Mixed 7 21.2
2 m. n=32	24 75	15.2 *	1:10 23 95.8 1:20 ▲ 1 4.1	----------	Speckled 22 91.6 Mixed 2 8.3
3m. n=24	21 87.5	34.4 *	1:10 20 95.2 1:20 ▲ 1 4.7	--------	Speckled 12 57.1 Rim 2 9.5 Mixed 7 33.3
4m n=22	19 86.3	32.6	1:10 15 78.9 1:20 3 15.7 1:40 ▲ 1 5.2	IgG 1 100	Speckled 11 57.8 Mixed 8 42.1
5 m. n=15	12 80	22.9	1:10 10 83.3 1:20 ▲ 2 16.6	----------	Speckled 6 50 Rim 3 25 Mixed 3 25
6m. n=15	6 40	38.5	1:10 5 83.3 1:40 ▼ 1 16.6	gG 1 100	peckled 3 50 rim 2 33.3 mixed 1 16.6

* ▲ increase, ▼ decrease ** Regression index % = 1- (Regression % after treatment / Regression %before treatment) x 100

Table 2: Antimicrosomal, antithyroglobulin antibodies and immunoglobulin isoytpes in head and neck cancer patients before and during S2 complex treatment and in healthy controls.

1ndividual	Anti Microsomal Abs +ve Reaction No %	Regression index %	Titer	no.	%	% iso type > 1:40	No %	Anti Thyro Globu Lin Ab +ve reaction No.%	Titer	no.	%	% iso type > 1:40
Control N=20	3 15 (1)	--	1:10	3	100	--	--	--	--	--	--	--
Before N=63	41 65	--	1:10 1:20	33 7	80.4 17.07	IgG	1 100	--	--	--	--	--

	(1)		1:40	1	2.4							
After N=51	45 88	35.5	1:10 1:20	42 3	89.3 6.6	--	--	1 1.9	1:20 1:100	--	--	--
1m n=45	27 60	7.7	1:10 1:20 1:40	25 1 1	92.5 3.7 3.7	IgG	1 100	--	---	--	--	--
2ms n=32	22 68	5.5	1:10	22	100	--	--	1 3.1	1:10 1:100	--	--	--
3ms n=24	19 79	21.5	1:10 1:20	18 1	94.7 5.2	--	--	--	--	--	--	--
4ms n=22	15 68	4.6	1:10	15	100	--	--	--	--	--	--	--
5ms n=15	10 66	2.3	1:10	10	100	--	--	--	--	--	--	--
6ms n=15	5 33	48.8	1:10	5	100	--	--	--	--	--	--	--

↑ Increase ↓ decrease (1) p<0.005

Table 3: Ant epithelial antibodies and immunoglobulin isotypes in head and neck cancer patients before and during S2 complex treatment and in healthy controls.

1ndividual	+ve Reac Tion No.	+ve Reac Tion %	Regre Ssion index %	Titer	no.	%	Iso Type >1:40	no.	%
Contr Ol N=20	---	---	-----	-----	-----	-----	----	----	-----
Before N=63	30 *(4)	47.6	-----	1:10 1:20 1:40 1:80 1:640	19 6 1 2 2	63.3 20 3.3 6.6 6.6	IgG mix	4 1	80 20
After N=51	24	47.05	1.1 ↓	1:10 1:20 1:80 1:160	20 1 2 1	76.9 4.1 3.9 3.8	IgG mix	2 1	66.6 33.3
1m n=45	31	68.8	44.5 ↑	1:10 1:20 1:40 1:80	23 6 1 1	74.1 19.3 3.2 3.2	IgG	2	100
2ms n=32	21	65.6	37.8 ↑	1:10 1:20 1:40 1:80	15 1 2 3	71.4 4.7 9.5 14.2	IgG mix	4 1	80 20
3ms n=24	22 *(1,2, 3,4)	91.6	92.4 ↑	1:10 1:20 1:40 1:80	13 6 2 1	59.09 27.2 9.09 4.5	IgG mix	2 1	66.6 33.3
4ms n=22	16 *(1)	72.7	52.7 ↑	1:10 1:20 1:40	11 4 1	68.7 25 6.2	IgG	1	100
5ms n=15	9 *(2)	60	26.05 ↑	1:10 1:20 1:160	6 2 1	66.6 22.2 11.1	IgG	1	100

6ms n=15	8 *(3)	53.3	11.9 ↑	1:10 1:20 1:80	6 1 1	75 12.5 12.5	IgG	1	100

*(1): not significant, (2): P<0.02, (3): P<0.01, (4):P>0.001

Table4: Antiparietal cell antibodies and anti smooth muscle antibodies and immunoglobulin isotypes in head and neck cancer patients before and during S2 – complex treatment and in healthy countrol.

1ndividual	Anti Parietal Abs No.	Anti Parietal Abs %	Regression index %	Titer	No.	%	Isotype >1:40 No.	Isotype >1:40 %
Control N=20	------	-------	-------	-------	--------	-------	--------	-------
Before N=63	12	19.4	------	1:10 1:20 1:40	9 1 2	75 8.3 16.6	IgG 2	100
After N=51	5	9.8	48.5 ↓	1:10 1:20	4 1	80 20	-------	------
1m n=45	5	11.1	41.7 ↓	1:10	5	100	--------	------
2ms n=32	-----	0	---- ↓	------	------	-----	-----	-----
3ms n=24	3	12.5	43.3 ↑	1:10	3	100	--------	------
4ms n=22	2	9.09	52.2 ↑	1:10 1:40	1 1	50 50	IgG 1	100
5ms n=15	3	20	5.04	1:10	3	100	------	--------
6ms n=15	1	6.6	65.3	1:10	1	100	-------	-----

1ndividual	Anti Smooth Abs No.	Anti Smooth Abs %	Regression index %	Titer	No.	%	Isotype >1:40 No.	Isotype >1:40 %
Control N=20	------	-------	-------	-------	--------	-------	--------	-------
Before N=63	15	23.8 (1)	------	1:10 1:40 1:60	11 3 1	73.3 20 6.6	IgG 4	100
After N=51	26	50.9	113.8 ↑	1:10	26	100	-------	-------
1m n=45	26	57.7	142.4 ↑	1:10	25	96.1	IgG 1	100
2ms n=32	11	34.3	44.1 ↑	1:10	11	100	----------	----------
3ms n=24	12	50	110.08 ↑	1:10	12	100	----------	--------
4ms n=22	12	54.5	7.07 ↑	1:10 1:20 1:40	10 10 1	83.3 8.3 8.3	IgG 1	10

5ms n=15	9	60	152.1 ↑	1:10	9	100	----------	----------
6ms n=15	5	33.3	39.9 ↑	1:10	5	100	----------	----------

(1) not significant

Table 5: Antibasement membrane , antiendothelial and antimitochondrial antibodies in head and neck cancer Patients before and during S2- complex and in healthy controls.

1ndividual	Anti Base Ment Membrane Abs+ve Reaction No. %	Regre Ssion index %	Titer	no.	%	Anti Endo Thelial Ab +ve reaction No. %	Regre Ssion index %	Titer	no.	%	Anti Mito Chon drial Abs +ve Reaction No. %
Contr Ol N=20	--	--	--	--	--	--	--	--	--	--	--
Before N=63	1 3.1 (1)	--	1:10	2	100	3 4.7 (4) (2)	--	1:10	3	100	--
After N=51	13 25.4 (1)	719.3 ↑	1:10	13	100	11 21.5 (3) (2)	357.4 ↑	1:10	11	100	--
1m n=45	14 31	903.2 ↑	1:10	14	100	3 6.6 (3)	40.4 ↑	1:10	3	100	--
2ms n=32	5 15.6	403.2 ↑	1:10	5	100	5 15.6	231.9 ↑	1:10	5	100	--
3ms n=24	8 33.3	974.1 ↑	1:10	8	100	5 20.8	342.5 ↑	1:10	5	100	--
4ms n=22	7 31.8	925.8 ↑	1:10	7	100	3 13.6	189.3 ↑	1:10	3	100	--
5ms n=15	2 13.3	329.03 ↑	1:10	2	100	3 20	325.5 ↑	1:10	3	100	--
6ms n=15	3 20	545.1 ↑	1:10	3	100	4 26.6	465.9 ↑	1:10	4	100	--

(1) : p<0.001 , (4) (2) : p<0.01 , (3) : p <0.05

Table6: Antireticular, anti Hassall's corpuscles and anti thymocytes antibodies in head and neck cancer patients before and during S 2 –complex treatment and in healthy controls.

1ndividual	Anti Reticular + reaction No.	Anti Reticular + reaction %	Regress Ion Index %	Anti Hassal's Corpus-cles Abs + Reaction No.	Anti Hassal's Corpus-cles Abs + Reaction %	Regress Ion Index %	Anti Thymo-Cyt Abs + Reaction No.	Anti Thymo-Cyt Abs + Reaction %
Contr Ol N=20	--------	-------	--------	--------	--------	--------	--------	-------
Before N=63	47	74.6	-------	15	23.8	------	-------	------
After N=51	34	66.6 (1)	10.7 ↓	23	45.09 (2)	89.4 ↑	8	15.6
1m n=45	31	68.8	7.7 ↓	24 (2)	53.3	123.9 ↑	2	4.4
2ms n=32	20	62.5	16.3 ↓	5	15.6	34.4 ↓	1	3.1

3ms n=24	13	54.1	27.4	8	33.3	39.9	2	8.3
4ms n=22	10	45.4	39.0	1	4.5	81.09	1	4.5
5ms n=15	4	26.6	64.3	----	-----	0	1	6.6
6ms n=15	1	6.6 (1)	91.1	-------	-----	0	2	13.3

(1): P<0.005 (2): not significant

Table7: Antierythrocytea antibodies in head and neck camcer patients before and during S2-complex treatment and in healthy controls.

1ndividual	+ reaction No.	+ reaction %
Control N=20	----------	----------
Before N=63	----------	----------
-After N=51	----------	----------
1m n=45	1	2.2
2ms n=32	----------	----------
3ms n=24	1	4.1
4ms n=22	----------	----------
5ms n=15	----------	----------
6ms n=15	----------	----------

Table8: The mean (pluse, minuse -,+) standered error mean (SEM) of anti leukocytes, monocytes, T and B cells antibodies in head and neck cancer patients before and during S2-complex treatment and in healthy controls.

Tests done	Control No.=20	Before No.=63	After 2 weeks No.=51	1m. No.=45	2Ms. N0.=32	3Ms. No.=42	4Ms. No.= 22	5Ms. No.=15	6Ms. No.=15
Positive leukocytes	2+0.1 -	129-+16.06	71-+12.8	47-+9.08	12-+6.11	6-+1.34	3-+1.07	2-+.07	37-+19.6
p-value	--------	1	1	---------	---------	---------	--------	----------	----------
% increase or decrease 0ver before treatment	------	--------	-44.9	-63.5	-90.6	-95.3	-97.6	-98.4	-71.3
Positive T cells	2-+.-0.5	15-+4.3	26-+6.1	31-+5.6	4-+0.7	2-+0.3	2-+0.4	2-+0.5	3-+0.5
P value	2	2	---------	--------	--------	----------	----------	----------	---------
% increase or decrease 0ver before treatment	---------	---------	+73.3	+106.6	-73.3	-86.6	-86.6	-86.6	-80
Positive B cells	3-+0.09	22-+4.7	21-+4.9	28-+6.9	8-+5.5	9-+7.3	2-+0.1	2-+0.2	6-+3.9
P value	3	3	--------	--------	----------	---------	----------	---------	--------
% increase or decrease 0ver before treatment	--------	---------	+4.5	+27.2	-63.6	-59.09	-90.9	-90.9	-72.7
Positive monocytes	5-+1.2	45-+6.01	52-+7.2	39-+6.6	72-+12.7	33-+7.4	24-+7.8	20-+6.7	33-+10

P value	4	4	----------	---------	----------	----------	---------	----------	----------
% increase or decrease 0ver before treatment	---------	--------	+15.5	-13.3	+60	-26.6	-46.6	-55.5	-26.6

(4,3): P>0.001 (1):P<0.02, (2):P<0.005
- :decrease, +:increase

Table 9: Incidence of autoantibodies in head and neck cancer patients with or without recurrences and without S2-complex immunotherapy.

Autoantibodies	Patients with recurrence No.=55 No. %	Patients free from recurrence No.=15 No. %	Control No.= 20 No. %
Antinuclear Abs	30 54.5 P=0.005	2 13.3 N.S.	1 5
Antismooth muscle Abs	20 40 P=0.005	2 13.3 N.S.	-- --
Antiglomerular Abs	6 10.9 N.S.	1 6.6 N.S.	-- ---
Antimitochonderial Abs	--- ---	--- --	-- ----

DISCUSSION:

The incidence of autoantibodies in head and neck cancer (HNC) patients is significantly higher than in the control group. Our results were in agreement with other investigators (7,8). The autoantibodies in sera of patients directed against: nuclear, thyroid microsomal, epithelial cells, gastric parietal cells, smooth muscles, leukocytes, T cells, B cells, monocytes, thymus reticular tissues and Hassall's courpuscles. Besides, antibodies (Ab) at low incidence towards glomerular basement membrane and vascular endothelial tissues were also detected. The concept that autoimmune abnormalities may facilitate tumor growth are present in animal model(9). In our study the participation of autoimmune status in the immune alterations in patients with HNC had not been previously suggested. The presence of these autoAbs in sera of HNC confirmed the previous finding that cytotoxic drugs, radiotherapy, surgery, alone or in combination induced cellular destruction leading to release and denaturation of self antigens (Ag)e.g. DNA (10). This medication might be involved in triggering the immune response to self Ags. The presence of high level of antinuclear Abs in agreement with other results (11). High incidence of antimicrosomal Abs in our patients are in agreement with other results in breast cancer after radiotherapy or interferone therapy (12). Antiparietal cells Abs. Were detected for the first time in HNC patients. Previous reports showed no remarkable differences in patients with breast cancer and contro group(13). In case of antismooth muscle Abs which present in HNC patients may be due to change in the malignant cell membrane (7). The other auto Ab which was detected in our study is antiepithelial autoAbs It adds to the list of malignancies (skin, thymus, prostate, bladder and chronic lymphocytic leukemia). This is due to a common antigenic determinants on malignant cells that might stimulate immune system to produce cross reactive Abs with both tumor and normal skin (14). In addition to that, there is no significant difference in the incidence of antimitochondrial and antithyroglobulinin HNC which is in agreement with other results in breast cancers (13). Anti red blood cells (RBC) Abs were not detected in HNC but only detected in ovarian tumor, bronchogenic carcinoma (15). The other autoAbs which were detected in small frequency were antiglomerular basement membrane and renal vascular endothelial Abs in HNC patients. In recent years, interest in the development of immunologic approaches to malignancies has increased and there is good evidence that the growth of renal cell carcinoma can be modulated by the host immune system using interleukin-2 and B7-1 gene modified tumor cell as vaccine in the treatment of this disease (20). Immune therapy is another method of treatment HNC in addition to other methods. It acts as adjuvant to other methods. In our study we used S2 –complex which is a low molecular weight, synthetic compound. It stimulates the immune system (16,17).S2-complex treatment inducing transient increase in the incidence of these

autoAbs. This is due to activation of local immunological reactions in the HNC area. This might be due to cross reaction with kidney tissues resulting in autoAbs stimulation. Our report document the occurrence of autoAbs to thymus compounds which may contribute in the HNC pathogeneses. There were also autoAbs reactive with peripheral leukocytes, T, B cells and monocytes in serum samples from advanced HNC patients treated by conventional modalities. These autoAbs were present in other diseases like systemic lupus erythematouses and rheumatoid arthritis (18). Other studies reported that antimitochondrial and glomerular autoAbs were formed as a consequence of hepatic damage of certain liver diseases (25). The most exciting observations in our study were the transient increase of various autoAbs after S2 –complex treatment which last in most cases between 2-8 weeks. Moreover, long term use of S2-complex resulted in inhibition of the various temporally stimulated autoAbs response which was pronounced after 6 months of S2-complex treatment. Our present data may seem to indicate that S2-complex probably may play a role in the positive control of lymphocytes infiltration and class II Ag expression in many tissues . We think that additional experiments and clinical studies were warranted to prove that S2-complex can induce class II Ags expression in head and neck as well as other tissues. The promotion of tumor by induction of autoAbs had been only reported in experimental models (19). Some autoAbs were examined for their presence in patients serum with HNC and without treatment as shown in table-9-. Patients with recurrence showed increased frequencies of these autoAbs while patients who were free from recurrence showed decreased in autoAbs level. These autoAbs were formed due to accelerated lysis either spontaneous or theraputically induced, these led to shed large quentities of related Ags. This led to formation spesific Abs that cross-react with normal tissue components and disequlibirium of the normal immunoregulatory balances (10). Interferon is one of immune modulator used in the treatment of many conditions as immune therapy . It had been reported that treatment with alpha interferon induced autoimmune lesion of thyroid in patients with chronic viral hepatitis (21) and dermatomyositis in patients with melanoma (22) . Treatment with alpha interferon also induced polyarthritis (seropositive rheumatoid arthritis) in patients with chronic myelogenous leukemia and hairy cell leukemia (23). In addition, treatment with beta interferon induced autoimmune thyroiditis in patients with multiple sclerosis (24). In our study , S2-complex were not induced such autoimmune diseases as a result of induction of autoantibodies.

CONCLUSION:

S2 - complex as a new immune modulator did not induce a long term high titer of autoantibodies which might induce autoimmune diseases as a result of poly clonal activation .

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The Location of Aerobactin Determinants of Uropathogenic *E. coli* in Association with Hemolysin Production Antibiotic Resistance and Patient Characteristics.

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ABSTRACT:

BACKGROUND:

Aerobactin, Hemolysin and the resistance to some antibiotics axe important Features of Uropathogenic *E. coli* (UPEC). The characteristics of patients from which we isolate the UPEC have associate with these Features and their determinants location.

AIM:

We determined the phenotypic expression and gene location of Aerobactin and phenotypic expression of Hemolysin among 60 UPEC isolates. We correlated the presence of Aerobactin system with antibacterial agents resistance. The expression of Hemolysin and the characteristics of patients.

METHODS:

Two methods of plasmid curin, sodium dodecyl sulfate and elevated temperature plasmid curing, are used then plasmid extraction and agarose gel electrophoresis are performed to determine the location of Aerobactin determinants and the size of Aerobactin plasmids, as well as the location of the determinants of some antibiotics resistance which were ampicillin (Am), Tetracycline (Tc), gentamicin (GM), Chloramphenicol (C) and co- trimazole (Co).

RESULTS:

Aerobactin and hemolysin expression among UPEC isolates were 93.3% and 35% respectively. The isolates of non compromised patients produce statistically higher rate of expressed hemolysin (90.5% , $p<0.01$). Plasmid- borne Aerobactin was absent in UPEC isolates of non compromised patients (89.5%, $p<0.01$). On the contrary compromised patient isolates express plasmid Aerobactin of 59.5% ($p<0.02$). We also found that Aerobactin determinants are located on a plasmid in compromised patient isolates and associated with the absence of chromosomal Hemolysin production (82.9%, $p<0.01$). Yet, The chromosomal aerobactin is associated with hemolysin production (61.9%, $p<0.02$). Furthermore, UPEC isolates of compromised patients carry relatively large plasmid of Aerobactin (85.7%. $p<0.05$) and these large plasmids carry either chloramphenicol (83.3%.$p<0.02$) or gentamicin determinants (100%. $P<0.01$) but not co- trimazole, tetracycline or ampicillin.

CONCLUSION:

The isolates of non compromised patients carry chromosomal Aerobactin and hemolysin. While the isolates of compromised patients express plasmid Aerobactin and do not express chromosomal hemolysin. Aeobactin plasmids are relatively large plasmids and carry either chloramphenicol or gentamicin resistance determinants.

KEY WORDS: Uropathogenic *E .coli*. Aerobactin. Hemolysin. Antibiotics. Compromised . Non compromised.

INTRODUCTION:

Aerobactin is a derivative of hydroxamic acid. Produced by enteric bacteria like *E. coli* [1,2]. *Shigella* spp and *Klebsiella* spp.[3]The determinants that encode the production of Aerobactin are located either on a plasmid or on the chromosome of uropathogenic *E. coli*, UPEC [1.4].

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The determinants of some antibiotics resistance like chloramphenicol, gentamicin, co- trimazole, ampicilin and tetracycline are carried on the Aerobactin plasmids [5.6]. Such plasmids are usually relatively large plasmids called P Col V plasmids.[7] Hemolysins (Hly) are group of phospholipases, produced by pathogenic *E.coli* strains and had been demonstrated as chromosomal occurance virulence factor (VF). The present study determine the prevalence extent of expression, chromosomal versus plasmid location of the

genetic determinants for Aerobactin and to characterize interrelationship between hemolysin and aerobactin. In addition, we sought to determine the presence and expression of aerobactin determinants among *E. coli* strains from patients with urinary tract infections (UTIs) who have compromising urological and medical conditions .We also investigated the association between the presence of the determinant of aerobactin and resistance to some antibiotics (ABs).

PATIETS, MATERIALS AND METHODS:

A total of 321 midstream urine samples were collected from hospitalized and non- hospitalized patients (of both sexes and different ages), complaining of UTI symptoms attending AL-Kadhymia Teaching Hospital- Baghdad. 238 patients had UTI symptoms without predisposing factors (PF_s) of infection and about 81 patients had UTI symptoms with PF_s (diabetes, n=4; urinary catheterization, n=4; urinary stones, n=49; prostate enlargement, n=4; leukemia, n=16; bladder CA, n=4). For isolation and identification of uropathogens, blood and MacConkey agars (Oxoid- UK) were used and a set of biochemical tests. Lambda phage DNA (Boehringer- Germany) serves as molecular marker during electrophoresis. A method of Dillon *et al*, 1985[8] and Ausubal *et al*, 1987[9] was used for plasmid DNA extraction. Minimum growth medium (M9) supplemented with 2,2 dipyridyl (BDH-UK), a chelating agent, was prepared according to Johnson *et al* (1988)[1] method. The detection of Hly production was performed using 5% blood agar plates. Disc diffusion test for AB_s susceptibility was applied according to Treagan and Pullian (1982)[10] and Vandepitte *et al* (1991)[11]. Elevated temperature at 43°c[12] and 10% sodium dodecyl sulfate (SDS)[13] methods were used for plasmid curing. The data were analyzed by chi- square test which was applied for the comparison among different groups when the enumerative data were qualitative.[14]

REULTS:

1.patients and culture results:

Out of 238 urine samples that obtained from patients suffering from the symptoms of UTIs without PFs, only 49 gave positive culture results. While urine samples that were obtained from patients who had symptoms of UTIs in association with PFs and showed positive culture results were 75. *E. coli* isolates were the most predominant bacteria, 60 isolates (48.4%) among uropathogens, 25 isolates are obtained from compromised patients (with PFs). While the other 35 isolates were from samples of non compromised patients.

2.Aerobactin production:

Out of 60 UPEC isolates that were tested for Aerobactin production, 56 (93.3%) isolates gave positive results.

3.plasmid curing:

All of 56 UPEC isolates were submitted to two methods of plasmid curing, elevated temperature method (at 43 °C) and 10% SDS plasmid curing method. Curing by elevated temperature depends on the differences in the replication rate between the plasmid and chromosomal DNA at 43°C.[15] While curing with 10% SDS relys on the principle of cell membrane disruption.[13] *E. coli* isolates that fail to grow on M9 supplemented media with 2,2 dipyridyl after curing, may carry Aerobactin determinants on a plasmid, on other hand. *E. coli* isolate that can grow on such media after its submission to the curing procedure, probably carring these determinants on its chromosome. Out of these 56 Aerobactin expressed isolates, 37 (66%) lose the aerobactin production phenotype, 26 of them were lost by both curing methods, three by SDS and eight by elevated temperature method (Table-1 and Figure 1).

Table1: The loss of aerobactin production feature of uropathogenic *E. coli* after plasmid curing with elevated temperature and sodium dodecyl sulfate (SDS) methods.

Loss of aerobactin production feature after plasmid curing with	No. of the isolates n=37
Elevated temperature	8
SDS only	3
Both of them	26

As shown in table 2, *E.coli* isolates of non compromised patients tend to preserve such feature after curing, and plasmid- borne aerobactin is absent in the isolates of non compromised patients (89.5%, $p<0.01$). On the contrary, the isolates of compromised patients express plasmid aerobactin (59.5%, $p<0.02$).

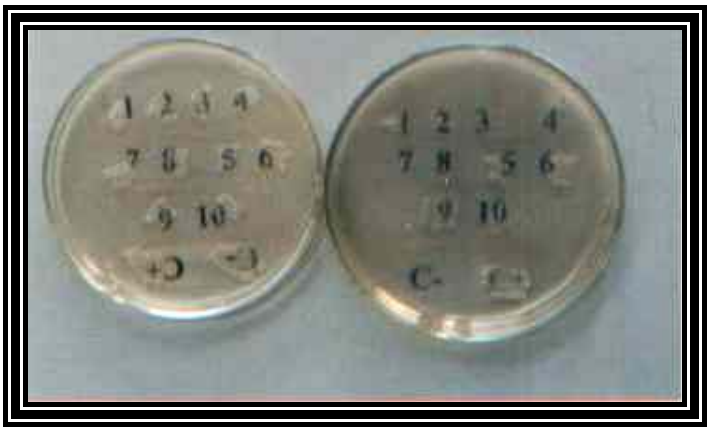

Figure 1: The loss of the aerobactin production feature of uropathogenic *E. coli* after plasmid curing with elevated temperature. The plate on the right contain M9 media supplemented with 2,2 dipyridyl whereas, the one on the left contains M9 media without 2,2 dipyridyl. C- = control negative
C + = control positive 1-10 = The streak of well- isolated colonies obtained from single colony submitted to the elevated temperature plasmid curing. Colonies number 2,3,4,7 and 8 cannot grow on medium containing a chelating agent due to the loss of aerobactin plasmid.

Table 2: plasmid- borne aerobactin in *E. coli* isolates of compromised and non compromised patients.

Patient groups	Plasmid aerobactin	
	Present n=37 (66%)	Absent n=19 (34%)
Non- compromised	15 (40.5%)	17 (89.5%)[a]**
Compromised	22 (59.5%) [b]*	2 (10.5%)

χ^2 test : [a] between patient groups : ** P < 0.01
[b] Within the group : * P < 0.02.

4. Hemolysin production: In table 3 only 35% of UPEC were Hemolysin, and were associated with the isolates of non compromised patients, (90.5%, p< 0.01). On the other hand, table 4 reveals that these isolates were associated with chromosomal aerobactin expression (61.9%, p< 0.02), and tend to be absent in the isolates that express plasmid aerobactin (82. 9%, p< 0. 01).

Table 3: Expressed hemolysin in uropathogenic *E. coli* isolates of compromised and non compromised patients.

Patient groups n=60	Hemolysin	
	Present n=21 (35%)	Absent n=39 (65%)
Non compromised	19 (90.5%)**	16 (41%)
Compromised	2 (9.5%)	23(59%)

Between the groups x^2 test: ** p< 0.01.

Table4: The association between the location of aerobactin determinant and the hemolysin expression feature in uropathogenic *E. coli*.

The location of aerobactin determinant	Hemolysin	
	Present n=21 (37.5%)	Absent n=35 (62.5%)
Chromosomal aerobactin	13 (61.9%)a*	6 (17.1%)
Plasmid aerobactin	8 (38.1%)	29 (82.9%) b**

χ^2 test: [a] between hemolysin expression and chromosomal aerobactin : * P < 0.02
[b] Plasmid aerobactin Vs hemolysin expression = ** P < 0.01.

5. The loss of antibiotics resistance feature: Antibiotic susceptibility to the co- trimazole (CO), tetracycline (Tc) gentamicin (GM), chloramphenicol (C) and ampicillim (Am) wad performed before and after plasmid curing for all of the 37 isolates that showed loss for aerobactin feature after plasmid curing, table 5 shows the loss of resistance to the five antibiotics in association with failure of aerobactin production and patient characteristics after plasmid curing.

Table 5: The association between patient characteristics and the loss of resistance to some antibiotics in association with failure of aerobactin production after plasmid curing.

Patients characteristics	Co-trimazole Resistance		Chloramphenicol Resistance		Ampicillin Resistance		Gentamicin Resistance		Tetracycline Resistance	
	L n=8 (23.5 %)	N n=26 (76.5 %)	L n=21 (91.3 %)	N n=2 (8.7 %)	L n=2 (5.7 %)	N n=33 (94.3 %)	L n=19 (79 %)	N n=5 (21 %)	L n=5 (19.2 %)	N n=21 (80.8 %)
Compromised	6 (75%)	14 (53.8 %)	17 (80.9 %)	1 (50 %)	1 (50 %)	20 (60.6 %)	14 (73.7%)	1 (20 %)	4 (80%)	14 (66.7 %)
Non-compromised	2 (25%)	12 (46.2 %)	4 (19.1 %)	1 (50 %)	1 (50 %)	13 (39.4 %)	5 (26.3%)	4 (80 %)	1 (20%)	7 (33.3 %)

L= The loss of the antibiotic resistance feature following aerobactin loss by plasmid curing .
N=No change in antibiotic resistance feature after curing of aerobactin plasmid.

6- Aerobactin plasmids: 26 *E. coli* isolates that lost the aerobactin production feature after plasmid curing with both methods of curing are submitted to the plasmid extraction and agarose gel electrophoresis before and after curing to screen the loss of the plasmids.21 isolates show the loss of just one plasmid in both cured extracts (table -6). The first or second band, from two to three bands of these isolates, the rest five loss two bands other than first or second bands. Figure 2 shows this fact, the first isolate (lane 2,3 and 4) show the loss of the second band in both elevated temperature (lane 3) and SDS cured extract (lane 4), the third isolate (lane 8,9 and 10) also lose the second which has the molecular size less than lambda phage DNA (48 kb). The second isolate (lane 5,6 and 7) lose the second band which has approximate molecular size of lambda phage DNA. The failure of aerobactin production after curing with both SDS and elevated temperature phenotypically is accompanied with the loss of just one large plasmid in both cured extracts and that mean that the lost plasmid is the aerobactin plasmid. Table 6 shows that the isolates of compromised patients tend to carry relatively large plasmid (85.7%, $p< 0.05$), as compare with those of non compromised (14. 3%).

Table 6: The size of aerobactin plasmid of uropathogenic *E. coli* isolates of compromised and non- compromised patients.

Patient groups	The aerobactin plasmid	
	Large n=21 (80.8%)	Small n=5 (19.2%)
Compromised	18 (85.7%)*	2 (40%)
Non compromised	3 (14.3%)	3 (60%)

Between groups : χ^2 test : * $P < 0.05$

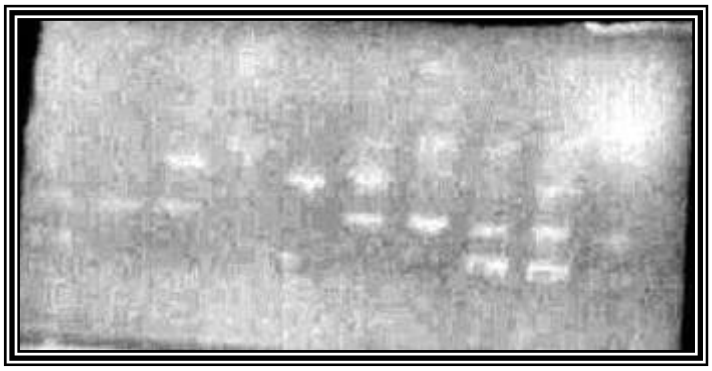

Figure 2: Agarose gel electrophoreticogram of the plasmids isolated from uropathogenic *E coli* (UPEC). Plasmids isolated from three isolates of compromised patients showing . **Lane 1:** Lambda phage DNA (48 kb) as a molecular marker marken. Lane 2,5 and 8, are the plasmid profile of three UPEC isolates before curing.Lane 3,6 and 9. plasmid cured of the previous isolates using temperature. of 43 ^{o}C. Lane 4,7 and 10 plasmid cured by dodecyl sulfate.

7-The plasmids of aerobactin and some antibiotic resistance: It was previously reported that aerobactin plasmid carry the determinants of some antibiotic resistance like C, Tc, Am, Co and GM[(6)]. Of these ABs only C (56.8%) and GM (51.4%) resistance loss were phenotypically associated with the loss of aerobactin (p< 0.05), but not with Co, Tc and Am (Table 7).

Table 7: The pattern of loss of some antibiotics (ABs) resistance following the loss of aerobactin production feature after plasmid curing with sodium dodecyl sulfate (SDS) and elevated temperature.

Antibiotics	Loss of antibiotic resistance following plasmid curing with						Total aerobactin cured isolate n=37	
	SDS only		Temperature only		SDS&temperature			
	No.	%	No.	%	No.	%	No.	%
Co-trimazole	1	12.5	0	0	7	87.5	8	21.60
Chloramphenicol	1	4.8	6	28.6	14	66.6	21	56.8*
Tetracycline	1	20	0	0	4	80	5	13.5
Ampicillin	2	100	0	0	0	0	2	5.4
Gentamicin	5	26.3	6	31.6	8	42.1	19	51.4*

X^2 test: The loss of resistance and aerobactin production feature VS the persistence * p< 0.05.

In table 8, the plasmids that carry the determinants of aerobactin production and chloramphenicol resistance are relatively large plasmids (85.7%) and associated with the isolates of compromised patients (83.3%, p< 0.02), Table 9 shows, that the plasmids that carry the aerobactin determinants accompanied with the determinants of GM resistance, were large and were obtained from compromised patients (100%, p< 0.01)

Table 8: The association between the size of the plasmid of aerobactin and chlormphenicol resistance with patient characteristics.

Patient groups (or characteristics)	Chloramphenicol resistance and aerobactin plasmid	
	Large plasmid n=12 (85.7%)	Small plasmid n=2 (14.3%)
Compromised	10 (83.3%)*	0 (0%)
Non compromised	2 (16.7%)	2 (100%)

Between groups X^2 test:* p< 0.02.

Table 9: the association between the size of the plasmid of aerobactin and gentamicin (GM) resistance with patient characteristics.

Patient characteristics	GM resistance and aerobactin plasmid Large n=7 (87.5%)	small n=1 (12.5%)
Compromised	7 (100%)**	0 (0%)
Non- compromised	0 (0%)	1 (100%)

Between groups X^2 test: ** p< 0.01.

DISCUSSION:

Aerobactin is a bacterial iron sequestration and transport system which enables *E. coli* to grow in iron- poor environments such as dilute urine. Iron is a vital trace element for bacterial growth, the functions of iron in the cells are to activate the electron transport systems,[3]and enzymes like ribonucleotide reductase, catalase, oxidases, superoxide dismutase as well as it enters in the composition of sulfer proteins.[16] Due to these critical functions the aerobactin production and utilization by UPEC reported in our study were high. The presence of direct repeats at both ends of aerobactin genes indicate that the transposition events are responsible for the plasmid and chromosomal location of aerobactin genes.[17] In the present study, plasmid aerobactin associated with the isolates of compromised patients, and the isolates of non compromised patients tend to preserve the feature of aerobactin production after plasmid curing and this confirms the chromosomal location of aerobactin determinants in the isolates of non compromised patients.

Hemolysins have tissue damaging properties which potentiate the bacterial toxicity and invasiveness[18], also increase the amount of iron available to the bacteria and liberate the iron that enhances the bacterial growth[19]. 35% of the tested isolates were hemolysin producers, also the isolates of non compromised patients express Hly as compared with those of compromised patients, and when the Hly is present chromosomal aerobactin is present, but when the Hly is absent plasmid aerobactin is present. Hybridazation studies have shown that several *E.coli* VF_s encoded by genes located on the same DNA gene cluster[20] and the deletion of one of them may also abolished the other[21] like aerobactin and Hly[1].

These findings support our observations, and the transposition events are responsible for such relationship[22]. We also conclude that the aerobactin plasmids of the isolates of compromised patients are relatively large ones and carry the determinants of either C or GM resistance. Bandereif and Neilands (1983)[7] estimated the size of aerobactin plasmid and called them P Col V plasmids in UPEC and found to be 144 Kb. In agreement with our observation, Perez- Casal and Crosa (1984)[5]reported that the determinants of some ABs are located on the aerobactin relatively large plasmids and associated with isolates of compromised patients. Johnson *et al* (1988)[1]explain such results by that the prior exposure of compromised patients to AB agents is responsible for increasing the resistance to some ABs in *E. coli* isolates from compromised patients. We believe that the presence of aerobactin operon composed of five genes[23]on the same plasmid with the determinants of resistance to one or more ABs make such plasmid a large one, and the presence of direct repeat at both ends of aerobactin genes is responsible for the transposition of aerobactin determinants to P Col V plasmids.

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The Laryngeal Mask Airway: technical guidelines and use in special situations

Ahmed Abdulameer SALIH

Summary:
Recent advances in airway management have changed the practice of anesthesia. Among these changes has been the introduction and increasing use of the Laryngeal Mask Airway (LMA). The laryngeal mask airway (LMA) is a novel device that fills the gap in airway management between tracheal intubation and use of the face mask.
This study describes the LMA, different methods of LMA insertion, uses, advantages, problems, complications and contraindications of LMA.
During eight years of LMA use in Al-Yarmook teaching hospital in Baghdad, the device was used for wide spectrum of surgical procedures successfully. We report some special series of cases were LMAs were used in providing a patent airway include LMA use with IPPV (intermittent positive pressure ventilation), for patient with history of failed intubation, for children who require general anesthesia for ultrasonic shock wave lithotripsy, the use of reinforce flexible LMA for nasal surgery and the use of total intravenous anaesthesia with LMA. Also we describe two methods of blind endotracheal intubation through LMA.
KEYWORDS: Equipment: laryngeal mask airway, Anaesthesia techniques: airway management.

INTRODUCTION:

The laryngeal mask airway (LMA) is a novel device that fills the gap in airway management between tracheal intubation and use of the face mask. The LMA is inserted blindly into the pharynx, forming a low-pressure seal around the laryngeal inlet and permitting gentle positive-pressure ventilation. It allows the administration of inhaled anesthetics through a minimally stimulating airway. It is relatively simple to insert and may have a useful role in management of the difficult or failed intubation [1]
The laryngeal mask airway(LMA) was developed and first used by Dr.Archie Brain [2](so some call it Brain airway [3]) in 1981. The LMA became commercially available in the United Kingdom in 1988 [1].The LMA represents one of the most important revolutions in the management of airway during anesthesia.

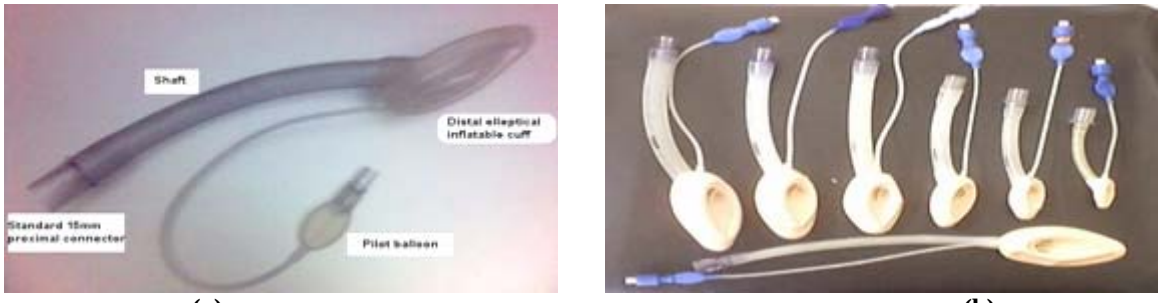
The classical LMA (Figure 1a) consist of:

1. A transparent silicone tube of wide internal diameter, the proximal end is a standard 15mm connection [4].

2. An elliptical "spoon-shaped" mask with inflatable rim "cuff"[5]. The mask resembles a small face mask and is inflated via a pilot balloon with a self-sealing valve.

3. There are slits at the junctions between the tube and the mask .These slits prevent the epiglottis from obstructing the LMA [6].

There are different sizes of the LMA (Figure 1b). Table 1 (below) shows the recommended LMA sizes (according to the weight of the patient), cuff inflation volume & the largest endotrachial tube (ETT) that can pass through each LMA).



(a) **(b)**

Figure 1. (a)The components of the Classical Laryngeal mask airway. (b) Different sizes of LMA.

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Table 1. The recommended LMA sizes, cuff inflation volumes and largest endotracheal tube (ETT) that can pass through it [6 - 8].

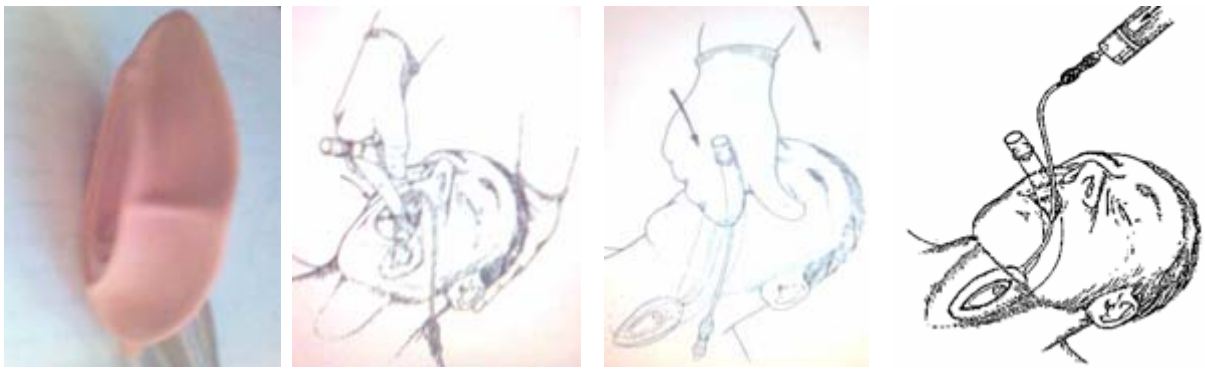
LMA Size	Patient Weight (kg)	Cuff Air Volume (ml)	Largest ETT (ID)
1	Up to 5	6	3.5
1½	5 - 10	10	4.0
2	10 -20	15	4.5
2½	20 - 30	21	5.0
3	30 – 50	30	6.0 cuffed
4	50 – 70	45	6.0 cuffed
5	70 – 100	60	7.0 cuffed
6	Over 100	75	7.0 cuffed

METHODS OF INSERTION OF LMA:

Standard method:

The deflated cuff is lubricated and inserted blindly into the hypopharynx, so that, once inflated, the cuff forms low-pressure seal around the entrance into the larynx. This requires an anesthetic depth slightly greater than that for the insertion of an oral airway [7], but the LMA tolerated at lighter level of anesthesia than the ETT [2]. Although insertion is simple, proper attention to detail will improve success rate (table 2 and figure 2).

Table 2 : Successful insertion of LMA depends upon attention to several details[1,7]:
1. Choose the appropriate size (table 1) and check for leaks before insertion**.**
2. The leading edge of the deflated cuff should be wrinkle-free and facing away from the aperture (figure 2a).
3. Lubricate only the back side of the cuff.
4. Ensure adequate anesthesia before attempting insertion.
5. Place patient's head in sniffing position (figure 2b).
6. Use your index finger to guide the cuff along the hard palate and down into the hypopharynx until an increased resistance is felt (figure 2c), the longitudinal black line should always be pointed directly cephalad (facing the upper lip).
7. Inflate with the correct amount of air (table 1).
8. Ensure adequate anesthesia depth during patient positioning.
9. Obstruction after insertion is usually due to a down folded epiglottis or transient laryngospasm
10. Avoid pharyngeal suction, cuff deflation or LMA removal until pharyngeal reflexes fully recovered and the patient is awake (e.g. opening mouth on command).



(a) (b) (c) (d)

Figure 2. Recommended LMA insertion technique. (a) Deflate cuff, ensure no wrinkles near the tip. (b) Place patient's head in sniffing position and press the LMA against hard palate. (c) Advance mask while maintaining pressure against posterior pharyngeal wall. (d) Cuff inflation while the LMA in position [1, 7].

Other methods of inserting LMA:

a. Under direct vision with the help of laryngoscope or fiberoptic bronchoscope (FOB) may prove beneficial in difficult cases [7, 8].

b. If difficult, insertion may be easier with the aperture facing backward, and the mask then rotated when in the pharynx [9].

c. An assistant grasp and draw the tongue forward using Magill forceps and then the LMA inserted by the anesthetist as in the standard method.

d. It has been suggested that LMA insertion is easier when the cuff of the device is partially or completely inflated. Although this technique may offer some advantages for an inexpert user, malposition of LMA is frequently encountered as well as partial airway obstruction; coughing or laryngospasm and the device frequently rest high in the larynx [2].

Uses and advantages of LMA:

LMA has been used safely and effectively in more than 100 million patients world wide and its clinical applications have greatly expanded to benefit virtually every subspecialty of anesthesia [2].

The use of LMA has been suggested;

1. To provide a clear airway without the need for anesthetist's hands to support a mask [4], or when holding a face piece may be difficult e.g. due to patient positioning or site of surgery [3].

2. To avoid the use of ETT during spontaneous ventilation [4], the LMA was found to be more effective as a ventilatory device than the facemask and to be less stimulant than the ETT [2].

3. For airway maintenance in difficult intubation in both previously suspected and known cases [7].

4. Emergency management of failed intubation, the LMA is a simple device that has been used to rapidly restore ventilation in the can not intubate / can not ventilate situation [8].

5. Cardiopulmonary resuscitation [3].

6. Assist blind intubation and FOB guided intubation in patient whose larynx could not be exposed by traditional rigid laryngoscope [8]. However, the position of the LMA around the larynx has a perfect central position only 45% to 60% of the time. Passage of FOB and ETT through the LMA has nearly 100% chances of success in most series, while the blind passage of an ETT through the LMA has 26% to 97% failure rate on the first attempt and 10% to 70% overall failure rate [8].The passage of ETT through the Intubating LMA"Fastrach" (a new modification of the classical LMA**)** has a better success rate (see below).

7. The LMA advocated for use in patient where pressure response to intubation would be detrimental. In patient with coronary artery disease or hypertension, who require GA for relatively short procedures; LMA insertion is associated with a lesser cardiovascular response than that seen during laryngoscopy and ETT insertion [2].

8. Ambulatory surgery for healthy adult and children: the use of ETT can be avoided, thus reducing the anesthetic requirements and shortening the emergency and recovery times [2].

9. For patient undergoing minor therapeutic or diagnostic procedures in location outside the operation room, e.g. radiation therapy, endoscopy and diagnostic and interventional radiology [2].

10. The LMA is also very useful for surgical procedures involving the head and neck area (e.g. eye surgery, ENT surgery and minor plastic surgery). The LMA use for intraocular procedures offers the advantages of a smooth induction and cough-free emergence, thus minimizing the risk of intraocular hypertension [2].

11. Positive pressure ventilation (PPV) can be usefully and successfully delivered through the LMA in patients with normal lung compliance. Tidal volumes of 6 to 8 ml/kg should be applied, while keeping the airway pressure between 15 and 20 cm H_2O to prevent gastric insufflation and oropharyngeal leak [1].

Problems and complication from LMA use:

1. If the inflatable balloon is not positioned properly, a large gas leak occurs around the mask, impairing ventilation. This leak is exacerbated by high airway inflation pressure [4] (see above).

2. The need to open the mouth widely to insert the device, thus obviating its use in any situation with temporomandibular joint dysfunction or oropharyngeal obstruction [10].

3. Despite the presence of slits, if the epiglottis is folded downwards by the tip of the mask on insertion, the airway may be obstructed [6, 9].

4. LMA may cause regurgitation and it does not protect against aspiration of gastric contents [2, 6].

5. Unlike the ETT, rotation of the LMA may result in complete airway obstruction. So in order to assess the LMA's orientation when inserted, a black line is present along the tube,

which should face the upper lip of the patient, when the LMA is in position [6].

6. Coughing, laryngospasm and even bronchospasm may occur [2, 5].
7. Post extubation stridor [5].
8. Failed insertion [2].
9. Kinking of the shaft of LMA [5].
10. Pharyngeal and laryngeal trauma, so the inflation pressure of the cuff should not exceed 60 cm H_2O [2].

Contraindications for LMA:

1. Patient with pharyngeal pathology (e.g. abscess) [7].
2. Pharyngeal obstruction [7].
3. A patient in whom regurgitation of gastric contents into the oesophagus is possible e.g. hiatus hernia [4].
4. Patient with full stomach or with any condition leading to delayed gastric emptying e.g. pregnancy (but the LMA has been used for emergency caesarean section when intubation proved impossible, where a cuffed ETT in the oesophagus may help prevent regurgitation and aspiration) [9].
5. Patient with high airway resistance (bronchospasm) [7].
6. Patient with low pulmonary compliance (e.g. morbid obesity), requiring peak inspiratory pressure more than 20 cm H_2O.
7. Where the surgical access (e.g. to the pharynx) is impeded by the cuff of LMA.
8. Airway obstruction at or below larynx [5].
9. Inability to extend the neck or open the mouth > 1.5 cm, making advancement of the LMA into the hypopharynx difficult (e.g., ankylosing spondylitis, severe rheumatoid arthritis, cervical spine instability) [1].
10. Inadequate depth of anesthesia to relax pharyngeal musculature [1].
11. One-lung ventilation [1].

Our experience with LMA in Al-Yarmook teaching hospital:

Although the LMA was introduced recently (mid 1998) in our hospital, we are using it widely in almost all the operation theatres. We use the LMA in orthopedics (e.g. anesthesia for upper and lower limbs surgery), urosurgery (e.g. anesthesia for cystoscopy and transurethral resection of the prostate , ophthalmology (e.g. anesthesia for cataract surgery), general surgery (e.g. anesthesia for head and neck operations, inguinal hernia surgery and anal surgery)and diagnostic radiology (e.g. MRI and CT-scan).

The following sizes are available: **#1 , #2 , #2.5 , #3 , #4 , #5** and **#4R**(reinforced flexible LMA)(see figure 1(b)). We use LMA for all age groups from neonates to geriatrics.

The resident anesthetists are well trained in using the LMA, and even some assistant anesthetist had used the LMA successfully.

I had tried all the above mentioned methods of insertion of the LMA successfully, except that the use of the FOB for the insertion of LMA because it is not available in our hospital.

Use of LMA in special situation :

During my eight years experience with LMA, I had report the use of LMA in the following special series of cases:

1. ***To deliver IPPV through the LMA*** successfully in a series of 12 cases(ten males and two females)

in different age groups (25 -76 years) and types of surgery, using non-depolarizing muscle relaxant, the LMA #3 was used to maintain the airway, tidal volumes 6-8 ml/kg and keeping the airway pressure between 15 and 20 cmH_2O to prevent gastric insufflation and oropharyngeal leak. For example, we apply this technique in orthopedics for a 76 years old female with fracture of left neck of femur (Osten Moore surgery for replacement of the head of the femur),which was a prolong operation(more than $2^1/_2$ hours). The LMA can be satisfactorily used for IPPV in patients with normal airway resistance and compliance who do not require high inflation pressures to produce normal tidal volumes [11].

Throughout its history, the LMA has provided a reliable method of assuring a patent airway. It has, however, encouraged the use of spontaneous breathing. Allowing a patient to breath without assistance has potential consequences that clinicians have attempted to avoid. Standard volume mode ventilators have proven difficult to manage with LMAs as a result of leaks, peak pressures, and gastric ventilation to name a few. Supporting patient ventilation with pressure modes may provide an answer. So we use the LMA to deliver IPPV with Pressure Controlled mode of ventilation. Initially an airway pressure of 13 cm H2O is selected, and then the pressure is adjusted to deliver a suitable tidal volume with no leak.

Spontanious breathing is the most popular mode of ventilation with the laryngeal mask airway (LMA), but provides less effective gas exchange than does positive pressure ventilation (PPV) [12]. Pressure support ventilation (PSV) is a form of partial ventilatory support in which each spontaneous breath is assisted to an extent that depends on the level of a constant pressure applied during inspiration [13]. PSV improves gas exchange in anesthetized intubated patients. PSV provides

more effective gas exchange than does unassisted ventilation with continuous positive airway pressure (CPAP) in anesthetized adult patients treated using the LMA [14].

2. ***For a patient with history of failed intubation:*** a 34 years old male 130 cm height (figure 3), with a history of failed intubation and then temporary tracheostomy was done for him to maintain anesthesia for splenectomy before three years ,tracheostomy was closed later on. On examination; there was no limitations of neck extension, thyromental distance= 6 cm, inter-incisors distance= 4 cm and Mallampatti's test Grade 3(only the soft palate could be seen). This patient was listed for left inguinal hernia repair. Inhalational induction of anesthesia with halothane was done, when the patient was deep enough, airway management became difficult, laryngoscopy was done which reveal long epiglottis, that cause airway obstruction and the larynx can not be seen(Grade -3-). Then the tongue drawn gently out using Magill's forceps and the LMA #3 was inserted in position successfully and the operation went smoothly.

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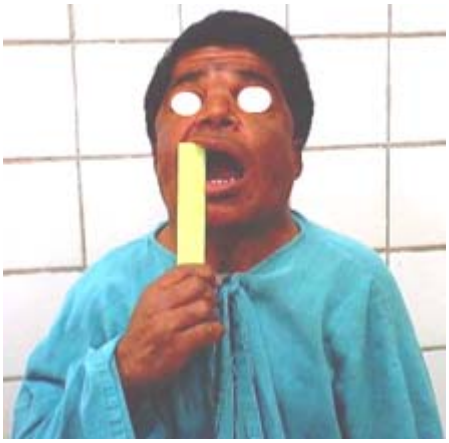


Figure 3. Patient with history of failed intubation

3. ***For children who require general anesthesia for ultrasonic shock wave lithotripsy***: Our series of 18 case reports of using the LMA for children (4 - 12 years) who require general anaesthesia for ultrasonic shock wave lithotripsy; as this procedure require light level of anesthesia (just keep the child still) and there is an exposure to X- ray for the anesthetist who may be away from the patient, or were a shield. Some times the procedure take a lot of time which make mask anesthesia unsuitable. So, we find that general anesthesia with spontaneous breathing using halothane and LMA to maintain the airway is satisfactory.
4. ***For nasal surgery using the reinforce flexible LMA (figure 4):*** We report a series of three cases.

E.g. a 25 years old male (70 kg) who underwent anaesthesia for reduction of nasal bone fracture. Thiopentone 250mg was used for the induction and halothane for the maintenance of anaesthesia , when the patient is deep enough a **Flexible LMA # 4** was inserted successfully from the first attempt (without the need for using a skeletal muscle relaxant).Then the cuff is inflated, the LMA fixed in position and connected to Magill circuit (figure 5a) so that making the surgical access easy (fig.5b).The operation went smoothly, and after the ending of surgery gentle suctioning of the mouth above the LMA was done, and when the cough reflex returned and the patient awake the LMA removed and the recovery was smooth. Figures (4a & b) shows that the back of the mask stained with blood, but the anterior part of the rim of the mask is partially stained with blood and there is no blood in the central part of the mask that face the larynx. So that no episode of hypoxemia related to blood inhalation occurred [15], and there is no need for the pharyngeal pack.

Protection of the lower airways from secretions, fluid or blood, arising above the LMA, would appear to be confirmed [16]

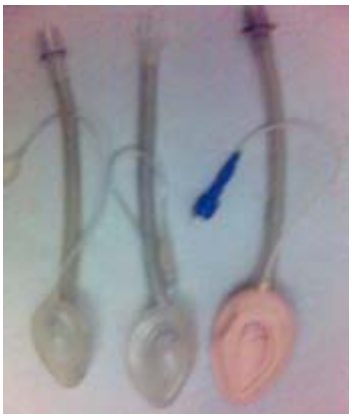
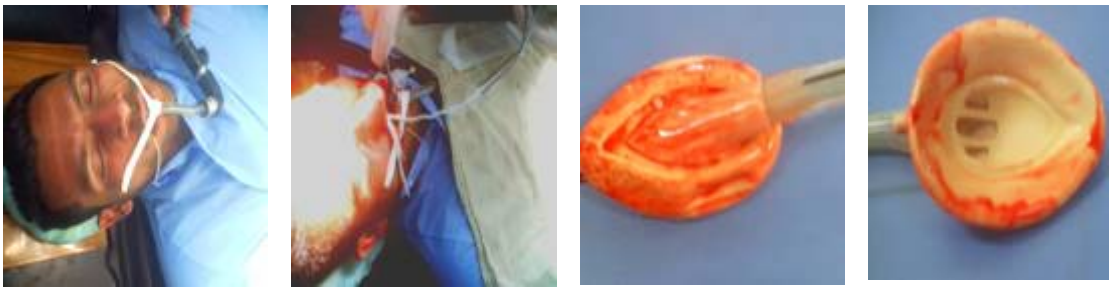


Figure 4. Reinforced flexible LMA.



(a) (b) (c) (d)

Figure 5. The use of flexible LMA for nasal surgery. (a) Flexible LMA in position connected to Magill circuit.(b) During surgery. (c) The back of the mask stained with blood. (d) The anterior part of the rim of the mask is partially stained with blood, but there is no blood in the central part of the mask that face the larynx.

5. Total intravenous anaesthesia (TIVA) and LMA:

Our series of case reports include six adult patients (21 – 58 years) e.g. a 58 years old male (75 kg) presented for removal

Of lipoma in the left shoulder (day surgery). While in right lateral position anaesthesia was induced with fentanyl 1µg/kg and propofol 2mg/kg then LMA # 4 was inserted successfully from the first attempt with no need to use skeletal muscle relaxant, and anaesthesia was maintained using propofol drip starting at 10mg/kg/hr for 10 minutes (170 µg/kg /min) then reduced to 8mg /kg/hr (130 µg/kg /min) .The operation went smoothly and the recovery was smooth and rapid.

Propofol is regarded currently as the most suitable anaesthetic agent for total intravenous anaesthesia (TIVA); it allows for rapid changes in anaesthetic depth and a rapid, clear–headed recovery [17]. Also, low context–sensitive half–time [18] makes it theoretically the best available agent for long procedures under TIVA. Furthermore, propofol attenuates airway reflexes to the extent that the laryngeal mask airway (LMA) may be positioned easily without neuromuscular block [19].

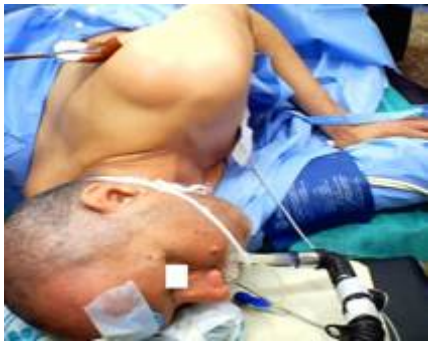


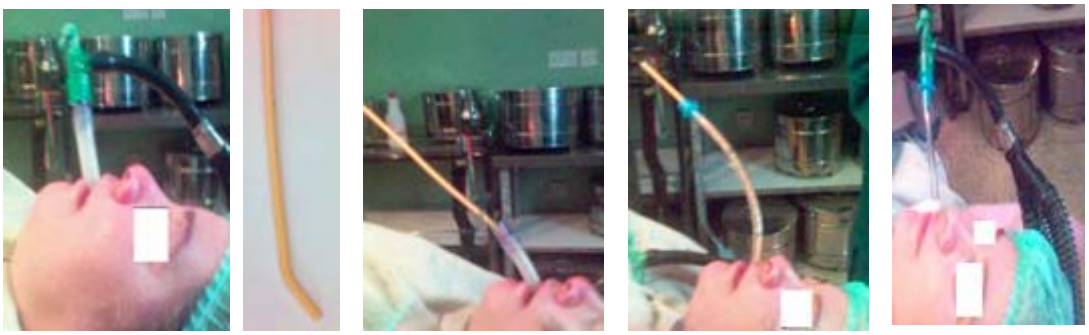
Figure 6. TIVA and LMA for day surgery.

6. Blind Intubation Techniques Using the LMA:

When the LMA is correctly inserted, its distal aperture sits directly over the laryngeal inlet, thereby allowing tracheal intubation by a variety of blind and fiberoptic techniques in awake or anesthetized patients. Because blind intubation can be accomplished rapidly with the LMA and does not require specialized equipment, it may play a role in both elective and emergent situations, even on awake patients. Placement of the LMA is facilitated if glottic reflexes can be obtunded by either deep anesthesia, topical anesthesia, or the use of muscle relaxants.

We report a series of four cases for whom blind intubation techniques using LMA. We use two different techniques that we describe down in details:

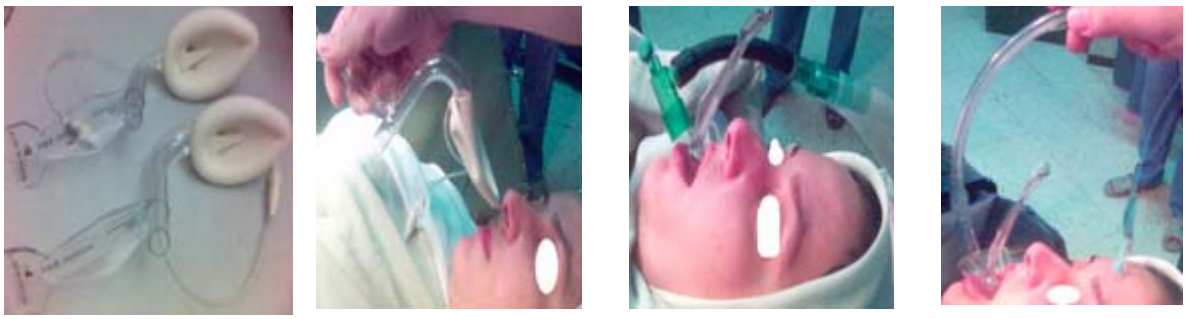
a. Blind intubation using a gum elastic bougie (GEB) through classical LMA: A 47years old female (85kg) was prepared for transabdomenal hysterectomy. After induction of anaesthesia with sleeping dose of thiopentone and pancuronium was used to facilitate the intubation procedure a classical LMA # 4 was inserted (Figure 7a). A gum elastic bougie (GEB) with an anterior angulation of its distal tip (figure 7b) was passed blindly through the LMA into the trachea of patient (figure 7c). Once the distal aperture of the LMA has been negotiated, Pennant JH recommends rotating the GEB 180° to facilitate its passage into the trachea [(1)]. The LMA was withdrawn and a tracheal tube "railroaded" over the GEB into the trachea (figure 7d). This maneuver permits removal of the LMA and the passage of any size of tracheal tube, and allows better surgical access to the oropharynx. The disadvantages are that the airway is neither protected nor controlled once the LMA has been taken out, and passage of a tracheal tube over the GEB may not be successful. The success rate in a study of 50 patients was 84–88% [(20)]**.** Malpositioning of the LMA was the usual cause of failure to successfully pass the GEB. Other investigators have reported success using this technique [(21)].



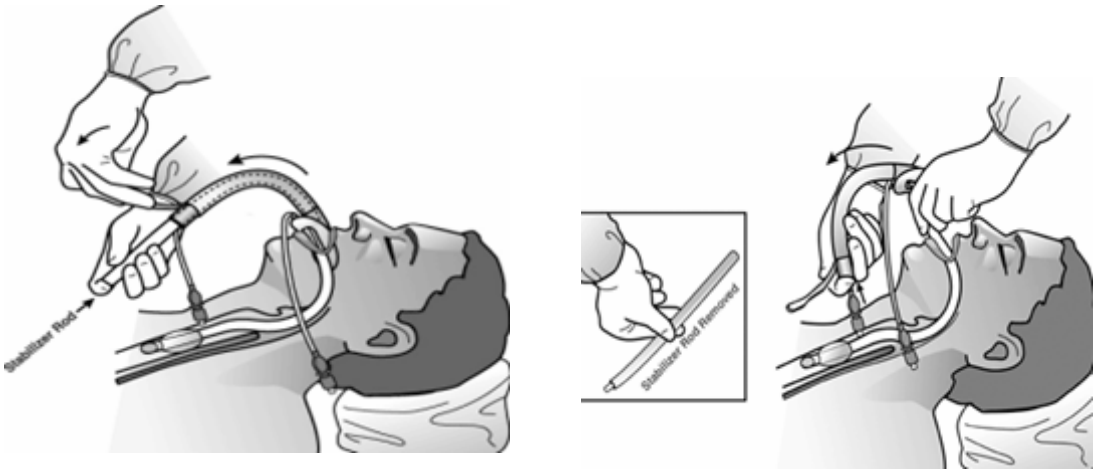
(a) (b) (c) (d) (e)

Figure 7. Blind intubation using a gum elastic bougie (GEB) through classical LMA. (a) a classical LMA was inserted and connected to ventilator. (b) A distal tip of GEB that has an anterior angulation. (c) A GEB was inserted to suitable depth through the LMA into the trachea. (d) The LMA was removed after deflating its cuff and then a cuffed endotrachial tube (ETT) railroaded over the GEB into the trachea. (e) The ETT in the proper position and connected to the ventilator.

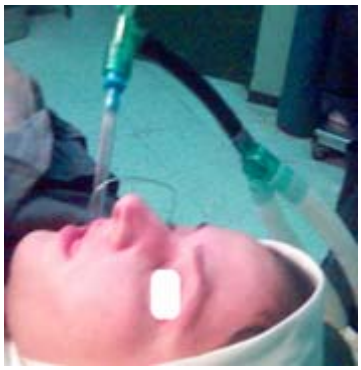
b. ***Blind intubation through the Intubating LMA Fastrach (ILMA) (figure 8a):*** A 27 years old female (75kg) prepared for appendicectomy. Anaesthesia was induced with ketamine 100mg and sleeping dose of thiopentone, pancuronium was used for skeletal muscle relaxation, then LMA Fastrach #5 was inserted(figure 8b) and connected to the ventilator after inflation of the cuff (figure 8c) an uncut, lubricated, 6.5-mm cuffed tracheal tube blindly passed through the shaft of the LMA Fastrach with the tracheal tube inserted in a reversed curve, instead of the conventional normal curve(Figure 8d) [(22)] , and into the trachea before the tracheal tube's cuff is inflated. Removing the LMA with the 6.5-mm tube in place is difficult, if there is no way of stabilizing the tracheal tube as the LMA is withdrawn; the tight fit between the tracheal tube and the inner wall of the LMA shaft tends to result in extubation of the trachea. The use of a *stabilizing rod* to the tracheal tube (figure 8e & f) before removal of the LMA is helpful in this situation. A study performed on 100 patients reported 90% success using this blind intubation technique [(23)]. Another study has demonstrated the safe and effective use of the LMA-Fastrach in 254 patients with Difficult Airway (DA). Insertion of the LMA-Fastrach and intubation through it were successful on the first attempt in a high percentage of patients with various types of DA [(24)].



(a) (b) (c) (d)



(e) (f)



(g)

Figure 8. (a) ILMA (Fastrach). (b) ILMA Fastrach insertion. (c) ILMA Fastrach connected to ventilator. (d) ETT insertion through ILMA. (e)& (f) Using a stabilizing rod to ETT while removing the ILMA. (g) ETT in position connected through breathing circuit½ to ventilator.

CONCLUSION:

The LMA improve the quality of the practice of anaesthesia and proved to be suitable alternative to ETT in many situations.

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Case Report

Adult Ebstein Anomaly with Right Atrial Myxoma

Hameed Al Ani*, Firas H. AL ANI, Wadhah Mahbuba*****

ABSTRACT:

BACKGROUND:

Ebstein Anomaly is rare in adults; a combination of this disease with cardiac myxoma has not been reported previously.

METHODS:

Surgery was performed by removal of the myxoma and replacement of the tricuspid valve and correction of the abnormal atrialization of the right ventricle.

RESULTS:

The patient had a smooth postoperative recovery and improved dramatically after wards.

CONCLUSION:

We have dealt with this rare condition successfully and the patient had good outcome.

KEY WORDS: Adult Ebstein Anomaly, Cardiac myxoma with Ebstein Anomaly, surgery on Ebstein Anomaly.

INTRODUCTION:

In 1866, Dr. Wilhelm Ebstein, a young physician in Breslau, Poland, described the unusual cardiac findings in a 19-year old laborer, who had died of cyanotic heart disease. The anterior leaflet of the tricuspid valve was enlarged and fenestrated and there was downward displacement of the posterior and septal leaflets in a spiral manner below the annulus, they were hypoplastic, thickened and adherent to the wall of the right ventricle [1].

Ebstein Anomaly (EA) is a rare cardiac anomaly that accounts for less than 1% of all congenital heart diseases; it involves both sexes equally [1]. *Although few patients reach advance age, life expectancy for most is limited*, and the most common cause of death is congestive heart failure, hypoxia and cardiac arrhythmia [1, 2, 3, 4, 5].

When diagnosis is made in infancy, the prognosis is worse; one-third to one-half of these patients will die before 2 years of age [4].

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Ebstein Anomaly has three characteristics [1, 2, 4]:

1. Downward displacement of septal and posterior leaflets into right ventricle and sail-like abnormality of the anterior leaflet, this displacement leaves a portion of the ventricle above the valve as an integrated part of the right atrium (atrialized ventricle).
2. An atrialized portion of the right ventricle between the tricuspid annulus and the attachment of the posterior and septal leaflets.
3. A mal formation of the right ventricle with a marked reduction in its size.

So according to Carpentier, it is classified into 3 types [4]:

A. Valve insufficiency with normal leaflet motion.

B. Valve insufficiency with leaflet prolapse.

C. Valve insufficiency with restricted leaflet motion.

Although important stenosis is uncommon, most Ebstein valves are incompetent, often severely so. This is contributed to by marked dilatation of the true tricuspid annulus and the right ventricle, as well as the morphologic abnormalities of the tricuspid valve [3].

The right atrium is enormously dilated in advance cases and usually an interatrial communication (cause paroxysmal embolization in adult) present in most cases as Patent Foramen Ovale (PFO), although ASD may be present and rarely ostium primum coexist [2, 3,].

Symptoms are related to severity of the incompetence of the tricuspid valve, presence or absence of associated Atrial Septal Defect (ASD), impairment of right ventricular function and presence of associated cardiac anomaly, like right cor-triatriatum and right overt accessory atrioventricular pathway [1, 2, 6].

In older patients, the predominant symptoms are fatigability, dyspnea on exertion and cyanosis. Palpitation in the form of paroxysmal atrial arrhythmia and premature ventricular beats are common, less frequently ascitis and peripheral edema are present [1, 2, 3, 7, 8].

Physical signs vary, heart sounds are usually soft, and a multiplicity of sounds and murmur is often

heard, all originating from the right side of the heart, a systolic murmur of tricuspid regurgitation may be heard along the left sternal border [1, 2, 3, 4]. Low intensity diastolic and presystolic murmur result from anatomic or functional tricuspid stenosis [1, 2, 3, 4].

Diagnosis of Ebstein Anomaly; usually follows the routine steps in diagnosing cardiac diseases, starting with the essential chest x-ray and ECG, to show the marked atrial dilatation and cardiomegally [1, 2, 3, 4]. Echocardiography, is essential to study the tricuspid valve and to show the displacement of the septal and posterior leaflets and to check the atrialized right ventricle and its size and the left ventricular function with the advanced use of the Live/real time three-dimensional transthoracic echo [9, 10].

Cardiac catheterization is reserved only for cases which seem difficult to assess by echo study alone, so we can study well the tricuspid valve and the severity of its incompetence and to check the coronaries for any associated lesions, especially in adult cases [1, 2, 3, 4].

CASE STUDY:

Male patient (A. J.) 45 years old referred to the surgical dept. in Ibn Al-Bitar Hospital for Cardiac Surgery on March 2005, with hemoptysis and features of severe right sided failure including; lower leg edema, ascitis, palpable liver and pleural effusion which necessitated frequent pleural aspirations.

On reception, routine investigations were done for him like chest x-ray & ct scan of the chest (fig. 1); showed a huge heart and an ECG which showed Atrial Fibrillation (AF).

Abdominal and chest Ultrasonography (fig. 2) showed hepatosplenomegaly, ascitis, left pleural effusion, engorged Inferior Vena Cava (IVC) and thickened Gall Bladder. Transthoracic Echocardiography -TTE (fig. 3) study showed the following data; Aortic root dimension 25mm, Left Ventricular Diastolic Dimension (LVDD) 45mm, Left Atrial dimension (LA) 33mm, Left Ventricular Systolic Dimension (LVSD) 30mm and Ejection Fraction of 62%.

Transesophageal Echocardiography (TEE) study labeled the patient as tricuspid valve regurgitation (Ebstein Anomaly) with sail-like anterior leaflet adherent to the wall, rudimentary posterior and septal leaflets with downward displacement of 28mm from the annulus. Cardiac catheterization (fig. 4), confirmed Ebstein Anomaly.

On May 2006, surgery was performed which showed two small atrial masses near the opening of the Superior Vena Cava (SVC) of about 1 x 0.5 cm and a very dilated and incompetent tricuspid valve (type C according to Carpentier).

A tissue valve (BICORE ™ – SJM size 33) was inserted according to the method described previously[11] which entitle utilizing the anterior leaflet of the tricuspid valve to attach the biological valve anteriorly and the opposite area of the atrialized ventricle is used for the attachment of the rest of the valve, and the rest of apparatus of the tricuspid valve was left without interference. The patient ran a smooth postoperative period apart from hypoproteinemia and high fluid demand which was corrected by intravenous albumin, plasma and colloids.

Early follow up after a week revealed marked improvement in the general condition with decrease in the edema and sound decrement in cardiac size by CXR and ECG (fig. 5), TTE (fig. 6) showed well functioning competent tissue valve and rebuilding of the atrialized part of the right ventricle and improvement in right ventricular contraction with good left ventricular function.

Later on, histopathology of the mass reported the presence of myxoma as a benign tumor in the right atrium (fig. 7).

DISCUSSION & CONCLUSION:

Ebstein Anomaly is a rare congenital heart disease and can be manifested in adults as well as in children [1, 2, 3, 4, 11]. Diagnosis of EA is usually a straight forward procedure and can follow the usual diagnostic steps of all cardiac diseases [1, 2, 3, 4, 9, 10].

Association of EA with other cardiac diseases is reported [1, 2, 3, 4,], and it may add to the severe symptoms of right sided failure and congestive heart failure, and in our

Patient, there were *a unique association of EA with Right Atrial Myxoma*, in which as far as our knowledge, is reported for the first time, as there was a report of an EA with cardiac rhabdomyomata [12].

Surgery for EA is recommended by many authors, and it ranges from repair of the tricuspid valve to total replacement or to new methods of inserting valves [1, 2, 3, 4, 11, 13, 14, 15].

Usually the results are good, and the patients seems to benefit well from the surgery in all types, apart from the expected possible complications of right sided failure which may take longer time to resolve on heavy diuretic therapy and cardiac inotropic support, also regarding right sided prosthetic valves thrombotic complications due to low pressure circulation [1, 2, 3, 4, 11, 13, 14, 15].

So in conclusion; we had dealt with unusual adult EA associated with atrial myxoma which was not diagnosed preoperatively, surgery was a success.

Legends:

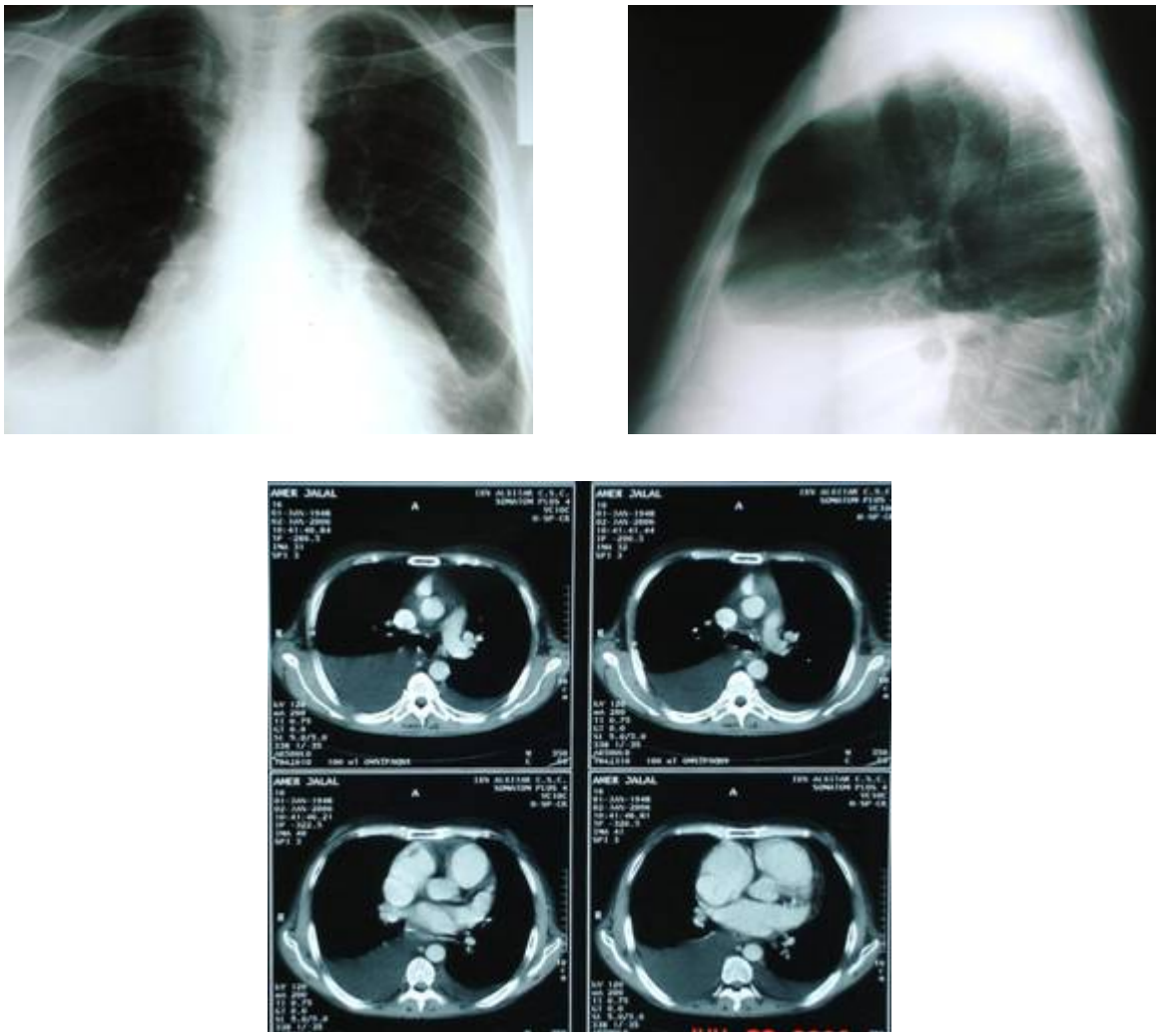

Fig. 1, Preoperative chest x-rays and ct-scan showing huge heart with with evidence of pleural effusion

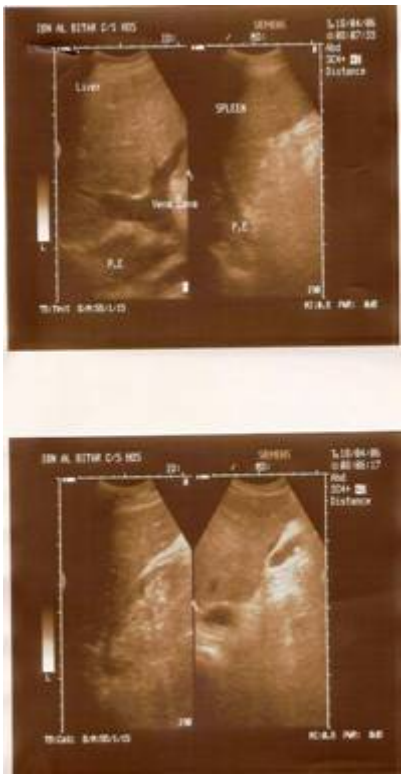

Fig. 2, Abdominal US showing engorged IVC and hepatomegaly

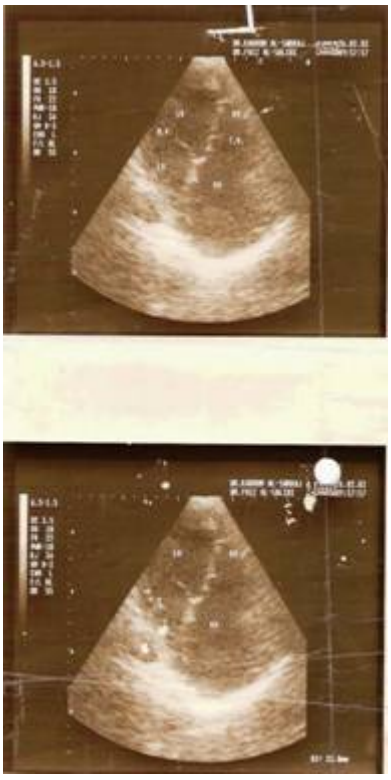

Fig. 3, Pre operative TTE

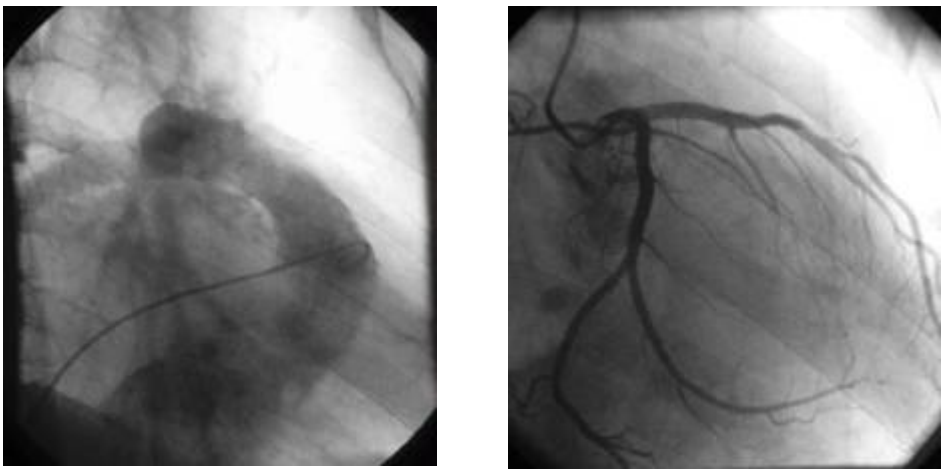

Fig. 4, Preoperative cardiac catheterization

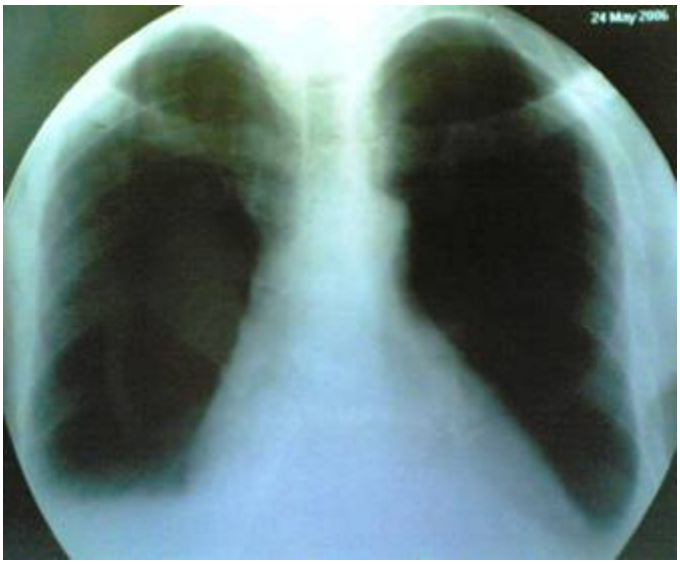

Fig. 5, Early Postoperative Chest X-ray

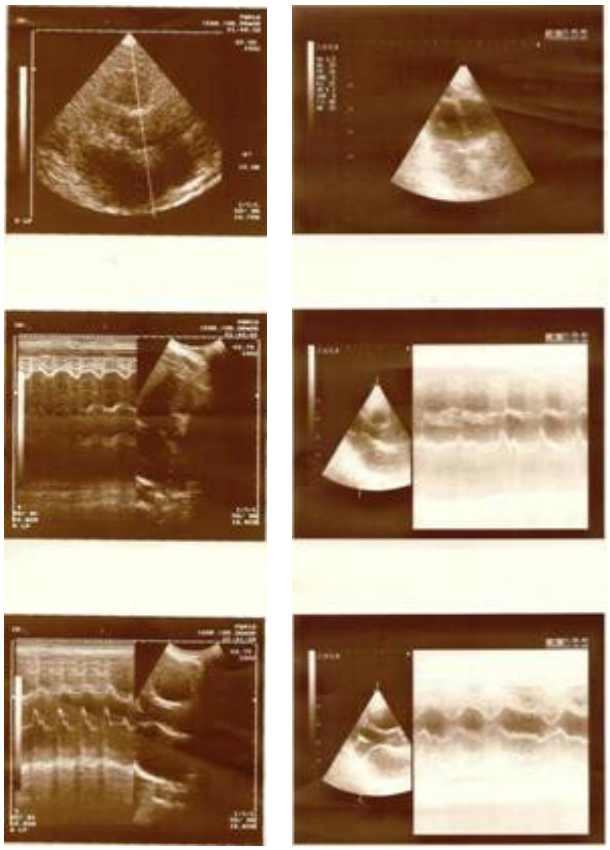

Fig. 6, Postoperative ECHO

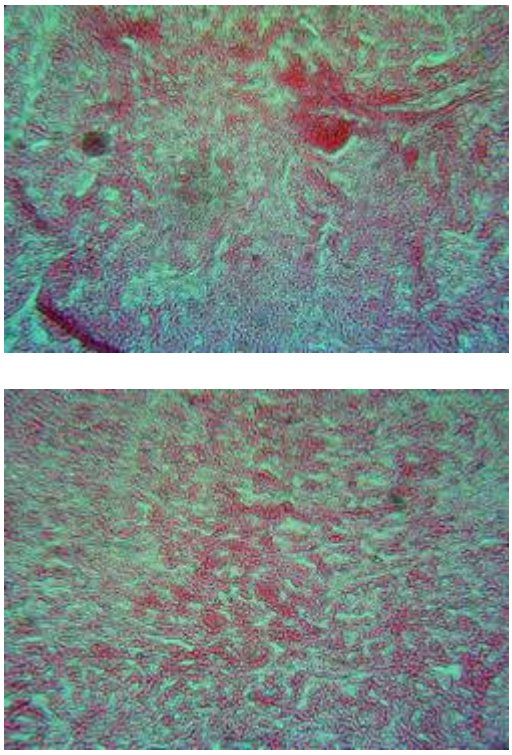

Fig. 7, Histopathological result of the right atrial mass showing the classical picture of myxoma

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