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Dissecting the Molecular Mechanisms of Intestinal Bacterial Translocation to Facilitate Definition of its Proposed Role in Systemic Sepsis

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Abstract

Intestinal translocation of bacteria is defined as the ingress of gastrointestinal microflora across the lamina propria to local mesenteric lymph nodes and thence to extranodal sites. Bacterial translocation has been long been considered as a possible direct cause of sepsis when under certain conditions bacteria cross the intestinal barrier, enter the systemic circulation and cause ageneralised inflammatory response syndrome. While this is an attractive hypothesis, which finds support from experimental models, evidence from clinical studies is equivocal in confirming that bacterial translocation is the primary cause of sepsis. Moreover, the underlying mechanisms by which gut bacteria gain entry to the systemic circulation are not well defined. This review provides a brief overview of bacterial translocation in the intestine, discusses our current understanding of the role it plays in the development of sepsis syndrome and suggests areas for future research to determine the molecular mechanism(s) involved in the aetiology of disease.

Keywords Bacterial translocation; mesenteric lymph node; gastrointestinal microflora; enteric bacteria; sepsis

List of abbreviation: MLN = mesenteric lymph nodes, E. coli = *Escherichia coli*, MODS = multiple organ dysfunction syndrome.

Introduction

An important function of the mammalian intestinal epithelial barrier is to prevent gut bacteria from invading systemic organs and tissues. However, in specific circumstances, gut bacteria may cross the epithelial barrier and appear in mesenteric lymph nodes (MLN) and possibly other organs. This movement is called bacterial translocation [(1)].

The anatomical site of bacterial translocation has been investigated in many studies with the consensus view that the rate of passage is greater in the small intestine(composed of the duodenum, jejunum and ileum) than it is in the large intestine (caecum, colon, rectum, and anus) [(2,3)]. This is related to the former location's role in food digestion and absorption of nutrients.

Three Primary Mechanisms Promoting Bacterial Translocation

As determined by investigation in animal models, translocation of enteric bacteria is considered to depend primarily on three influences:

1. *Disturbance of the ecological equilibrium of the indigenous microflora.* This may be through factors that regulate bacterial population size, such as impaired coordination of gene expression via quorum sensing or exposure to ingested antibiotics and other chemotherapeutic agents, in each

case resulting in overgrowth of certain bacteria, including opportunistic pathogens [4]. In mice with normal intestinal barrier function, bacterial translocation may occur if certain enteric bacteria reach or exceed a local population density of 10^{9-10} bacteria/gram of caecal content or stool [5].

2. *Physical disruption of the gut mucosal barrier.* Increased permeability of the epithelial barrier may be a consequence of a breakdown of tight junctions or loss of cell integrity, thus increasing bacterial passage to underlying tissue structures [6].
3. *Impaired host immunity.* The large and diverse microbial communities that exist in the gastrointestinal tract repeatedly challenge the mucosal immune system. This is a complex, multi-factorial network that interacts to maintain organisms at a normal level and/or eradicate potential pathogens that may cross the protective barrier [7]. Thus, a breakdown in immune function can lead to survival and overgrowth of bacteria that usually would be maintained at a healthily balanced population density [8].

When all three factors occur simultaneously, bacterial translocation occurs at a higher rate than when only one or two components are present [9]. In addition to these host-related conditions, bacteria may be implicated directly in this process. Species that are able to control the expression of virulence genes, along with amassing an overwhelming cell population density, both have a significant survival advantage and possess the capability to overrun an immune response before it is fully initiated [10,11].

Additionally, there are a number of physical events, which promote translocation. Accumulating evidence from human and animal studies indicates that events such as haemorrhagic stress, burn injury, trauma, endotoxaemia, malnutrition, fasting and intestinal obstruction promote bacterial translocation [12-16]. It is presumed that the stress response of the host leads to an increase in the three conditions discussed above, thereby causing an enhanced rate and/or absolute level of bacterial translocation.

Bacterial Survival Strategies

After passing through the mucosal barrier, translocating microorganisms can either enter the portal circulation or be carried to MLN by macrophages [17]. The MLN-thoracic duct-circulation course was first demonstrated to be a major route of bacterial dissemination in a model of experimental acute pancreatitis [18], a finding, which has found subsequent support in humans [19]. Since MLN are rich in lymphocytes and macrophages, they should be able to eliminate invading bacteria through phagocytosis. However, *Escherichia coli* (E. coli) strains can survive in MLN for several days [20]. Factors that enhance such survival could include a reduced host immune function but other possible influences have yet to be elucidated. It is postulated that bacteria which display phase-variable surface proteins may have a better ability to circumvent the immune response. For example, Ag43 of *E. coli* is a cell surface protein that enhances immune avoidance, thus promoting *E.coli* survival and colonisation of immunocompetent cells [21]. Once growth is established, bacteria may then move from MLN via the blood to organs such as the spleen and liver [19]. The net outcome is a systemic inflammatory response, induced sepsis and multiple organ dysfunction syndrome.

Experimental colonisation of gnotobiotic mice [22] with single strains of bacteria has demonstrated clearly that not all bacteria are able to translocate at the same rate and that Gram-negative enteric bacilli translocate more efficiently to MLN than do Gram-positive cocci and obligate anaerobes. For example, in a mouse model 89% of Gram-negative isolates translocated to MLN between 1-3 weeks [23]. These bacteria were also found in the spleen, liver, kidney and peritoneal cavity. In contrast, Gram-positive bacteria translocated to MLN in only 43% and 50 % of mice after weeks 1 and 3, respectively, with translocation to the abdominal visceral organs and peritoneal cavity similar to

that of Gram-negative organisms [23]. Among Gram-negative enteric bacteria, *E. coli* strains translocate at a higher rate than do other gut organisms due to their ability to produce continuously new phenotypes that facilitate adaptation to a multiplicity of environments [24].

Pathogenic *E. coli* may be divided into groups that cause intestinal diseases and those that produce disease elsewhere in the body [6,25]. Pathogenic *E. coli* are not only the major cause of gastroenteritis globally, but are responsible for almost 85% of community-acquired urinary tract infections, of which 50% are transmitted nosocomially. These bacteria are also one of the five leading causes of bloodstream infections and are the principal source of Gram-negative meningitis in neonates [24]. Disease is produced by strains that possess specific somatic (O-antigen) determinants and virulence-associated characteristics [24]. Virulence determinants of *E.coli* strains may be patho type-specific, such as toxin production and adhesive properties in the case of diarrhoeagenic strains, or include a variety of properties necessary for invasion and survival inside the human body [6,25].

Non-pathogenic *E. coli* form part of the normal flora of the gastrointestinal tract. When the gut is in homeostasis, these bacteria engage with other microorganisms to metabolise ingested food [26]. Although regarded as non-pathogenic, under conditions of host stress and/or bacterial overgrowth, they have the ability to adhere to the gut epithelium and translocate from the gastrointestinal tract to extra-intestinal sites [6,7]. The trigger for, and mechanism by which, this apparent change in behaviour occurs are not understood. Neither is it clear whether strains of *E. coli* found in MLN of animal models or in the blood of septic patients with gut-associated bacteraemia are better able to cross the mucosal barrier or have an increased ability to survive in the hostile environment of lymphoid tissues.

Determining Mechanisms of Bacterial Translocation

In order to gain a better understanding of this process, several mechanisms require investigation [27]. The route of translocation should be assessed to determine if it is a transcellular, paracellular or phagocytic cell-mediated occurrence. Mechanisms that promote translocation need to be elucidated. This includes utilising already known virulence characteristics of *E. coli* to ascertain if translocating strains possess any of these determinants. The survival and colonisation of an organism within MLN may be addressed to ascertain the mechanisms that enhance bacterial survival in this phagocyte-rich environment.

Light may be shed on the mechanisms of bacterial translocation of *E. coli* and its survival in MLN by:

a. identification of genes involved in adherence of these bacteria to intestinal epithelial cells and by elucidation of the process of translocation;
b. identification of genes involved and mechanisms by which the translocating strains of *E. coli* survive the hostile environment of MLN;
c. verification of the role of genes involved in adhesion, translocation and survival of both wild-type and mutant strains using gnotobiotic and conventional mice;
d. investigation of the presence of translocating genes among epidemiologically unrelated *E. coli* strains isolated from septicaemic patients.

Future Research Directions

Both translocating and non-translocating strains of *E. coli* isolated from animal models for their adherence and translocation characteristics may be studied using conditioned monolayers of the polarised gut epithelial cell line Caco-2 [28,29]. Originating from a colorectal adenocarcinoma, Caco-2, when grown to confluence, expresses properties similar to those expressed in the human gut, i.e. relevant membrane potential, ion conductance and permeability [30]. Cells polarise significantly, are joined by tight junctions, form domes on impermeable substrates (including apical to basal ion transport), have well developed apical microvilli

and express several disaccharides and peptidases typical of normal small intestinal villous cells. These properties make Caco-2suitable for exploring intestinal functions and bacterial translocation [31,32]. The K12 strain of *E. coli*, known to be non-translocating, may serve as a negative control.

The adhesion of *E. coli* isolates to Caco-2 cells and the route and process of translocation may be observed by electron microscopy. Transmission electron microscopy is used to ascertain if translocation is an intracellular or extracellular phenomenon using confluent Caco-2 cells lines inoculated with bacteria [33]. Adhesion and host cell structure may be observed by scanning electron microscopy [34].

Translocating strains may be subjected to a phagocytosis assay to identify their ability to survive in MLN [9,35]. Bacteria and phagocytic cells are grown in serum, after which extracellular bacteria and macrophages are separated by centrifugation. Macrophages are lysed and seeded onto plates to permit growth of internalised bacteria. The number of colony forming units is a representation of the surviving bacteria. This method also permits quantification of bacterial phagocytosis over time [35].

Comparative genome analysis, using the technique of next generation sequencing, may be utilised to investigate genetic differences between translocating and non-translocating *E. coli* strains. Whole genomic DNA is extracted (including both chromosomal and plasmid DNA), sequenced and assembled using publicly available assembly data [36]. Publicly available *E. coli* genomic data (and also plasmid-specific databases) are used to analyse the sequence data of translocating strains and to compare them to non-translocating strains (*E. coli* JM109). In addition to identifying genes unique to translocating *E. coli*, such as strains HMLN-1 in humans, it is also possible to examine the sequences of genes involved in the translocation process [37]. Once target genes have been identified, primers can be developed to isolate these and validate further their involvement in translocation via PCR across many strains of translocating bacteria.

Finally, the presence of genes involved in translocation and/or survival of *E. coli* may be investigated among epidemiologically unrelated *E. coli* strains isolated from patients with septicaemia. Control groups comprise *E. coli* strains isolated from healthy human faeces and urinary tract infections. Hybridisation probes are designed for genes involved in translocating and/or survival and their presence in bacteria is determined by probing dot blots of translocating genes after digesting whole chromosomal DNA with specific endonuclease [38].

Clinical Significance

Mortality and morbidity that are due to systemic bacterial infections, especially among critically ill patients, represent a very significant public health problem. Neonatal sepsis caused by *E. coli* and other Gram-negative bacteria is a noted concern in Iraq and various Middle East countries [39]. Hospitalised patients at highest risk for Gram-negative septicaemia include immunecompromised patients (e.g. cancer patients and organ transplant recipients), patients in post-surgical recovery and those suffering trauma. The mortality associated with systemic *E.coli* infection is consistently higher than that caused by other Gram-negative bacilli, ranging from 18-20% in cases of community-acquired infection to 23-40% in cases of hospital-acquired bacteraemia [40]. In hospitals, patients in intensive therapy units, where there is heavy dependence on antibiotics, are at greater risk. These individuals can be colonised by naturally resistant microorganisms and develop a potentially life-threatening bacteraemia. In addition, immunocompromised patients readily develop infection with bacteria of low virulence, which may invade, often causing bacteraemia. For instance, around 30,000 blood isolates are reported from laboratories in the UK each year [41]. The source of most systemic infectious complications and the multiple organ dysfunction syndrome (MODS) in surgical and intensive therapy unit patients is now known to

be indigenous gut bacteria translocating to extra-intestinal sites by passing through the intestinal epithelial barrier [42].

Conclusions

While the role of bacterial translocation in pathogenesis of sepsis has received a great deal of attention, most translocation studies have focused on the function of the intestinal barrier and less research has been performed to elucidate the role and/or properties of microorganisms in this process [43]. Future studies should aim to investigate bacterial properties that are directly involved in overcoming the function of the intestinal epithelium and immune defence mechanisms. Research to date suggests strongly that translocation, at least in *E. coli* strains, is principally a bacteria-related phenomenon rather than due to a host-associated mechanism. Identification of genes involved in this process should establish a platform for investigating further the relative importance of bacteria and of host defence to the translocation process. Furthermore, such insights may facilitate the adoption of an improved strategy for the management of bacterial sepsis.

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The Relation of Hypokalemia to Hypertensive and Non-Hypertensive Ischemic Stroke

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Abstract

Background Several clinical and laboratory observations are consistent with the hypothesis that hypokalemia might be a risk factor for the development of ischemic stroke in humans.

Objectives To evaluate the level of serum potassium in ischemic stroke patients and its relation to non-stroke patients and those with hypertension.

Methods Serum potassium was estimated from one hundred newly discovered first-life ischemic event of acute ischemic stroke patients and one hundred control patients with an attempt to evaluate the its level in ischemic stroke and the relation to hypertension. Normal value of serum potassium in both sexes was considered as 3.5-.5.5 mmol/L.

Results Mean serum potassium level of stroke patients was significantly lower than that of control group (3.89±0.67 versus 4.19±0.56, p=0.0001). Hypokalemia was found in 23 (23%) patients with stroke compared to 7 (7%) of the control group. The Mean serum potassium in hypertensive patients was significantly lower than those without hypertension (3.91 ±0.65 versus 4.2 ±0.54). The mean serum potassium for hypertensive stroke patients was significantly lower than non-hypertensive stroke patients (3.79±0.78 versus 4.26±0.72), while there was no significant difference among the control hypertensive and non-hypertensive subjects (4.05±0.57 versus 4.43±.40).

Conclusions Serum potassium should be taken in consideration as low levels are significantly associated with ischemic stroke with hypertension.

Keywords Serum Potassium, ischemic stroke

List of Abbreviation: K^+ = Potassium, IS = ischemic stroke, HT = hypertension, BP = blood pressure, CVD = cardiovascular disease.

Introduction

Stroke is defined by the WHO as the clinical syndrome of rapid onset (usually seconds or minutes) of focal (or global, as in subarachnoid hemorrhage) cerebral deficit, lasting more than 24 hours or leading to death, with no apparent cause other than a vascular one [(1)].

Pathologic process is given an inclusive meaning, namely, occlusion of the lumen by embolus or thrombus, rupture of a vessel, an altered permeability of the vessel wall, or increased viscosity or other change in the quality of the blood flowing through the cerebral vessels [(2)].

Stroke is the first leading cause of disability in developed and developing countries [(3)]. Stroke is the third most common cause of death in most western populations, after coronary heart disease and cancer [(4)]. Information on incidence, prevalence, and mortality of stroke is extremely important in the assessment of priorities for dealing with this disease, in the recognition of its occurrence, and hence the design of programs for prevention and control. Such information is

limited in the developing world [4]. Humans evolved ingesting a potassium-rich, sodium-poor diet, and mechanisms developed to retain sodium and excrete potassium (K^+). The sodium-rich diet of modern humans produces sodium overload and K^+ depletion [2]. There are no specific interventional studies on whether stroke incidence could be affected by K^+ supplementation. Since serum K^+ is kept within a narrow range, it may be clinically difficult to monitor whether K^+ supplement is adequate for an individual [5]. Hypokalemia contributes to the pathogenesis of cardiovascular disease (CVD), and many CVD and drugs aggravate hypokalemia. Hypokalemia is therefore a common, reversible factor in the natural history of CVD [6].

Numerous studies have found that low K^+ intake and low serum K^+ are associated with increased stroke mortality, but data regarding stroke incidence have been limited. A lower stroke mortality rate has been found with higher K^+ intake, and higher stroke rate with low K^+ intake [5].

In two population-based studies, individuals on a low K^+ diet had a 40 to 50 percent increase in the risk of stroke, independent of other risk factors such as the systemic blood pressure (BP) [7]. A similar effect has been demonstrated in stroke-prone hypertensive rats, in which the incidence of both stroke and renal vascular disease can be diminished by a high K^+ diet [8].

In one study, hypokalemia in the year before a stroke of treated hypertensive patients was associated with an increased risk of incident ischemic and hemorrhagic stroke independent of diuretic use when compared to normal serum K^+ levels [5].

Increasing dietary K^+ reduces neointimal formation after angioplasty and reduces atherosclerotic load. K^+ ameliorates oxidative stress by reducing free-radical formation, impairing vascular smooth muscle cells proliferation, and reducing monocyte adherence to vessel walls. Thus, K^+ retards the progression of atherosclerosis [6].

The aims of the study is to evaluate the level of serum K^+ in ischemic stroke (IS) patients in relation to non-stroke patients and the relation to hypertension (HT).

Methods

Study setting and design: The study was conducted in the wards of Internal Medicine/ Neurology of Al-Imamain Al-Kadhmiyain Medical City. A hospital based case control study with an attempt to evaluate the level of serum K^+ in IS patients and the relation to HT. The period of data collection was one year started from Jan. 2012 to the end of Jan. 2013.

Selection of the study sample: A total number of 100 IS patients were examined from both sexes (50 males and 50 females), aged from 45-80 years old, who have an acute IS, were examined for serum K^+, on (fasting) morning blood samples.

One hundred (50 males and 50 females) were selected from those suffering from other medical and neurological diseases other than stroke and cardiovascular events and considered as control group.

Baseline Examinations: The patients were assessed with full medical clinical examination for the diagnosis of stroke and exclusion of mimics, using a Computerized Tomography, Magnetic Resonance Imaging for Stroke confirmation and IS selection, complete blood count was done to exclude hematological disorders, basic biochemical investigations including fasting, random blood sugar, lipid profile (depending on total serum cholesterol, triglyceride, high density lipoproteins and low density lipoprotein for hyperlipidemia assignation), blood urea and serum creatinine to assess renal function.

Data collection: A Questionnaire was used to get information from studied population, which included general information from the patients and duration of stroke. Serum K^+ was measured with an autoanalyzer and the normal value of K^+ in both sexes was considered as 3.5-.5.5 mmol/L. [6]

Statistical analysis

The collected data was organized, tabulated, and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 15. Values were expressed as mean ± SD. A comparison of continuous variables was performed by unpaired t-tests were used for categorical variables. The strength of associations between K^+ and acute stroke was assessed by comparing stroke and control subjects. Significance levels were set at P values < 0.05 in all cases.

Results

The mean age of patients with IS was significantly higher than that of control group (63±8.96 versus 53.86±9.29; *P* < 0.001). There was no significant association in distribution of cases according to gender (*P* = 0.832).

The mean serum K^+ of patients with IS was significantly lower than that of control group (3.89±0.67 versus 4.19±0.56, *P* = 0.0001) as seen in fig. 1 and table 1.

The mean K^+ for hypertensive stroke patients was significantly lower than non-hypertensive stroke patients (3.79±0.78 versus 4.26±0.72 *P* < 0.001), while there was no significant difference in K^+ levels among the control hypertensive and non-hypertensive subjects (4.05±0.57 versus 4.43±.40; *P* > 0.05) table 2, 3.

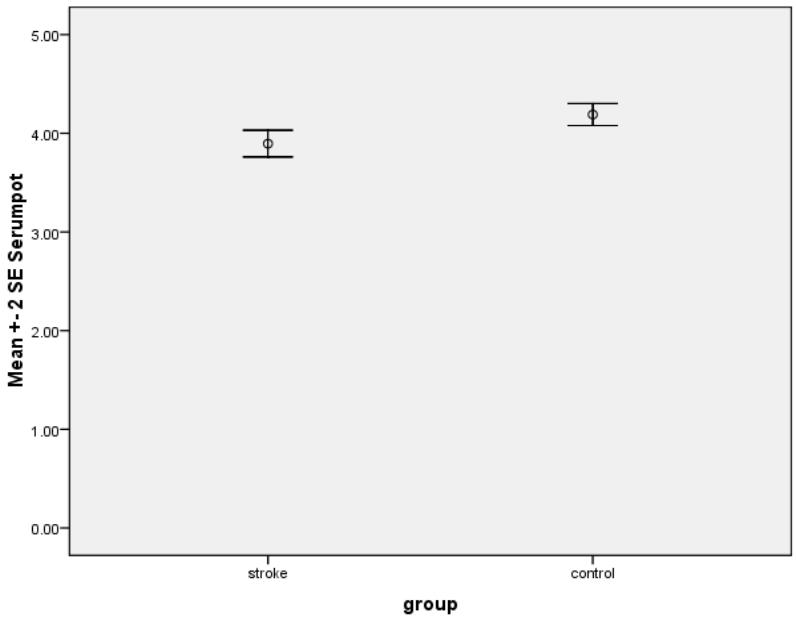

Fig. 1. Comparison of mean serum potassium between stroke group and control group

Table 1. Incidence of low serum potassium level in stroke and control groups

Serum potassium (mmol/L)	Stroke patients	Control group	Total
≥3.5	93	77	170
<3.5	7	23	30
Total	100	100	200

P = 0.002

Table 2. Comparison of mean serum potassium in patients with stroke versus control group

Serum Potassium mmol/L	Control Group		Stroke patients	
	non-hypertensive N = 35	Hypertensive N = 65	non-hypertensive N = 43	Hypertensive N = 57
Mean± SD	4.26±0.43	3.79±0.57	4.43±0.72	4.05±0.78
Minimum	3.80	2.80	2.80	2.10
Maximum	5.60	5.50	6.00	5.60
P Value	> 0.05		< 0.001	

Table3: Distribution of serum potassium level in stroke and control groups

Serum potassium (mmol/L)	Control		Stroke patients		Total
	non-hypertensive	Hypertensive	non-hypertensive	hypertensive	
≥3.5	35	58	38	39	170
<3.5	0	7	5	18	30
Total	35	65	43	57	200

P < 0.001

Discussion

In this study, the relation of K^+ with stroke was examined. The study tried to compare epidemiological data from other studies that had linked K^+ to stroke in human. Previous data revealed that K^+ is an independent predictor of hypertension. Hypertension remains the second most common modifiable risk factor for stroke in the general population, including the elderly which exhibits that K^+ levels were significantly lower in hypertensive than non-hypertensive patients (3.91±0.65 versus 4.2±0.54, $P < 0.0001$) consistent with the previous studies [9]. This study has shown that K^+ levels were significantly lower in patients with ischemic stroke than the control group for other risk factors (3.89±0.67 versus 4.19±0.56, $P = 0.0001$). These results can suggest that hypokalemia may be an independent risk factor for the development of ischemic stroke. This study has shown the mean K^+ for hypertensive stroke patients was significantly lower than non-hypertensive stroke patients (3.79±0.78 versus 4.26±0.72; $P < 0.001$), while there was no significant difference in K^+ levels among the control hypertensive and non-hypertensive subjects (4.05±0.57 versus 4.43±0.40; $P > 0.05$). These findings might be contributed that hypertension was the leading risk factor in this sample (57% of stroke patients were hypertensive). These data point that hypokalemia should be seriously considered in the hypertensive population as it was associated with significant increase in the risk of stroke in this particular group.

In 1997 Gariballa *et al* concluded that hypokalemia post stroke is common and may be associated with a poor outcome. They also found that on survival analysis, a lower plasma K^+ on admission to hospital was associated with an increased chance of death, independent of age, stroke severity, history of hypertension, blood pressure level, or smoking history (hazard ratio 1.73 (95% CI: 1.03-2.9) for a 1 mmol/L lower plasma K^+ concentration) [11]. These results were also suggested by Moussavi *et al* in 2010 as they have found that patients with serum K^+ levels below normal levels initially and at discharge have worse outcomes, especially in elderly patients [12].

Serum K^+ has a fundamental role in BP regulation, and there is evidence highlighting the importance of K^+ homeostasis in hypertension. Pikilidou *et al* have concluded in their study the reverse relation between serum K^+ and BP supports a close pathophysiological connection between serum K^+ and essential HT. Moreover, they found that diuretic therapy is a significant cause of hypokalemia and requires systematic monitoring [(9)].

The other important point needed to be taken in consideration is the effect of diuretics as a treatment for hypertensive patients. In 2003, Smith *et al* found that in adults with treated HT, hypokalemia in the year before a stroke was associated with an increased risk of incident ischemic and hemorrhagic stroke independent of diuretic use when compared to normal serum K^+ levels.[(10)]

Speculating on the mechanisms underlying their findings, they note that high serum K^+ levels are thought to inhibit processes such as free radical formation, platelet aggregation, and arterial thrombosis, thereby exerting a cardioprotective effect. Conversely, there is evidence that hypokalemia may increase the risk of cardiac arrhythmias in patients with underlying coronary disease. "A combination of these mechanisms may explain the association between hypokalemia and an increased IS risk," Smith *et al* conclude [(10)].

In 2004, Drs. John Macdonald and Allan Struthers of Ninewells Hospital in Dundee, UK have produced an excellent summary of many consequences of hypokalemia in relation to cardiovascular disease. Among the highlights of their findings was High blood levels of K^+ inhibit platelet aggregation and thus help prevent IS and that adequate K^+ levels retard the progression of atherosclerosis [(6)].

In 1999, Hiroyaso *et al* published a study that concluded low calcium intake, and perhaps low K^+ intake, may contribute to increased risk of IS in middle-aged American women [(13)].

In contrast, K^+ supplementation appears to modestly lower the BP in some normotensive and hypertensive patients. The magnitude of benefit was illustrated in two meta-analyses of randomized trials [(14, 15)].

In the evidence of the above data and the findings of this study, it was shown that low serum K^+ is associated with IS and HT. Serum K^+ monitoring should be considered in populations who have other risk factors for stroke such as HT especially those groups treated with diuretics which may predispose them to develop hypokalemia. These groups may benefit from oral K^+ supplements to keep their serum K^+ within the acceptable range.

One of the most important limitations of this study was the sample size which reduced the power of the study. It is recommended that in the future to enroll a larger sample size and that patients may be followed over a period of time to estimate how serum K^+ levels may predict the development of IS especially in patients who are considered at high risk.

The measurement of serum K^+, although easily accomplished, is seldom standardized. Indeed, the normal range for K^+ values is itself highly variable between laboratories; the lower limit fluctuates between 3.5 and 3.8 mmol/L and the upper limit between 5.0 and 5.5 mmol/L. Interpretation of a specific serum K^+ value initially requires an understanding of sampling conditions. For example, a serum K^+ value derived from a serum sample (red-top tube) is typically 0.1 to 0.3 mmol/L higher than one obtained from a plasma sample (green- or purple-top tube). Blood samples obtained using poor technique can also falsely increase serum K^+ values (pseudohyperkalemia). Prolonged use of a tourniquet above a venipuncture site or extended fist clenching produces tissue hypoxia and promotes the escape of K^+ from tissues into the plasma compartment [(2)]. Serum K^+ values also show evidence of a circadian rhythm (average peak-to-trough difference ≈0.60 mmol/L, with lowest values at night).[(16)]

In conclusions, there was significant association between IS and low serum K^+ levels. Low serum K^+ level was significantly more evident in hypertensive than non-hypertensive patients.

Serum K^+ should be taken in consideration as low levels are significantly associated with IS. A low serum K+ level in patients with is needed to be taken in consideration in hypertensive patients. Early detection and correction of low serum K^+ in high risk patients for is recommended. Further studies are required to assess the value of K^+ supplements in patients with other risk factors for IS.

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Author contribution

Dr. Haider A. Husain: acquisition of data, analysis or interpretation of data, statistical analysis. Dr. Hasan Azeez Al-Hamadani: revising the manuscript, study concept or design, study supervision. Dr. Munther T. Hamzah: collection of data.

Conflict of interest

No potential conflicts of interest.

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Correlations between Symptoms, Nasal Endoscopy and Computed Tomography in Patients with Chronic Rhinosinusitis

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Abstract

Background Rhinosinusitis is the inflammation of nasal and paranasal sinus mucosa and is associated with mucosal alteration ranging from inflammatory thickening to gross nasal polyp formation. Concha bullosa is the term used for an aerated middle turbinate or a cell found within the middle turbinate. These structures appear as a widened area of the middle turbinate, and they may obstruct the ostio meatal complex. In the absence of a history of sinusitis, the incidental finding of widened middle turbinate during endoscopy or concha bullosa on computed tomography (CT) does not mandate further investigation.

Objectives To determine the symptoms described at presentation, radiological findings, endoscopy and to compare endoscopy and computed tomography scan findings of the nose and paranasal sinuses in patients with chronic rhinosinusitis before endoscopic sinus surgery.

Methods Forty three patients with chronic rhinosinusitis were studied. Physical history, ear, nose and throat examinations plus endoscopic examinations of nose and paranasal sinuses were performed. Computed tomography scan of the sinuses and ostiomeatal complex were done for all patients.

Results The patients aged 17 to 53 years (32.44 ± 9.83 years), and male to female ratio was 1.15:1. Headache is the commonest symptom (69.76%). The duration of symptom was 1-5 years in 44.18% of patients. Septal deviation was the most common finding 46.51% by endoscopic examination. Mucosal thickening present in all patients (100%) by CT-scan. Between endoscopic and CT scan findings There is a significant statistical difference for enlarged ethmoid bulla but not for septal deviation, abnormal uncinate proces and hypertrophy of inferior turbinate.

Conclusion Endoscopy and CT-scan of the nose and paranasal sinuses are mandatory before endoscopic sinus surgery of nose and paranasal sinuses in patients with chronic rhinosinusitis.

Keywords Chronic sinusitis, endoscope, CT-scan

List of abbreviation: OMC = ostio meatal complex, CT = computed tomography, RS = rhinosinusitis, CRS = chronic rhinosinusitis, ESS = endoscopic sinus surgery

Introduction

The cilia of the maxillary and frontal sinuses transport mucus in specific patterns only toward the natural Ostia, despite the presence of accessory Ostia. Rhinosinusitis (RS) is usually preceded by a viral upper respiratory infection that impedes mucociliary clearance, causing blockage of the natural sinus Ostia. Allergies, likewise, can cause mucosal inflammation and edema. Anatomic obstruction of the Ostia can be caused by septal deviations, concha bullosa, paradoxical middle turbinate, infraorbital cells, agger nasi cells, and nasal polyps. Cystic fibrosis, ciliary dyskinesia, and immunodeficiency can impair mucociliary clearance and predispose to chronic rhinosinusitis (CRS) [(1)].

Messerklinger pioneered the study of the endoscopic anatomy and pathophysiology of the

paranasal sinuses, publishing his experience with endoscopic sinus surgery (ESS) in 1978. He highlighted the role of the ostiomeatal complex (OMC) in the pathophysiology of rhinosinusitis and directed attention to it during surgery [2]. The Caldwell-Luc procedure remained popular as the primary choice of treatment in CRS through the early 20th century; although Hirschman conducted the first endoscopic examination of the nose with a modified cystoscope in 1901.

Understanding of the anatomy and pathophysiology of each disease process is necessary before one can embark on surgery [3]. Each patient is individually assessed to determine the site of pathology and obstruction, and surgery is tailored to address them [4].

Anatomic Variations

Anatomic variations include structures such as a concha bullosa, agger nasi cells, infraorbital (Haller) cells, sphenoethmoid (Onodi) cells, and paradoxical middle turbinate. Concha bullosa is the term used for an aerated middle turbinate or a cell found within the middle turbinate. These structures appear as a widened area of the middle turbinate, and they may obstruct the OMC. In the absence of a history of sinusitis, the incidental finding of widened middle turbinate during endoscopy or concha bullosa on CT does not mandate further investigation [5].

Most patients with such variations remain asymptomatic. In a review of 172 coronal sinus CT scans, a concha bullosa was found in 28% of patients with sinus disease and in 26% of patients without sinus disease [6].

The objectives of this study were to determine the symptoms described at presentation, radiological findings, endoscopy and to compare endoscopy and computed tomography scan findings of the nose and paranasal sinuses in patients with chronic rhinosinusitis before endoscopic sinus surgery.

Methods

A prospective study of 43 selected patients at Al-Imamain Al-Kadhymain Medical City (Baghdad) for one year duration. A thorough history, ear, nose and throat examinations and endoscopic examinations of the nose and paranasal sinuses under local anesthesia were performed in the outpatient clinic. All patients were sent for CT scan within one week after endoscopy.

CT scan was done using (Seimens multi detector 64 slices (Germany), coronal and axial view 4 mm slices thickness for imaging the sinuses and 2 mm coronal view for ostiomeatal complex were done for all patients.

Each patient who is diagnosed as having chronic rhinosinusitis was included in this study after failure of medical treatment for at least 4 weeks (Amoxicillin + Clavulanic acid 625 mg/three times daily. Clarithromycin 250 mg /twice daily in penicillin sensitive patients, Budesonide intranasal spry /2 times daily) and these patients prepared for Endoscopic Sinus Surgery.

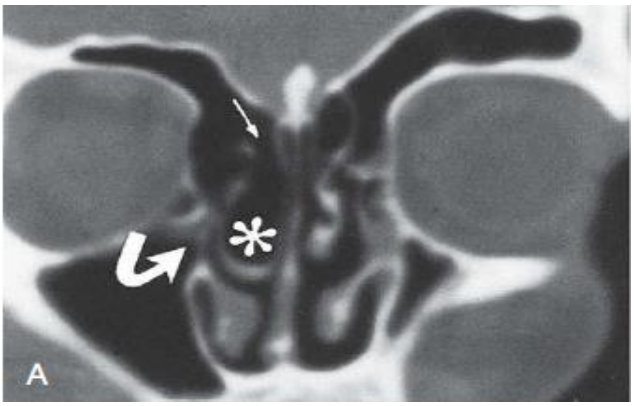

Fig. 1. CT scan- Concha bullosa [7]

Endoscopic technique

Under local anesthesia and vasoconstriction: (Lidocaine nasal spray 4% and Xylometazoline) nasal spray for few minutes. The patient was in a sitting position, the nasal cavity examined with 0^o, 30^o and 70^o 4 mm rigid endoscope sometimes with fiberoptic nasopharyngoscpe. The endoscope examinations were done by classic 3 passes.

First pass carefully along the floor of the nose, while the septum, inferior meatus, inferior turbinate, middle turbinate and nasopharynx are inspected.

Second pass the endoscope is reinserted between the inferior and middle turbinate.

While advancing in a posterior direction, the inferior portion of the middle turbinate and middle meatus, the fontanelles and any accessory maxillary Ostia are examined. The sphenoethmoid recess is visualized by passing the scope medial to the posterior aspect of the middle turbinate and rotating it superiorly. The examiner will often be able to visualize the superior turbinate and the natural sphenoid ostium.

The telescope is then gently withdrawn and re-passed again in a direction directly toward the middle meatus passing between the middle turbinate and lateral wall of the nose inspecting the uncinate process, bulla ethmoidalis, hiatus semilunaris and maxillary ostium (OMC area).

Third pass while withdrawing the endoscope. The scope is rotated laterally beneath the posterior aspect of the middle turbinate to gain access to the deeper areas of the middle meatus. Visualization of the bulla ethmoidalis, hiatus semilunaris and infundibular entrance is obtained, and as the scope is withdrawn even further, an excellent view of the uncinate process and its overlying mucosa is obtained.

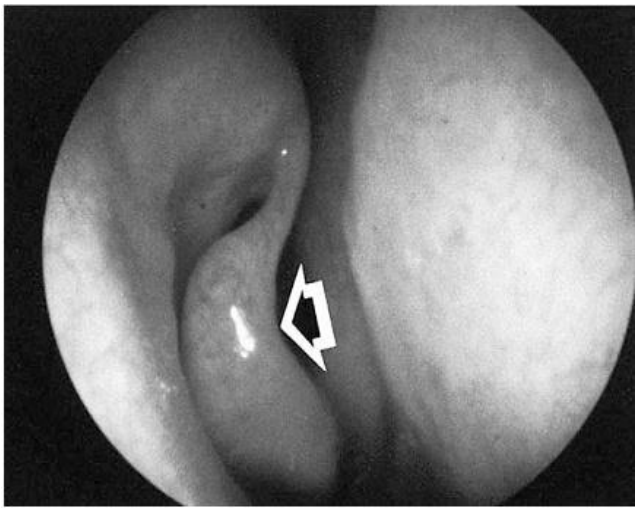

Fig. 2. Endoscopy - Paradoxical Middle Turbinate [7]

Statistical analysis

The chi-square (x^2) test was used to evaluate the association between categorical variables; to determine whether association was significant, the *P* value was calculated for the results as follow; significant (S) when it equals ≤ 0.05 and not significant (NS) when it equals ≥ 0.05

Result

The age range of the patients studied was 17 to 53 years, the mean age is (32.44 ± 9.83years) the commonest age group affected was between 31-40 years (34.88%) as seen in table 1.

Table 1. Age distribution

Age (years)	No.	%
11-20	4	9.3
21-30	13	30.23
31-40	15	34.88
41-50	8	18.6
51-60	3	6.97

The males to females ratio was 1.15:1 (Table 2).

Table 2. Gender distribution

Gender	No.	%
Male	23	53.48
Female	20	46.51

The duration of symptoms was between 1-5 years in 44.18% of the patients (Table 3).

Table 3. Duration of Symptoms

Duration (years)	No.	%
<1	16	37.2
1-5	19	44.18
6-10	6	13.95
>10	2	4.65

Headache was the commonest symptom and encountered in 30 patients (69.76) and cacosmia was the least symptom noticed in only 2 patients (4.65%) as seen in table 4.

Septal deviation was the commonest endoscopic finding (20 patients 46.51%) and paradoxical middle turbinate was the least findings (one patient 2.32%) as shown in table 5.

Diseased OMC and mucosal thickening of the sinuses was the CT-scan finding in all patients 43 (100%) and the least was Haller's cells 3 patients 6.97% as observed in table 6. Septal deviation by

endoscopy was observed in 46.5% versus 48.8% by CT scan while enlarged ethmoid bulla in 4.65% by endoscopy versus 20.93% by CT scan (Table 7).

Table 4. Occurrence of symptoms

Symptoms	No.	%
Headache	30	69.76
Nasal obstruction	29	67.44
Postnasal drip	18	41.86
Sore throat	9	20.93
Facial pain/ pressure	17	39.53
Rhinorrhea	13	30.23
Sneezing	11	25.58
Snoring	8	18.60
Hyposmia	14	32.55
Cacosmia	2	4.65
Otalgia	2	4.65

Table 5. Endoscopic examination findings

Finding		No.	%
Mucopus within OMC		10	23.25
Abnormal uncinate pross.		2	4.65
enlarged middle turbinate		13	30.23
Septal deviation	Mild	9	46.51
	Moderate	6	
	Severe	5	
Enlarged ethmoid bulla		2	4.65
Polyps		14	32.55
Paradoxical mid. turbinate		1	2.32
Hypertrophy inf. turbinate		18	41.86

OMC = ostio meatal complex

Discussion

The average age of our patients was low as compared to the study done by Ling and Kountakis (2007) (8) which was 49.4 years (range 18-80 years). This difference because the older ages in our study carries some medical co-morbidity that makes the surgical operation risky on their life while the male-to-female ratio was 1.1:1 which is similar to our findings. On the contrary, Abdul Aziz et al (9) when 160 male, 68 female and 18 pediatric found that the adult male: female ratio was 2.35:1 which was higher than in the present study which may be due to large number of patients they studied while their patients' age was in accordance with this study.

In the current study, the common symptoms in order of frequency was headache in 30 patients (69.76%); nasal obstruction in 29 patients (67.44%); post nasal drip in 18 patients (41.86%); rhinorrhea in 13 patients (30.23%); hyposmia in14 patients (32.55%); facial pain/pressure in 17 patients (39.53%); sore throat in 9 patients (20.93%); sneezing in 11 patients (25.58%); snoring in 8 patients (18.60%); and otalgia in 2 patients (4.65). In study by Netkovski J, *et al* [10] the leading symptom were nasal obstruction in 93.7% and post nasal discharge in 86.2% of the patients. Furthermore, patients reported anterior nasal discharge (Rhinorrhea) in 72.5%, headache in 65% and hyposmia in 62.5% of the patients. This may be due to the fact that their patients are more concerned with nasal obstruction that lead***s*** to sleep disturbance and its impact on the daily activity. While headache is the commonest problems that obligate the patients for seeking a medical advice in our society.

In a study performed by Chester *et al* [11], they noticed facial pain/pressure in 35%; facial congestion / fullness in 10%; nasal obstruction / blockage in 42%; nasal discharge / purulence /discolored postnasal discharge in 47%; hyposmia/anosmia in 35%; and ear pain / pressure/fullness in 6%.

These differences may be due to variation in the Demography in which these studies were done and the large sample size of the study of patients selected.

Endoscopic findings

Endoscopic findings in this study were as following: Septal deviation in (46.51%), polyps (32.55%), enlarged middle turbinate (30.23%), Mucopus within ostiomeatal complex in (23.25%), abnormal uncinate process (4.65%), enlarged ethmoid bulla (4.65%) and paradoxical middle turbinate (2.32 %).

In study done by Danielsen and Olofsson [12] included 230 patients, enlarged middle turbinate

8.69%, prominent ethmoidal bulla 38.69%, paradoxically bent middle turbinate 15.21 % and septal deviation 5.65% were the commonest finding, the difference from current study is likely due to larger number of patients were included in the last study.

In 1989, Kamel [13] studied 100 patients and reported that Mucopus within ostiomeatal complex found in 27.21%, abnormal uncinate process 17.72%, enlarged middle turbinate 5.69% enlarged ethmoid bulla 19.62% polyps 33.53% (polyp in frontal recess, ant. ethmoid region, maxillary ostium 9.49%, 15.18%, 8.86% respectively) paradoxical middle turbinate 6.65% (1.89% diseased sinus, 4.76% disease free sinuses).

Table 6. CT-scan findings

Finding		No.	%
Septal deviation		21	48.83
Mucosal thickening of sinuses	Total	43	100
	Maxillary	34	79.06
	Ethmoid	39	90.69
	Sphenoid	8	18.6
	Frontal	10	23.25
Concha bullosa		12	27.90
Abnormal uncinate process		5	11.62
Enlarged ethmoid bulla		9	20.93
Haller's cells		3	6.97
Diseased OMC		43	100
Hypertrophied inferior turbinate		18	41.86

Table 7. Comparison between endoscopic and CT-scan findings

Finding	Examination	
	Endoscopic	CT-scan
Septal deviation	46.5%	48.8%
Enlarged ethmoid bulla	4.65%	20.93%*
Abn. uncinate process	4.65%	11.62%
Hypertro. inf. turbinate	41.86%	41.86%

* $P = 0.024$

CT-scan findings

All the patients (100%) in the current study had diseased OMC and mucosal thickening of sinuses (maxillary 79.06% ethmoid 90.69% sphenoid 18.6% frontal 23.25%), septal deviation (48.83%), hypertrophied inferior turbinate (41.86%), concha bullosa (27.90%), and abnormal uncinate process (11.62%), enlarged ethmoid bulla (20.93%), and Haller's cells (6.97%).

Tezer *et al* (2006)[14] in their study on 399 patients, the CT findings was maxillary sinus opacification (48.85%) ethmoidal sinus opacification (43.60%) frontal sinus opacification (27.56%) and sphenoid sinus (18.8%), OMC disease (38.09%), concha bullosa (31.07%) and Haller's cell (12.78%).

In other study done by Bernholz (2000) [15], finds mucosal thickening in 43% of the patients, septal deviation in 65% and concha bullosa in 20%.

Comparison between endoscopic and CT scan findings

In the current study, there is significant difference for enlarged ethmoid bulla but not significant for septal deviation, abnormal uncinate process and hypertrophy of inferior turbinate (Table 7). Shahizon *et al* [16], in a study of 40 patients found eighteen patients (45%) had concha bullosa detected on CT and only 10 patients (25%) were detected to have concha bullosa on nasal endoscopy. Three patients (8%) presented with paradoxical turbinate on CT all of which were not detected endoscopically. Seven patients were noted to have paradoxical turbinate on endoscopy. None of these patients had paradoxical turbinate on CT. Of these patients, five had concha bullosa and two patients had normal middle turbinate. Eighteen patients (45%) had nasal polyps on endoscopy of which only five patients were reported to have polyps on CT. Ten patients (25%) were noted to have septal deviation on endoscopy, of these patients; nine patients had nasal septal deviation on CT-scan. On CT-scan, the diseased mucosa, polyps and Mucopus are non specific findings which are better assessed endoscopically.

It was clearly evident that CT was superior in detecting OMC involvement, presence of concha bullosa, and paradoxical turbinate. Paradoxical turbinate was not easily detected on nasal endoscopy and was easily mistaken for concha

bullosa by an inexperienced endoscopist. This study also found that polyps, diseased mucosa and Mucopus in the middle meatus had no specific features on coronal CT, while endoscopy had an essential role in accurately diagnosing these pathologies. Understanding of the disease process and its presentation in correlation to nasal endoscopic findings will assist radiologist in interpreting the CT findings. Functional interactive partnership between the radiologist and otolaryngologist is likely to yield a positive outcome.

In conclusion, endoscopy and CT-scan of the nose and paranasal sinuses are mandatory before Endoscopic Sinus Surgery of nose and paranasal sinuses in patients with chronic rhinosinusitis.

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Conflict of interest

The author declare no conflict of interest

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Misoprostol Efficacy in Labor Induction

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Abstract

Background The process of normal human childbirth is categorized in three stages of labor: the shortening and dilation of the cervix, descent and birth of the infant, and delivery of the placenta. Oxytocin is the most commonly used agent for induction, and is used to induce uterine contractions.

Objective To estimate the efficacy of oral misoprostol for labor induction.

Methods This randomized, controlled trial study was comparing intravenous oxytocin to a 25-microgram dose of oral misoprostol every 3-4 hours. A woman who had cervical dilation of 0-2 cm then undergoes labor induction. Our outcome was recorded.

Results we found when we used misoprostol the time duration was significantly less specially in primigravida and when os closed, the side effect approximately same as oxytocin.

Conclusion Oral misoprostol is an effective agent for inductionof labor.

Keywords Induction of labor, misoprostol, oxytocin

List of abbreviation: ARM = artificial rupture of membrane, ACOG = American College of Obstetricians and Gynecologists, C/S = caesarean section, NICU = Neonatal intensive care unit.

Introduction

Induction of labour can be defined as the artificial initiation of labour, before its spontaneous onset for the purpose of delivery of the fetoplacental unit. Prostaglandins and oxytocin are the principal hormones which can both be produced synthetically and be given to pregnant women to induce labour [(1)].

Induction of labor is commonly performed in clinical practice, compared to spontaneous labor the main risks of induction are ineffective labor and excessive uterine activity, which may cause fetal hypoxia. In woman with previous cesarean sections or uterine scars there also appears to be higher incidence of uterine rupture. Labor may induced using medical methods (oxytocin or prostaglandins) or mechanical methods (e.g. extra-amniotic balloon catheters or artificial rupture of membrane [ARM]) the most common methods in hospital practice worldwide are oxytocin (combined with ARM where possible) and vaginal prostaglandins [(2)].

The naturally occurring prostaglandin E series was first discovered to inhibit gastric acid secretion in 1967 by Robert et al., and was first used for the induction of labor with a dead fetus in 1987 [(3)].

The uterotonic and cervical softening effects on the female genital tract were considered as side effects rather than therapeutic effects when misoprostol was first introduced. However, it is because of these effects that misoprostol is so widely used in obstetric and gynecological practice today [(4)].

After a single dose of oral misoprostol there is increase in uterine tonus to produce regular contractions, however a sustained plasma level of misoprostol is required and this requires repeated oral doses [(5)].

The American College of Obstetricians and Gynecologists (ACOG) guidelines recommend a full evaluation of the maternal-fetal status, the status of the cervix, and at least a 39 completed weeks (full term) of gestation for optimal health of the newborn when considering elective induction of labor. Induction is also considered for logistical reasons, such as the distance from hospital or psychosocial conditions, but in these instances gestational age confirmation must be done, and the maturity of the fetal lung must be confirmed by testing. The ACOG also note that contraindications for induced labor are the same as for spontaneous vaginal delivery, including vasa praevia, complete placenta praevia, umbilical cord prolapse or active genital herpes simplex infection [6].

The objective of this study was to estimate the efficacy of oral misoprostol for labor induction

Methods

Two hundred forty women with early labor were admitted to maternal and pediatrics teaching hospital in Al-Diwanyia city or labor induction between April 2011 to March 2012. A standardized data sheets were prepared for collection of information including name, age, body weight, height, maternal history. Cervical dilation of 0-2 cm. if decided to induce labor we approached any women with a full term pregnancy at least 40 weeks' gestation. We then obtained written informed consent. We excluded women with a "favorable" cervix (defined as a modified Bishop score of ≥ 7), any contraindication to vaginal birth, previous uterine surgery (including caesarean section), or ruptured membranes.

Those women divided into two groups: first group .121 patients started with misoprestol 25 microgram oral misoprostol every 3-4 hours (59 patients of them were primigravida) and second group 119 (60 patients of them primigravida) patients on oxytocin infusion and monitoring every patient by partogram and continuous cardiotogography.

Our primary outcome measures duration of labor induction till delivery (including women who achieved vaginal birth after 24 hours and those women who required a caesarean section), caesarean section (all and for heart rate tracing indicating fetal distress), and uterine hyperstimulation with changes in fetal heart rate.

We defined uterine hyperstimulation as uterine tachysystole (with five or more contractions in a 10 minutes period for two consecutive 10 minute periods) or uterine hypertonus (a uterine contraction lasting for more than two minutes). The changes in fetal heart rate that we considered abnormal included persistent late, or variable decelerations, fetal tachycardia (fetal heart rate > 160 beats per minute), fetal bradycardia (fetal heart rate < 100 beats per minute) and abscent variability. A single investigator blinded to the treatment allocated reviewed all fetal heart rate tracings from an induced labour to maintain consistency in interpretation.

Results

Two hundred forty cases of women with labor were admitted to the hospital. There were 121 (50.5%) patients started with misoprestol (59 patients (48.7%) of them were primigravida) and 119 (49.5%) (60 patients (50.4%) of them primigravida) patients on oxytocin infusion as shown in table 1.

Table 1. Distribution of study groups

Patients	Treatment	
	Misoprestol	Oxytocin
Primigravida	59 (48.7%)	60 (50.4%)
Multigravida	62 (51.2%)	59 (49.5%)
Total	121 (50.5%)	119 (49.5%)

The time duration for delivery with primigravida patients on misoprostol shown that 29 patient (49.1%) take about 8-10 hours and 2 patients (3.38%) take more than 14 hours. The primigravida patients on oxytocin shown that 38 patients (63.33%) take about 10-12 hours and 19 patients (31.66%) take more than 14 hours (p = 0.01) . as shown in table 2.

The multigravida patients on misoprostol shown that 27 patients (43.5%) take about 8 - 10 hours for delivery while 1 patient take more than 14 hour (1.61%).The patients on oxytocin shown that 26 patients (44.06%) take about 10 - 12 hours and 7 patients (11.86%) take more than 14 hours. (p = 0.01) as shown in table 3.

Table 2. Mean duration for delivery (primigravida)

Misoprestol		Oxytocin	
No.	Time (hours)	No.	Time (hours)
10	<8	8	<8
29	8 - 10	14	8 – 10
21	10- 12	38	10 -12
2	>14	19	>14

Table 3. Mean duration for delivery (more than one pregnancy)

Misoprestol		Oxytocin	
No.	Time (hours)	No.	Time (hours)
14	<8	10	<8
27	8 - 10	16	8 – 10
20	10- 12	26	10 -12
1	>14	7	>14

Patients on the misoprostol group show uterine tachysystole and hypertonus compared with women on the oxytocin (76% compared with 63%, respectively; *P* = 0.01). There was no significant difference between two groups regarding non reassuring fetal heart rate (*P* = 0.20) or need a cesarean delivery. Patients on misoprestol shown meconium stained liquor (*P* = 0.02). No difference in need to admission to NICU (Table 4).

Table 4. Maternal and perinatal outcome

Outcome	Misoprestol	Oxytocin
Tachysystol & hypertonus	92 (76%)	75 (63%)*
Non-reassuring fetal HR	23 (19%)	22 (18.4%)
Required c/s	26 (21.8%)	27 (22.6%)
Meconium stained liquor	32 (26.44%)	20 (16.8%)†
Admission to NICU	27 (22.3%)	25 (21%)

* *P* = 0.01, † *P* = 0.02, HR = heart rate

Discussion

Nowadays, induction of labor is more widely used than ever before [7,8]. Recent studies have shown that this increase is mainly due to a rise of inductions for marginal or elective reasons. Women may experience distress when labor has not started by the expected date [9] and obstetricians have to withstand pressure from these patients as well as the temptation to use prostaglandins earlier. Appropriate evaluation of the pregnancy and consultation with such patients will lead to the correct selection of those who will benefit most from a labor induction.

In this study, the time duration for delivery with primigravida patients on misoprostol shown that 29 patient take about 8-10 hours while the primigravida patients on oxytocin shown that 38 patients take about 10-12 hours. The multigravida patients on misoprostol shown that 27 patients take about 8-10 hours for delivery while patients on oxytocin shown that 26 patients take about 10-12 hours .This is in agreement with a study of Alfirevic [10]. Who done trial on 80 randomized women with prelabour rupture of membranes at term showed that, compared with placebo; oral misoprostol reduces the need for oxytocin infusion from 51 percent to 13 percent (relative risk 0.25, 95% confidence interval (CI) 0.1 to 0.6) and shortens delivery time by 8.7 hours.

To the best of our knowledge, Aalami-Harandi [11] estimates the efficacy of oral misoprostol for labor induction. He shows that misoprostol is a safe and effective drug with low complications for the induction of labor. Failure is seen less with misoprostol and caesarean sections are less frequently indicated as compared to oxytocin. Maternal and fetal complications were comparable between groups except gastrointestinal symptoms which were encountered more frequently in the misoprostol. Of particular concern are several reports of uterine rupture following misoprostol use in woman with and without previous caesarean section. Adverse effects were reduced, within lower rates of uterine

hyperstimulation and a tend to fewer admissions to neonatal intensive care unit [12, 13].

The finding of a significantly more meconium stained liquor with misoprostol is of interest. Wing et al suggested the possibility of meconium passage in response to uterine hyperstimulation or a direct effect of absorbed misoprostol metabolites on the fetal gastrointestinal tract [14].

They have previously observed an increased rate of meconium stained liquor in woman who has ingested casteroil, though causality was not proven, and suggested a possible direct effect of the caster oil metabolites on fetal bowel [15]. It is unlikely that the small amount of hydrogenated caster oil found in misoprostol tablets would have any pharmacological effect, but the possibility that misoprostol metabolites may directly stimulate fetal bowel is of interest [16].

Attempting an explanation to the afore-mentioned side effects of misoprostol use and taking into account other reports, it appears that the increase in clinically relevant adverse effects is not only misoprostol related but it may be dose dependent [17,18].

In conclusion, this study show oral misoprostol is an effective agent for induction of labor. We recommend use of misoprostol for induction of labor depend on selection of patient, like non scaring uterus and the compliance of the patients with good monitoring of ongoing process of labor and regarding its safety because of a relatively high rate of uterine hyperstimulation. Further studies needed regarding the dose, and use in scaring uterus.

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Author contribution

Dr. Asma Z. Fadhil writes the article and the assistant Prof. Edwar Z. Khosho materially participated in the research and article preparation.

Declaration of interest

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The Effect of Foeniculum Vulgare Alcoholic Extract on Some Metabolic Changes in Liver and Kidneys of Alloxan Diabetic Mice

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Abstract

Background Some people used herbal extracts as a treatment for diabetes mellitus worldwide. Such treatment of considerable benefit especially during the early stages of the illness**.** Many phytoconstituents responsible for anti diabetic effects have been isolated from hypoglycemic plants; one of which is *Foeniculum vulgare*.

Objective To investigate the effects of alcoholic extract of *Foeniculum vulgare* on some biochemical parameters in liver and kidneys of alloxan-induced diabetic mice, in order to use this herb as natural products to the management of diabetes mellitus.

Methods Twenty four female mice (18 diabetic mice and 6 control) were divided into 4 groups, each contain 6 mice (I = control, II = alloxan-treated miceI, III and IV = extract treated mice). Oral administration of *Foeniculum vulgare* extract given for 30 days to group III and IV in doses of 1.2 mg/kg BW) and 2.2 mg/kg BW, respectively. The serum glucose, insulin, lipid profile, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkalinposphatase, creatinine, urea and calcium, were measured in fasting state.

Results As compared with group II, serum glucose was significantly reduced and serum insulin significantly elevated in groups III and IV following oral administration of extract for 21 days. Following 30 days, significant reduction in the total cholesterol, triglycerides, low density lipoprotein- cholesterol, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, very low density lipoprotein- cholesterol, and creatnin, whereas high density-cholesterol and calcium were significantly increased.

Conclusion Foeniculum vulgare extract have good hypoglycemic, antihyperlipidemic in Alloxan- induced diabetic mice, in addition to the improvement in the glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, creatinin, urea and calcium.

Keywords Diabetes militates, Antihyperlipidemia, Hypoglycemic effect, alloxan, medical plants, Foeniculum Vulgare.

List of abbreviation: ALP = Alkaline phosphatase, ANOVA = Analysis of variance, BW = Body weight, Ca = Calcium, CAMP = cyclic adenosine mono phosphate, DM = Diabetes mellitus, FV = Foeniculum vulgare, GOT = Glutamate oxaloacetate transaminase, GPT = Glutamate pyruvate transaminase, HDL-C = High density lipoprotein-cholesterol, LDL-C = Low density lipoprotein- cholesterol, T.Chol. = Total cholesterol, TG = Triglycerides, VLDL-C = Very low density lipoprotein- cholesterol, WHO = World health organization

Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in production of insulin by beta cells of pancreas, or by the ineffectiveness of the insulin. It is an extremely common metabolic disorder affecting carbohydrate, fat and protein metabolism characterized by hyperglycemia, glucose urea and negative nitrogen balance. It is mainly due to lack of insulin secretion, or resistance to insulin action or both [(1)]. Two types of DM were noticed type 1 and type 2 [(2)]. It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million People and is set to rise to 300 million by 2025. The disease

takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa [1,3]. DM causes number of complications like retinopathy, neuropathy and peripheral vascular insufficiencies [4]. The detrimental effects of diabetic complications are mainly mediated through oxidative stress [5]. DM is still not completely cured by using anti diabetic agents. Insulin therapy is the only satisfactory approach in it, even though it has several drawbacks like insulin resistance, anorexia and fatty liver in prolong treatment [6]. Synthetic anti diabetic agents can produce some side effects and they are not suitable for use in all patients. Treatment of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with anti diabetic activity and fewer side effects [7]. Furthermore, after the recommendation made by World Health Organization (WHO) on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important. WHO has estimated that 80% of population of developing countries still relies on traditional medicines mostly plant drugs for their primary health care needs and ensure patient safety by upgrading the skills and knowledge of traditional medicine providers [8].

Herbal drugs or natural products are gaining popularity in the treatment of DM, it prescribed widely considering their safety, low incidence of side effects, effectiveness, availability and low cost [5,6]. The anti diabetic activity of herbs depends upon variety of mechanisms such as; Pancreatic beta cell potassium channel blocking, cyclic adenosine mono phosphate (cAMP) stimulation, inhibition of renal glucose re absorption, stimulation of insulin secretion from beta cells, reduction in insulin resistance , providing certain elements for the beta-cells like calcium, zinc, magnesium, manganese and copper, regenerating of pancreatic beta cells, stimulation of insulin secretion, stimulation of glycogenesis and hepatic glycolysis, protective effect against the destruction of the beta cells, inhibition of β-galactocidase and α-glucocidase, cortisol lowering activities, inhibition of alpha-amylase and preventing oxidative stress in beta cell [6].

Several medicinal plants are used in the management of DM [7]. Many phytoconstituents responsible for anti diabetic effects have been isolated from hypoglycemic plants [9]. One of such plants is *Foeniculum vulgare* (*FV*). It is a member of family of Apiaceae. This plant is an aromatic plant [10-13]. The name Foeniculum was given by Romans to this plant and is derived from a latin word (Foenum) which means (hay) perhaps because smell of fennel rell of fennel resembles that of hay. Fennel is a native of Mediterranean region and Europe but is commonly cultivated throughout India especially in Assam, Maharashtra, Punjab and Gujarat [14]. An analysis of fennel shows it to consist of moisture 6.3%, protein 9.5%, fat 10%, minerals 13.4%, fiber 18.5% and carbohydrates 42.3%. Its mineral and vitamin contents are calcium, phosphorous, iron, sodium, potassium, thiamine, riboflavin, niacin and vitamin C. Main components of *FV* are trans-anethol (50-70%), estrogen-dianthol. Flavonoids and organic acids [10,14]. Fennel is chiefly known as culinary herb but it is a commonly used household remedy for various medicinal purposes. Fruits are used as spice and condiment, as carminative and stimulant, also employed as flavoring agent in culinary preparations, confectionary etc. Fennel is often added to purgatives in order to allay their tendency to cause gripe. In a study carried out on rats, *FV* has shown a protective effect against ethanol induced gastric mucosal lesions. Fennel has shown anti-inflammatory; antioxidant, anti platelet and antithrombotic; antispasmodic activities.

It has also been reported to possess bronchodilatory; diuretic; hepatoprotective; hypotensive; insecticidal; nematicidal; and oculohypotensive properties; and pain reliever in primary dysmenorrhoea. Anethole has a chemical structure similar to a chemical substance called dopamine, naturally present in the body. Dopamine is known to have a relaxing effect on

the intestine and perhaps, explains why fennel has a beneficial effect on infantile colic. Also *FV* have antimicrobial properties. Therefore it is used in traditional medicine as antibacterial and antiviral [10,14,15].

The present study was undertaken to investigate the effects of alcoholic extract of *Foeniculum vulgare* on some biochemical parameters in liver and kidneys of alloxan-induced diabetic mice, in order to use this herb as natural products to the management of diabetes mellitus.

Methods

FV were purchased from the local gardens and identified in a Biotechnology Research Centre, Al-Nahrain University. The leaves were cleaned and finely powdered and extracted by: 50 gm of plant was extracted with 250 ml of methanol by soxhlet apparatus for 6 hour at 40-60 ^{o}C, then the cooled solution was evaporated to dryness by rotary evaporator at 40 ^{o}C, and and crude extract was kept until used [16].

The doses prepared from the extracted material with a concentration of (1.2 and 2.2 mg/kg BW) this methanol extracts, administrated daily for 30 days.

Healthy adult albino females of Swiss albino strain were obtained from animal house of Biotechnology Research Center, Al-Nahrain University. Twenty four mice were used in this study, the age of the mice were in the range of 2.5 to 3 months old, and the weight in the range 25-30 grams. The animals were housed in small plastic cages, which were cleaned weekly by washing with soap and tap water and sterilized with 70% ethyl alcohol throughout the period of the study. The room temperature was maintained at 24 ± 2 ^{o}C, and the animals were exposed to 14 hours light program. The period of treatment extended from 20th Jun. 2011 till 20th Jul. 2011.

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrated (5% w/v) in physiological saline at a dose of 150 mg/kg BW in a volume of 0.1 ml. The diabetic state was confirmed 48 hours after alloxan injection by weight loss and hyperglycaemia. There was 75% mortality in animals treated with alloxan. Surviving mice with a fasting blood glucose level higher than 200 ml/dl were included in the study. Four groups consisting of six animals for each group were maintained as follows [17].

In the present investigation, total of 24 female's mice (18 diabetic surviving mice and 6 normal mice) were taken and divided into four groups of 6 mice each.

Group I: Normal untreated mice (control),

Group II: Alloxan-induced diabetic mice,

Group III: Alloxan-induced diabetic mice treated with *FV* alcoholic extract (1.2 mg/kg BW) daily for 30 days, and

Group IV: Alloxan induced-diabetic mice treated with *FV* alcoholic extract (2.2 mg/kg BW) daily for 30 days.

At the end of 15 day and 30 day, the mice fasted over night and killed by cervical dislocation, and then blood was collected from heart puncture. Fasting blood (glucose and insulin) level was evaluated at the day 15 and 30 for (control, diabetic and treated groups). Lipid profiles, Ca^{2+}, creatinin, urea, serum glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) and serum alkalin phosphatase (ALP) were evaluated at the day 30 for all groups.

All the above biochemical parameters were assayed by the reported methods in their kits, while low density lipoprotein - cholesterol (LDL-C) concentration are most commonly calculated by using the Friedwald formula which was based on the assumption that very low density lipoprotein - cholesterol (VLDL-C) is present in serum at a concentration equal to one fifth of the TG concentration.

Friedwald formula as follow [18]:

LDL-C mg/dl = total cholesterol – (HDL-C + TG/5), when all concentrations are given in milligrams per deciliter.

Data were analyzed by 1-way analysis of variance with ANOVA test is presented as means ±SE. The level of significance was $P < 0.05$ [19].

Results

The alloxan-induced diabetic mice (group II) in the present study showed a significant increase in the

mean of serum glucose level and significant decrease in the mean of serum insulin after 21 days. Also the results showed after 30 days significant increase in the means of GOT, GPT, ALP, T.Chol., TG, LDL-C, VLDL-C, creatinine and urea, while the means of HDL-C and Ca were significant decrease as compared with control (group I), with $P < 0.05$, (Tables 1,2,3 and 4).

After oral administration of FV extract in doses (1.2 and 2.2 mg/kg BW) for groups III and IV respectively, the results showed after 21days significant decrease in the means of serum glucose and significant increase in the means of serum insulin. After 30 days the results showed significant decrease in the means of cholesterol, TG, LDL-C, GOT, GPT, ALP and creatinine, in contrast to a significant increase in HDL- C and Ca but there were no significant differences in the means of urea, as compared with group II at $P < 0.05$ (Tables 1,2,3 and 4).

There was no significant difference in the mean of VLDL-C in group III as compared with group II at $P < 0.05$ (Table 2), while there was significant difference in the mean of VLDL-C in group IV as compared with group II.

Table 1. Effect of methanol extract of *Foeniculum vulgare* on serum glucose and serum Insulin level in alloxan-induce diabetic mice

N	Groups	Serum Glucose (mg/dl) (mean ± SD)		Serum Insulin (mg/dl) (mean ± SD)	
		21 days	30 days	21 days	30 days
I	Control	A , a 98.53±4.72	A , a 93.75±10.05	A , a 20.23±3.27	A , a 26.59±5.40
II	Alloxan	B , a 308.22±17.83	B , a 320.41±25.91	B , a 8.51±2.94	B , b 5.21±1.08
III	Alloxan + *FV* (1.2 mg/kg BW)	C , a 204.60±12.28	C , a 192.74±12.41	C , a 13.22±2.73	C , a 11.45±3.27
IV	Alloxan + *FV* (2.2 mg/kg BW)	D, a 166.20±9.85	D, b 132.69±9.72	C , a 14.68±3.01	D, a 18.40±5.54

Letters A,B,C are significant at $P < 0.05$ (comparison between columns), Letters a,b,c are significant at $P < 0.05$ (comparison between rows).

Table 2. Effect of methanol extract of *Foeniculum vulgare* on serum lipid profiles in alloxan-induced diabetic mice

N	Group	T Chol. mg/dl (mean±SD)	TG mg/dl (mean±SD)	HDL-C mg/dl (mean±SD)	VLDL-C mg/dl (mean±SD)	LDL-C mg/dl (mean±SD)
I	**Control**	A 133.28±25.05	A 101.24±16.93	A 32.57±6.83	A 20.25±4.22	A 80.46±13.04
II	**Alloxan**	B 251.28±31.06	B 212.24±37.62	B 19.48±8.33	B 42.45±7.05	B 189.35±36.41
III	**Alloxan+ *FV* (1.2 mg/kg BW)**	C 174.29±31.76	C 178.46±25.72	C 25.08±4.82	B 35.69±8.71	C 113.52±21.01
IV	**Alloxan + *FV* (2.2 mg/kg BW)**	C 157.03±19.22	D 130.96±13.94	AC 30.12±6.77	C 26.19±7.29	C 100.72±15.48

FV = Foeniculum vulgare

Differences letters A,B,C are significant at ($P < 0.05$) to compare columns

Table 3. Effect of methanol extract of Foeniculum vulgare on serum biomarkers in alloxan induced diabetic mice

N	Groups	GOT IU/ml (mean±SD)	GPT IU/ml (mean±SD)	ALP IU/ml (mean±SD)
I	Control	A 174.36±21.26	A 76.24±6.84	A 82.19±13.38
II	Alloxan	B 286.26±19.05	B 132.51±24.46	B 161.34±20.55
III	Alloxan + *Foeniculum vulgare* (1.2 mg/kg BW)	C 203.05±33.84	A 84.76±8.93	C 103.61±16.53
IV	Alloxan + *Foeniculum vulgare* (2.2 mg/kg BW)	A 185.25±20.73	A 80.33±12.04	AC 91.75±11.79

Differences letters A,B,C are significant at ($P < 0.05$) to compare columns.

Table 4. Effect of methanol extract of *Foeniculum vulgare* on serum creatinine, urea, Ca^{2+} in alloxan-induced diabetic mice

N	Groups	Creatinine mg/dl (mean±SD)	Urea mg/dl (mean±SD)	Ca mg/dl (mean±SD)
I	Control	A 0.56±0.031	A 20.13±3.38	A 14.53±2.94
II	Alloxan	B 0.84±0.068	B 32.58±6.40	B 12.68±2.74
III	Alloxan + *Foeniculum vulgare* (1.2 mg/kg BW)	C 0.72±0.044	B 28.22±5.11	A 15.18±1.94
IV	Alloxan + *Foeniculum vulgare* (2.2 mg/kg BW)	A 0.59±0.039	AB 23.14±4.02	A 16.09±2.33

Differences letters A,B,C are significant at ($P < 0.05$) to compare columns.

Discussion

In the present study oral administration of *FV* extract reduced serum glucose. This result were in agreement with the results of Abou El-Soud *et al*, in which they reported that the reduction was observed in blood glucose in diabetic rats treated with the tested essential oil of FV Mill. Also they reported that research studies on the effect of fennel oil on blood glucose are not numerous [15]. A single study by Barros et al reported that fennel can improve rat glucose tolerance obviously [20]. The antidiabetic effect of methanol extract of *FV* may be due to the presence of more than one antihyperglycemic principle and their synergistic effects [17]. Abou El-Soud *et al*. reported that the chemical constituents of *FV* for anti-diabetic are (flavonoids and terpenoids) [15].

It is well known that in uncontrolled diabetes mellitus, there will be an increase in total cholesterol, Triglycerides and LDL-C associated with decrease in HDL-C and contributes to coronary artery disease, which is related with significant changes in lipid metabolism and structure. Although abnormalities in cellular cholesterol level in diabetes occur, the precise mechanisms underlying these enzymatic changes have not been elucidated. Such a significant increase in TG may be due to the lack of insulin under diabetic condition, while insulin activates the enzyme lipoprotein lipase and hydrolyze TG under normal condition [17]. In the present study the total cholesterol, triglycerides and LDL-C increased in diabetic group (II) and it was reduced after 30 days treatment with *FV* extract as well as the HDL-C level was

significantly increased. This may be due to saponin in *FV* which is reduces the uptake of certain nutrients including glucose and cholesterol at the gastro intestinal tract. Hence, it has been reported to have hypocholesterolemic effect and thus may aid lessening metabolic burden that would have been placed in the liver; this suggests that the extract may inhibit the pathway of cholesterol synthesis and increased HDL-C / LDL-C ratio may be due to activation of LDL-C receptors in hepatocyte, which is responsible for taking up LDL-C into the liver and reduce the serum LDL-C level [(21)].

Some studies found in a trial of fenugreek *FV* in twenty-five patients with type II diabetes, a trial group of thirteen patients received one gram per / day of an evaporated hydro- alcoholic extract of fenugreek seeds for two months. At the end of the period, blood sugar response to meal was significantly lower in fenugreek group. Insulin secretion was also lower, as were serum TG, HDL- C was improved, and a standard measure of insulin sensitivity showed increased insulin sensitivity. Several trials showed that evaporated hydro-alcoholic extract as effective as the aqueous extract [(22)]. Other study of Oulmouden *et al*, in which they found that the administration of the methanol extract of fennel caused a hypolipidemic and anti-atherosclerotic effect in reducing the lipids concentrations of serum and liver [(23)]. These results agreed with the results of glucose and lipid profile in the present study.

Serum GOT and GPT levels increase as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis, liver cancer and inflammatory conditions including diabetes. In some studies and in the present study, it was observed that the levels of serum GOT and GPT in alloxan induced diabetic mice were elevated. It may be due to leaking out of enzymes from the tissues and migrating in to the circulation by the adverse effect of alloxan. The transaminase enzymes were used as markers to assess the extent of liver damage in streptozotocin induced diabetic mice [(17)].

In the present study oral administration of *FV* extract reduce serum GOT, GPT, ALP. These results were in agreement with the results of Selvan *et al* (2008) who found that hepatic damage was restored and the elevated transaminase activities were significantly reduced by hypoglycemic plant. The diabetic complication such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activities [(6)].

Renal disease is a common complication in diabetic patients. More than ten percent of people with diabetes die from renal complications as (renal failure) [(4)]. Significant elevations in serum creatinine and urea indicate impaired renal function in diabetic animals [(24)].

The recovery of renal function with treatment of fennel oil can be explained by the regenerative capability of the renal tubules. Similar results have been observed with the treatment of Streptozotocin induced diabetic rats with other herbal extracts as fenugreek alkaloid extract and onion oil. The role of fennel oil in reversing the diabetic state at the cellular level besides the metabolic normalization further proves its potential as an anti diabetic assert [(15)].

These results are in agreement with the results of the present study in which *FV* extract can partially inhibit Alloxan renal toxicity as observed from serum urea and creatinine levels Table 4.

Also *FV* extract correct the concentration of calcium in group III and IV as shown in Table 4. This result were in agreement with the result of Mohamed *et al* (2006) who reported that some herbs Provide certain necessary elements like Calcium, Zinc, Magnesium, Manganese and Copper for the beta- cells. Therefore the hypoglycemic effect of these herbs is probably due to stimulation of insulin release via modulation of intracellular Ca^{2+} concentration in pancreatic beta-cells [(25)].

Finally, the results about the effects of *FV* extracts in this study were in agreement with the results of Abou El-Soud *et al* (2011) who reported that essential oil of *FV* mill corrected the hyperglycemia and pathological abnormalities in diabetic rats which could be in part through its antioxidative effect and restoring redox homeostasis. This makes the possibility for included it as in anti diabetic drug industry [(15)].

So we can conclude from all the above biochemical parameters investigated that, *FV* extract showed exhibit anti-hyperglycemic, hyperlipidemic effects on alloxan diabetic mice.

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Declaration of interest

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Detection of *Pneumocystis carinii (jiroveci)* from Iraqi Patients with Lower Respiratory Tract Infections

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Abstract

Background *Pneumocystis carinii* is one of the rare fungi which cause pneumonia in immunocompromised patients. It is important to detect the fungus from the clinical specimens of suspected patients by laboratory tests.

Objective To identify *Pneumocystis carinii* from immunocompetent and immunocompromised patients with lower respiratory tract infections.

Methods This study included 300 patients suffering from lower respiratory tract infections of both immunocompetent (150) and immunocompromised (150) patients attending the Teaching Hospital in Mosul/Iraq. The clinical specimens collected were samples of sputum (247), and bronchial wash (80). The identification of *Pneumocystis* staining methods.

Result The organism was detected from 8 immunocompromised patients with pneumonia. Seven out of the 8 patients had carcinoma.

Conclusion *Pneumocystis carinii* is an opportunistic fungus which is an important pathogen in immunocompromised patients.

Key words *Pneumocystis carinii (jiroveci),* pneumocystis pneumonia, respiratory tract infection.

List of abbreviation: LRT = lower respiratory tract, AFB = acid fast bacilli, PCP = pneumocystis carinii pneumonia, HIV = human immunodeficiency virus, AIDS = acquired immunodeficiency syndrome

Introduction

Pneumocystis carinii was originally thought to be a protozoan when first described in the early 1900, but the advent of molecular techniques has now firmly established *P. carinii* as a member of the fungal kingdom [1]. The name *P. jiroveci*, to distinguish the organism found in human from physiological variants of *pneumocystis* found in other animals, was first proposed in 1976, in honor of Ottojiroves [2].

The occurrence of *Pneumocystis carinii* is worldwide, except in Antarctic, and is commonly found in the lungs of healthy individuals [3]. Most children are believed to have been exposed to the organism by age 3-4 years [4,5]. The organism causes pneumocystis pneumonia [2]. It affects only people with weakened immune system, especially people who are human immunodeficiency virus (HIV) positive [6]. The use of combination immunosuppressive agents is associated with reports of *P. jiroveci* pneumonia [7]. Infection occurs following the inhalation of spores, or by the reactivation of a latent infection [8].

The disease form when defects exist in both cellular and humoral immunity [5]. Once inhaled, the trophic form of the organism attaches to the alveoli and starts replication, then gradually fills the alveoli [9]. The organism is found in three distinct morphological stages. The trophozoite or trophic form, the sporozoite which is a precystic form and the cyst, which contain several intracystic bodies (2-8) or spores [5,10].

The aim of the present study is to identify *Pneumocystis carinii* from immunocompetent and immunocompromised patients with lower respiratory tract infection, using direct detection procedures namely different stains, and fluorescent microscopy.

Methods

This prospective study was conducted from April 2007 to June 2008 on 300 patients suffering from lower respiratory tract infections. Males were 175 (58.3%) and females were 125 (41.7%). Patient's age ranged from 1 to 89 (55.44 ± 17.9) years. The subjects included were of equal number, 150 apparently immunocompetent and 150 presumably immunocompromised patients.

Immunocompromised status was suspected in patients with different types of carcinoma and leukemia (46.0%), uncontrolled diabetes mellitus of > 5 years duration (25.3%), old tuberculous patients with negative acid fast bacilli (AFB) (10.7%), and chronic diseases under long-term corticosteroids therapy (18.0%).

Studied samples

A total of 327 specimens were collected from patients in the Ibn Sina Teaching Hospital (Respiratory Care Unit, Bronchoscopy Unit/ Wards) and from the Oncology and Nuclear Medicine Hospital, Mosul, Iraq.

The samples consisted of 227 sputum and 80 bronchial wash (27 patients with both sputum and bronchial wash).

The sputum of each patient was shaken by a vortex for 3-5 minutes, and the bronchial wash was centrifuged for 5 minutes, then the sediment was used for direct microscopical examination [11].

Pneumocystis carinii was identified by direct microscopical examination with different stains. Three slides were prepared from each specimen. One, wet mounted slide with 20% KOH solution and calcofluor stain (Becton Dickinson, USA), then examined under fluorescent microscope to detect the cysts. The other two fixed smears stained with Giemsa and Toludine blue stain to detect trophozoites and intracystic bodies by Giemsa stain and cysts by Toludine blue stain under 100x magnification of light microscope [6].

All the participants had given consent to participation in the research work which approved by Department of Microbiology / University Research in 21/6/2007 (4S/1329) and Teaching Hospitals (confirmed by the Center of Continuous Medical Education, No. 8021 in 2/7/2007).

Results

The patients were categorized according to the clinical entities. The most frequent clinical entity was pneumonia (49.6%). *Pneumocystis carinii* was identified in 8 cases with pneumonia. The organism was detected in bronchial wash and/or sputum of immunocompromised patients only. The patients were 6 males and 2 females, four of them were farmers, and 7 had malignancies under radiation and/or cytotoxic therapy (Table 1).

The wet prepared slide showed the cysts under 40X lens of fluorescent microscope (Figure A). Stained smear with Giemsa stain showed the intracystic bodies and trophozoites (Figure B and C). The third slide was stained with toludine blue stain for the appearance of the cysts also (Figure D). The organism was identified from 5 cases singly (without other fungus), and in the other 3 cases were mixed with yeast species. However, in 7/8 of these cases, heavy growth of bacteria appeared when the specimens inoculated on blood and MacConkey's agars.

Discussion

Pneumocystis carinii is a rare cause of infection among the general population, but is a major pulmonary pathogen for the immunocompromised patients mainly those with acquired immunodeficiency syndrome (AIDS) and malignant diseases [12,13]. The organisms were detected in immunocompromised patients from cases of pneumonia. The main predisposing factor for these patients was malignancy, which was diagnosed in 7 out of the 8 patients (Table). A previous study mentioned that *Pneumocystis*

carinii pneumonia (PCP) is a common opportunistic infection in patients with lymphoma and leukemia [14]. In a more recent report, the immunocompromised patients with no AIDS and at risk for PCP include individuals with hematological malignancies [15]. Furthermore, opportunistic organisms including *P. carinii* caused experimental infection in immunosuppressed mice [16].

Table 1. Clinical data of the patients with *Pneumocystis carinii*.

N	Sex	Age (yr)	Occupation	Predisposing factors	specimen examined	Main symptoms & signs
1	♂	80	farmer	carcinoma (cytotoxic therapy)	sputum & bronchial wash	Cough (8/8) Dyspnia (7/8) Fever (6/8) Haemoptysis (5/8)
2	♀	65	farmer	bronchial carcinoma	bronchial wash	
3	♂	35	unemployed	lymphoma (cytotoxic & RT)	sputum	
4	♂	60	farmer	asthma (steroid therapy)	sputum	
5	♂	26	unemployed	leukemia (cytotoxic therapy)	sputum	
6	♀	42	housewife	leukemia (cytotoxic therapy)	sputum	
7	♂	49	worker	leukemia (cytotoxic therapy)	sputum	
8	♂	65	farmer	leukemia (cytotoxic therapy)	sputum	

RT = Radiotherapy

The organism was identified from bronchial wash and/or productive sputum of 8 patients out of the 300 cases studied. A reported study showed that 24 (11.8%) of 204 clinical specimens (bronchial aspirate, induced sputum) were positive for *P. carinii* [17]. During the study, the productive sputum was examined, not the induced type because this study not only for detection of *P. carinii*, but for the isolation of other fungi in the lower respiratory tract (LRT), and may be affected by the hypertonic saline used for sputum induction.

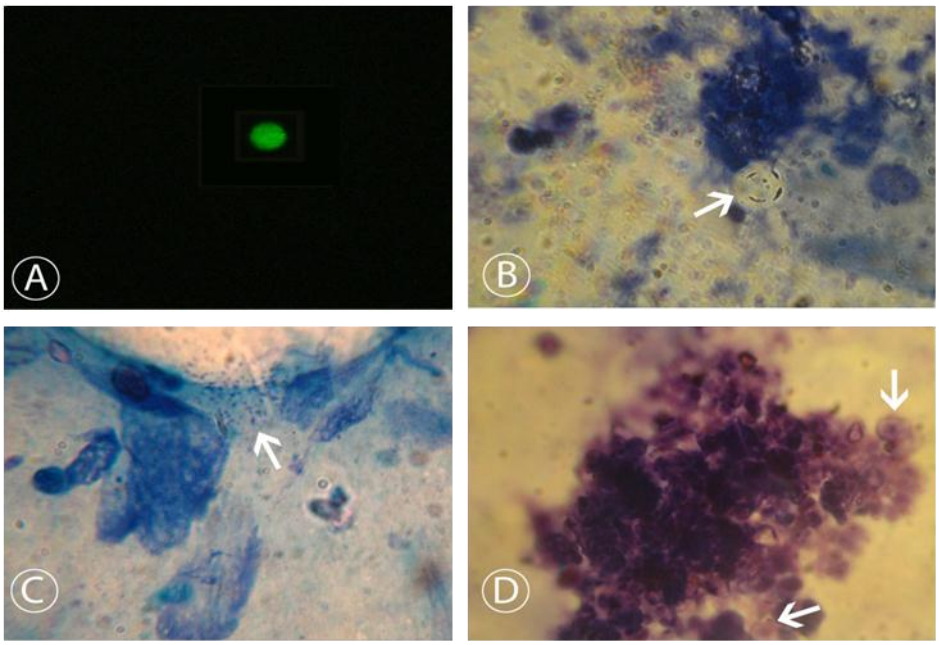

Fig. Sputum & bronchial wash showing *Pneumocystis carinii* (A) 20% KOH and calcofluor stain (spherical cyst; cyst wall and thickening intensely flurescent) 40X. (B) Giemsa stained smear (4 purple intracystic bodies -arrow) 100X. (C) Giemsa stained smear (small extracellular blue trophozoites - arrow) 100X. (D) Toludine blue stained smear (many spherical violet cysts - arrow) 100X.

The symptoms and signs of PCP are non-specific because the infection occurs in debilitated patients with other primary diseases [18]. However, the diagnosis of such cases was

difficult. The identification of the organisms during the study depended on the direct examination of each clinical specimen by different stains, because *P. carinii* cannot be cultured [19]. Previous studies reported that calcofluor white is a fungal cyst-wall stain [6]. Furthermore, toludine blue stain also allows diagnosis of *P. carinii* cysts [20], and trophic forms can be detected with Giemsa stain [6].

The isolation of concomitant bacteria and yeast from the sputa of most pneumocystic cases was also reported by other investigators [21]. Large amount of sputum was produced by patients who had *P. carinii* mixed with positive bacterial cultures. Such findings had been also reported by others [5,6].

In conclusion, *Pneumocystis carinii* was detected in immunocompromised patients with lower respiratory tract infections by direct microscopic examination of the clinical specimens with different staining methods.

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Author contributions

Dr. Manahil PhD student write the manuscript and Prof. Zainalabideen supervised the research work.

Declaration of interest

The authors declare no conflict of interest.

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In vitro study on using bacteriophages in the treatment of pathogenic *Escherichia coli* in Iraq

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Abstract

Background Bacteriophages, or phages, are virus-like agents that infect bacteria. Lytic phages can be used as biological antimicrobials and can kill bacteria.

Objective To formulate therapeutic phage cocktails able to overcome multiple-drug resistant *Escherichia coli* in Iraq.

Methods *Escherichia coli* were isolated from Iraqi hospitals and were characterized in terms of site of isolation, patient's age, sex, and disease. Antibiotics sensitivity test was used to evaluate antibiotics effectiveness. Accordingly, isolated bacteria were grouped in terms of resistance to antibiotics, infection type, and infection site. Wild phages specific to *Escherichia coli* were isolated from different areas. The isolated phages were optimized and their biokinetics were measured.

Results A total of 10 samples of *Escherichia coli*, 7 samples of them revealed specific phages. The formulated phage cocktail to *Escherichia coli* was shown to remarkably minimize the bacterial resistance to individual phages.

Conclusion Bacteriophage cocktails are useful to tackle the problem of MDR bacteria.

Keywords Multi-drug resistance, bacteriophage, phage therapy, *Escherichia coli.*

List of abbreviation: MDR = multiple drug-resistant, *E. coli* = *Escherichia coli*, BT = burst time, BS = burst size, IP = infective percentage

Introduction

Nowadays, disease-causing microbes that have become resistant to drug therapy are considered as an increasing public health problem and is one of the great challenges for modern healthcare [(1)].

Bacteriophages, or phages, are viruses whose hosts are bacterial cells. Like all viruses, phages are metabolically inert in their extra-cellular form, reproducing only after infecting suitable host bacteria. Discovered over 80 years ago, they have played a key role in the development of modern biotechnology. Their initial isolation appeared to offer the first therapeutic for the control of infectious disease [(2)].

The discovery of antibiotics in the 1940s eclipsed bacteriophage-based therapies. However, the increasing rate of emergence of multiple drug-resistant (MDR) bacteria led scientists and health bodes in the world to re-evaluate phages as the basis of new therapeutic strategies.Phage therapy is the use of phages as antimicrobial agents for the control of pathogenic and other problem bacteria [(3)].

The main problem of phage therapy is the rapid development of bacterial resistance which is much faster than that against chemical antimicrobials. However, the strength point in

phage therapy is that phages are endless source of new anti-pathogen phages[4].

Therefore, it has been conceived that formulating a cocktail of specific lytic bacteriophages against certain bacteria would mask the rapid development of bacterial resistance against attacking phages. Moreover, bacteriophage cocktail can be used very efficiently in combination withantibiotics. Since mechanisms of bacterial resistance against antibiotics are different from that against phages, combinational phage-antibiotics therapy can be the last resort of holding up the inevitable point when human beings reach the post-antibiotic era which is expected to reach 15 to 25 years from now[5].

The objectives of this study was to formulate therapeutic phage cocktails able to overcome multiple-drug resistant (MDR) *Escherichia coli* in Iraq.

Methods

Bacterial sampling:

Isolates of *Escherichia coli*(*E. coli*) were collectedfrom 10 samples(4 urine, 3 blood, 2 ear swab and one knee fluid).These samples were obtained from Al-Kadhimyia Teaching Hospital, Central Teaching Hospital for Children, and Al-Kindi Hospital in Baghdadduring the period from December 2012 to January 2013.

Bacteriophage sampling:

Different crude samples for phage isolation obtained from sheep faeces, cattle faeces, cattle manure, cattle farms, farms soil, sewage, mastitis discharge of human and cattle, burns, wounds, and hospital wards were collected from different regions in Baghdad

Isolation, preparation, and propagation of bacterial samples:

The bacterial samples were cultured by spreading on the nutrient agar;MacConkey agar and blood agar plates. Plates were incubated overnight at 37^{o}C. Cultures were then examined,refreshed, subcultured, and finally stored in 20% glycerol as backup samples.

Antibiotic susceptibility tests:

Antibiotic susceptibility tests were carried out on isolated and identified colonies of bacterial isolates using commercially prepared antibiotic sensitivity discs[6]. Antibiotics used were:amikacin (10 µg), ampicillin (10 µg), gentamcin (10 µg), vancomycin (30 µg), ciprofloxacin (10 µg), cefotaxime (10 µg), methicillin(30 µg), tetracycline (30 µg), ceftazdime (30 µg), rifampicin (5 µg), amoxicillin (25 µg), nitrofurantion (100 µg), cloxacillin (10 µg), clindamycin (2 µg), chloramphenicol(10 µg) and imipenem (10 µg).

Bacteriophages isolation:

Wild phages specific for *E. coli* can be isolated from different areas e.g. sewage. Phage-amplification assays were used by mixing culture broth of the *E. coli*with mixture of specimens in which specific phages were suspected. The mixture of E. *coli*broth and specimens were incubated at 37^{o}Covernight. Next day, if any phage was there, a phage amplification reaction was taken place giving rise to high titer of specific phages. Bacterial lawns were prepared to apply the phage-lawn assay as a screening test for detecting the presence of any specific lytic phages. Phages detected in bacterial lawns were picked up and isolated using the chloroform-shaking method. Isolated phages were kept in lambda buffer.Phage-based plaque assay were done to confirm the detected phages as well as to characterize phages in terms of plaque size, shape, opacity, edge, and depth, this assay was used to maximize the titer of the isolated phages.

Phages optimization and measurement of biokinetics of phages:

The plaque assay for phages, an optimization process, was used to apply phage passage in vitro for obtaining the best of the best plaque forming phages, i.e. pick up the best plaques in terms of size, clarity, depth.The biokinetics of the optimized lytic phages were measured using the novel patented single master tube biokineticassay. The biokinetic measurements are bursttime (BT), burst size (BS) and infective percentage (IP). Gradually build up a mixture of optimized (vertically bred) lytic phages specific

to a single target bacterial species, this mixture named as phage cocktail.Build up and increase the number of effective lytic phages in phage cocktails for *E.coli*in a way it was highly preferred that target bacteria is covered by more than one phage in order to minimize the development of resistance against attacking phages.

Results

Characteristics of *E. coli* samples:

A total of 10 samples of *E. coli*, 7 samples of them were enrolled in the present study because of revealing specific phages. The specimens, from which *E. coli* were isolated, were as follows: blood 2/7 (28.6%), knee fluid 1/7 (14.3%), urine 3/7 (42.9%) and ear swab 1/7 (14.3%).The patients' age ranged between one day to 80 years. This means that different age groups could be suitable source for *E. coli* isolates and good source for corresponding specific lytic phages. Sex ratio was females 4/7 (57.1%) and males 3/7 (42.9%).

Antibiotic susceptibility tests:

The results showed that different isolates of *E. coli* had different antibiotic profiles and these isolates were resistant to many important antibiotics; for example, all of the 7 isolates of *E. coli* were resistant to cefotaxime, rifampicin, amoxcillin and nitrofurantion. Moreover, the isolates were resistant to many antibiotics that have frequently been used in Iraq and as follows 1/7 (14.2%) amikacin, 6/7 (85.7%) ampicillin, 1/7 (14.2%) gentamicin, 2/7 (28.6%) imipenem, 2/7 (28.6%) ciprofloxacin, 7/7 (100%) cefotaxime, 6/7 (85.7%) tetracycline, 3/7(28.6%) ceftazdime, 6/7 (85.7%) ceftriaxone, 7/7 (100%) rifampicin, 7/7 (100%) amoxicillin, 7/7 (100%) nitrofurantion, 6/7 (85.6%) clindamycin and 4/7 (57.1%) chloramphenicol (Table 1).

Table 1. Antibiotic susceptibility test to phage-matched *Escherichia coli* bacteria.

Antibiotic	E1	E2	E3	E4	E5	E6	E7
Amikacin (AK)	*S	S	**R	S	***I	S	S
Ampicillin (AM)	R	R	R	R	R	R	I
Gentamicin (CN)	S	I	S	R	S	S	S
Imipenem (IPM)	S	R	I	S	S	R	S
Ciprofloxacin (CIP)	S	S	R	S	R	S	S
Cefotaxime (CTX)	R	R	R	R	R	R	R
Tetracyclin (TE)	R	R	R	R	R	R	S
Ceftazdime (CAZ)	S	S	S	R	R	S	R
Ceftriaxone (CRO)	R	R	R	S	R	R	R
Refampycin (RA)	R	R	R	R	R	R	R
Amoxcillin (AX)	R	R	R	R	R	R	R
Nitrofurantion (F)	R	R	R	R	R	R	R
Clindamycin (DA)	R	R	R	S	R	R	R
Chloramphenicol(C)	I	R	R	S	R	S	R

*S: Sensitive, **R: Resistant, ***I: Intermediate.

Characteristics of the isolated and optimized phages:

The nature of source specimen and plaque assay-based characteristics of the specific and lytic phages isolated and optimized to the 7isolates of *E. coli*. The characteristics of these phages are shown in terms of the type of phage specimen, size of plaques, shape of plaques, clarity of plaques, and plaques' margin cut as seen in table 2.The primary phages to *E. coli* were isolated as follows: the EP1-EP7 phages were isolated for E1-E7isolates, respectively. When these phages were optimized, each bacterial isolate was recognized by more than

the one primary phage; the newly recognizing phages were called secondary phages and as follows: the secondary phages to E1 were EP3, EP5 and EP7; the secondary phages to E2 were EP3 and EP6; the secondary phages to E3 were EP1 and EP6; the secondary phages to E4 were EP2 and EP7; the secondary phages to E5 were EP1, EP3 and EP4; the secondary phages to E6 were EP5 and EP7;the secondary phages to E7 were EP5 and EP6 (Table 3).

Table 2. The source specimen and plaque assay characteristics of the isolated phages to *E. coli*.

Bacteriophage name	Crude specimen of the phage	Plaques Size (mm)	Margin cut	Plaques clarity	Plaques shape
EP1	Chicken litter	1	Irregular	Semi –clear	Oval
EP2	Sewage	7	Regular	Clear	Circular
EP3	Sewage	4	Irregular	Clear	Oval
EP4	Sheep stool	5	Regular	Semi-clear	Circular
EP5	Sewage	3	Regular	Clear	Circular
EP6	Sewage	2	Regular	Semi-turbid	Oval
EP7	Sheep stool	0.8	Irregular	Turbid	Circular

Table 3. Plaque assay characteristics of the secondary phages to *Escherichia coli*.

Bacterial isolate	Secondary phage	Plaques Size (mm)	Margin cut	Plaques clarity	Plaques shape
E1	EP3	0.3	Irregular	Semi-turbid	Oval
	EP5	0.2	Irregular	Semi-turbid	oval
	EP7	0.5	Irregular	Turbid	oval
E2	EP3	2	Regular	Semi-clear	Circular
	EP6	0.9	Irregular	Turbid	Oval
E3	EP1	0.7	Irregular	Turbid	Oval
	EP6	0.6	Irregular	Turbid	Oval
E4	EP2	5	Irregular	Semi-turbid	Circular
	EP7	0.6	Irregular	Turbid	Circular
E5	EP1	0.5	Irregular	Semi-turbid	Oval
	EP3	3	Regular	Semi-clear	Circular
	EP4	2	Irregular	Semi-turbid	Oval
E6	EP5	1	Irregular	Turbid	Oval
	EP7	0.3	Irregular	Turbid	Oval
E7	EP5	1	Irregular	Semi-turbid	Oval
	EP6	0.6	Irregular	Turbid	Oval

The titers of the specific and lytic phages isolated and optimized to the studied bacterial isolates were measured by using top layer plaque assay; the highest titers of the isolated and optimized phages shown in table 4.

As demonstrated in fig. 1, the optimized highly lytic phages are shown to be able to lyse completely target bacteria in whatever manner of phages application.

Biokinetics of the isolated and optimized bacteriophages:

The mean BS of isolated phages to *E. coli* ranged between 103.4± 0.06 to 326± 10.92 phage progeny. The mean IP ranged between 64.6±

1.18 to 96.2± 0.18 %.The minimum BT was 30 min, while the maximum one was 35 min (Fig. 2).

Table 4. The titer of the specific and lytic phages to *Escherichia coli*.

Bacteriophage name	Titer (PFU/ml)
EP1	1.3×10^7
EP2	7×10^9
EP3	6×10^{11}
EP4	9×10^7
EP5	2.2×10^7
EP6	5×10^8
EP7	8×10^7

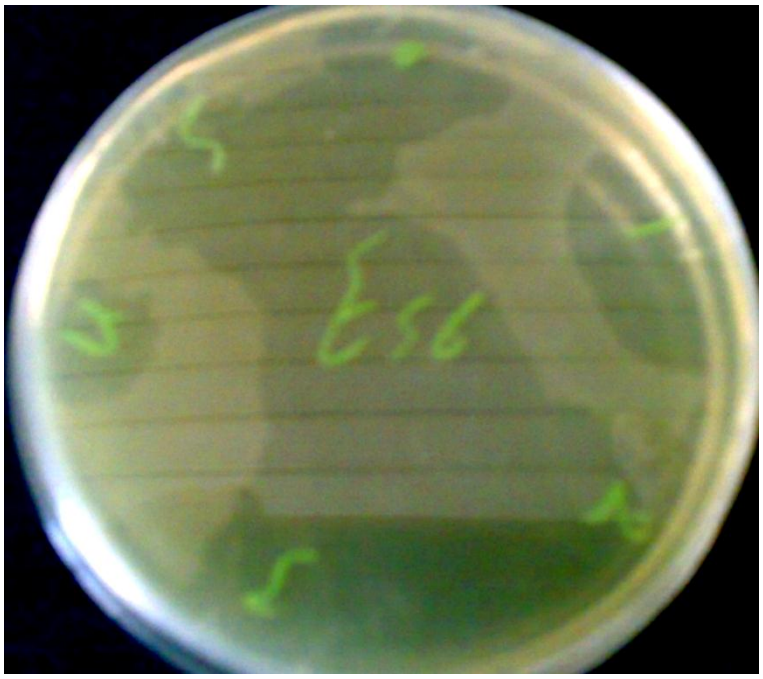

Fig. 1. Phage spot lysis plaque assay of bacteriopahge to *Echerichia coli*.

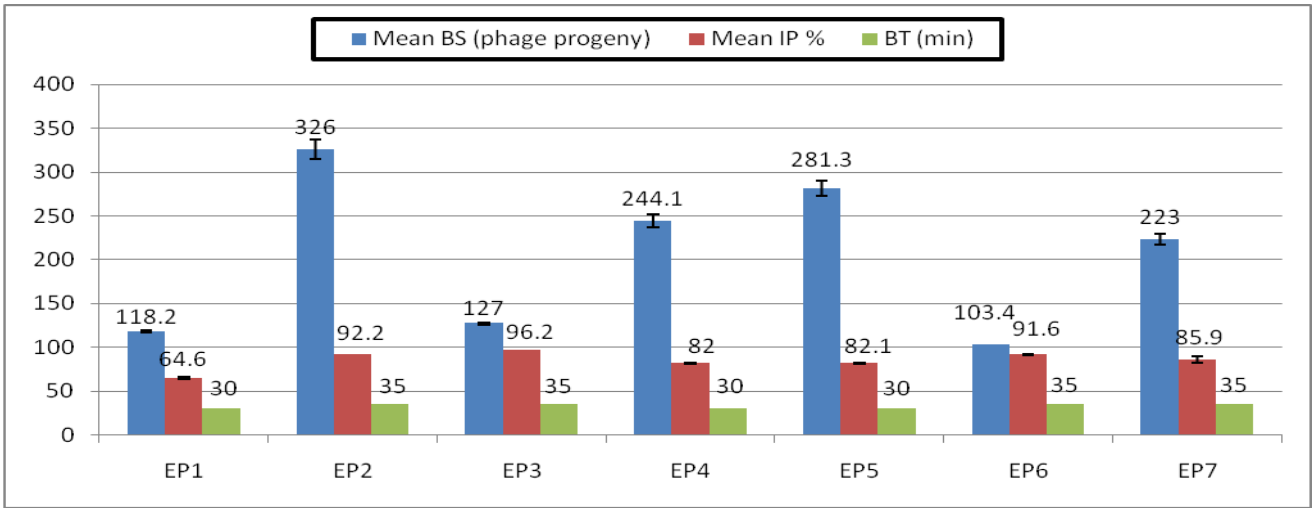

Fig. 2. Biokinetics, BS, BT, and IP%, of isolated phages to *Escherichia coli.*

Phage cocktails and challenge of developing bacterial resistance to phages:

The phage cocktails specific to each bacterial isolate were shown to remarkably minimize the bacterial resistance to individual phages. This finding solidifies our primary objective for using phage cocktails in phage therapy (Table 5).

Table 5. Resistance rate of *Escherichia coli* to phage cocktails versus individual phages.

	EP1	EP2	EP3	EP4	EP5	EP6	EP7	Cocktail
E1	$1:7.5\times10^5$	-	$1:1.5\times10^5$	-	$1:8.5\times10^5$	-	$1:1\times10^6$	$0:6\times10^8$
E2	-	$0:5\times10^7$	$1:5\times10^7$	-	-	$1:1.6\times10^7$	-	$0:1.3\times10^9$
E3	$1:2\times10^9$	-	$0:8\times10^9$	-	-	$1:8\times10^9$	-	$0:6\times10^{10}$
E4	-	$1:4\times10^5$	-	$1:2\times10^5$	-	-	$1:1.5\times10^5$	$0:7\times10^5$
E5	$1:6.6\times10^5$	-	$1:2\times10^6$	$1:7.5\times10^5$	$1:5.7\times10^6$	-	-	$0:4\times10^8$
E6	-	-	-	-	$1:8\times10^7$	$0:8\times10^7$	$1:2.6\times10^7$	$0:9.7\times10^8$
E7	-	-	-	-	$1:3.7\times10^6$	$1:1.8\times10^6$	$1:5\times10^6$	$0:8.6\times10^7$

Discussion

The emergence of antibiotic resistant strains constitutes a worsening global health problem; however, this problem is strikingly more obvious in Iraq. Globally, there are many reasons for this problem that can be explained by several hypotheses such as, chromosomal change or exchange of the genetic material by plasmids, antibiotic pressure- driven bacterial evolution, the dominant spread of mutant strains over sensitive strains, inappropriate use of antibiotics represented by the self-medication with wrong antibiotics, and the use of insufficient dosages, or subjected to unnecessary therapy. The current study revealed a remarkable MDR nature of *E. coli* isolated in Baghdad, Iraq (Table 1). Previous reports from Iraq and neiboring countries revealed similar MDR nature to what we have found in our study [7-10]. This necessitates serious work out of MDR problem in this region of the world as few studies focused on this problem are conducted in this region of the world. One of the best solutions can be taken is the use of bacteriophages as alternatives to progressively failing antibiotics.

In the current study, the phages to *Escherichia coli* were isolated from sewage, as like as other previously published studies [11-13], chicken litter [14], and sheep stool [15]. Sewage water was observed to be the best environmental source to get lytic phages with aggressive infective qualities; this might be attributed to the fact that phages from sewage tolerate hard conditions in the sewage; thus, these phages show high degree of lysis with high tolerance to harsh physical environment. Therefore, lytic phages from sewage showed larger plaques, higher titers, and clearer plaques than others.

Other sources that provided good lytic phages were chicken litterand sheep stool. The different sources of phages' isolation and the finding that each phage showed unique profile of size, shape, clarity, and margin cut of plaques provided preliminary evidence that isolated phages are unique and no phages are identical to each other.

The seven *E. coli* isolates, recognized by the primary phages at the beginning, were later recognized by more than one secondary phage. Some phages at the beginning showed very small and hazy plaques, but after serial top-layer plaque assay- based optimization, these phages showed enhanced infective characteristics to its primary host and moreover, the optimized phages showed positive infective capabilities towards bacterial hosts other than the primary host, called, secondary hosts and these phages are named secondary phages. When the bacterial isolate was recognized by more than one phage, it is a good indication to decrease or eliminate the bacterial resistance to phages; this

help confer greater chance to destroy the bacterial host.

The rate of 103-326 phage particles amplification per a cycle during 30-35min is much higher than the duplication time of bacterial hosts which is just 2 new daughter cells in approximately 25-30 min. Therefore, lytic phages showed dominant infective potential over bacterial cells. This gives a series of clues. First, lytic and specific phages to bacterial pathogens are able to eradicate bacteria in a short period of time. Second, lytic phages amplify specifically at the site of infections only while other parts of the body are spared. This is totally different from the kinetics of chemical antimicrobials where their concentration in the body is the same at both infected and non-infected sites. This criterion is considered as one of the main advantages of phages over using antibiotics and this makes therapeutic phages are largely safe to humans and animals. Third, the infection-oriented self-amplification of therapeutic phages allows administering only single dose rather than repeated doses of phages over a long course of time. Fourth, the self-amplification of therapeutic phages is followed by self-termination after eradicating the target bacterial pathogen.

An important notion from the results of the biokinetics is that each isolated phage showed unique set of biokinetic values. The results of the current study showed that each isolated phage has its own bacterial recognition profile which is different from other isolated phages. Besides, each bacterial isolate has its own set of attacking phages. This indicates that the bacterial isolates and attacking phages are all of different entities.

The successful phage cocktail must be composed of different lytic phages sharing no single receptor for bacterial recognition. Therefore, we hypothesized that each bacterial isolate which is recognized by more than one phage (several phages in addition to the primary phage) is attributed to that each bacterial isolate has more than one receptor and each receptor is specific for different phage to bind and infect. In addition, the resistance rate of bacterial isolates to the attacking phages was shown to be different to each other.

Accordingly, we picked up the resistant bacterial clone to each recognizing phage and tested its resistance rate to the other members of the phage cocktail which recognizes the same bacterial host. If the resistance was universal to all of the members of the phage cocktail, this means that phages, even though are different, share the same receptor of bacterial recognition which is not favored for the successful use of phage cocktails.

The current study showed remarkable findings that once bacterial isolate develops resistance to one member of phage cocktails, the isolate is still sensitive to other phages in the cocktail. One of the most haunting adverse effects of using phages in therapy is the rapid development of resistance by bacteria to infecting phages. The resistance rate to phages is much higher than that of antibiotics. Therefore, successful phage therapy needs to fix this weak point. Hence, this study succeeded in applying phage cocktails in reducing or eliminating the development of resistance to therapeutic phages. The current study compliments what our team achieved in a previous patented study outside Iraq where phage cocktails could minimize the development of bacterial resistance to phages [4,5]. In addition, many international studies have shown the same advantage of using phage cocktails [16-18].

In conclusion, phage cocktails are useful to tackle the problem of bacterial resistance to bacteriophages, and are good candidates for combating MDR bacteria whether used alone or in combination with antibiotics. More elaborated studies on phage cocktails are needed to find, formulate and design the proper phage cocktails to the endemic bacteria.

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Author Contribution

Marwa Bassim: conducted the sampling, phage isolation and in vitro and in vivo work. Ahmed Sahib designed the research, guided the protocols of bacteriophage related research, did the statistical analysis and finished writing and editing.

Conflict of Interest

We declare that there is no conflict of interest among authors of this study or with other authors or research teams.

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Protective Effects of N-acetylcysteine against 5-Fluorouracil-Induced Pulmonary Toxicity in Albino Rats

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Abstract

Background 5-fluorouracil (5-FU) is a potent chemotherapeutic drug widely used in the treatment of cancer and acts by blocking DNA synthesis. N-acetylcysteine (NAC) is a pharmaceutical drug and nutritional supplement represents the rapidly absorbed form of the amino acid L-cysteine and acts as antioxidant.

Objective The current study aims to investigate the protective role of N-acetylcysteine administration against 5-FU induced pulmonary toxicity in albino rats.

Methods The study was conducted on 18 healthy adult female and male Wistar albino rats which were randomly selected and equally distributed into three groups of 6 rats for each. Group I served as a control group. Group II received 5-FU (20 mg in 2ml normal saline per kg body weight) by intraperitoneal injection for 7 consecutive days. Group III received intraperitoneal injections of N-acetylcysteine (200 mg/kg) 24 hour prior to each intraperitoneal injections of 5-FU for 7 consecutive days. The specimens of lung tissue of the three groups were extracted and prepared for light microscopic examination. The tissue sections were stained with Harris Hematoxylin and Eosin (H&E) stain and with Masson's trichrome stain.

Results Structural changes were observed in Group II (5-FU recipient group compared to Group I (control group)including emphysematous dilatation of the alveoli, proliferation of BALT (bronchus associated lymphatic tissue), thickening of alveolar walls with mononuclear inflammatory cells infiltration, in addition to congestion and hemorrhage of pulmonary interstitium. Pretreatment with N-acetylcysteine effectively reduces the changes induced by 5-FUon the lung and reverts the abnormal pulmonary structure to become nearer to the norms.

Conclusion Treatment with N-acetylcysteine prior to 5-fluorouracil effectively attenuated 5-FU induced pulmonary damage and reverted the abnormal structural changes to near normal. Thus NAC has a protective potential in ameliorating 5-fluorouracil induced pulmonary toxicity.

Key words 5-Fluorouracil, N-acetylcysteine, rats, lung, emphysema.

List of abbreviation: 5-FU = 5-fluorouracil, TS = thymidylate synthase, ROS = reactive oxygen species, DPD = dihydropyrimidine dehydrogenase, NAC = N-acetylcysteine, LD_{50} = Lethal dose, BALT = bronchus associated lymphatic tissue, COAD = chronic obstructive airway disease.

Introduction

Chemotherapeutic drugs have been used worldwide for the treatment of a variety of human neoplasms given as a single or combined treatment protocol [1].

5-fluorouracil (5-FU) is pyrimidine analogue belongs to the family of antimetabolites. It is S-Phase specific drug which principally inhibits thymidylate synthase (TS) enzyme resulting in a decreased DNA synthesis. Moreover, it interferes with RNA processing and protein synthesis [2].

Cytotoxic effects of 5-FU may be exerted by generation of reactive oxygen species (ROS) resulting in apoptosis (programmed cell death)

or necrosis [3]. The metabolism of 5-fluorouracil occurs mainly in the liver and results in degradation products (e.g., carbon dioxide, urea, a-fluoro-*B* -alanine).It has a half-life of approximately 10 min, approximately 15-20% of the administered dose is excreted unchanged in the urine, the remaining 80-85% of the administered dose is excreted as carbon dioxide via expiration [4].

5-FU is degraded by the hepatic dihydropyrimidine dehydrogenase (DPD) which is the initial and rate limiting enzyme in 5-FU catabolism thus 5-FU.

Toxicity may be decreased if the catabolism is blocked by a genetic defect of DPD in the liver [5].

5-FU is used for the treatment of advanced colorectal cancer, breast cancer, carcinoma of the stomach, head and neck and pancreas [6], and topically (as a cream) for treating actinic keratoses and basal cell carcinoma [7] and in ophthalmic surgery [8].

Administration of 5-FU produces some adverse effects including stomatitis, mucositis and diarrhea in addition to leucopenia, hemolytic anemia and thrombocytopenia [9].

However, extensive investigations have been conducted on the toxicity of 5-FU including hepatotoxicity [10], cardiotoxicity and neurotoxicity of 5-FU [3,11] but there are limited information that concerned with the effects of 5-FU on the histology of the lung tissue.

5-FU causes excessive generation of ROS and induces a decrease in the antioxidant defense mechanism against oxidative damage resulting in cellular damage either as apoptosis (programmed cell death) or necrosis. Thus oxidative stress is an essential mechanism by which chemotherapy and radiotherapy work to kill cancer cells [12].

N-acetylcysteine (NAC) is the N-acetyl derivative of the amino acid L-cysteine. It exhibits direct antioxidant effect through its free sulphydryl (thiol) group which can reduce the free radicals [13].

In addition, NAC exerts an indirect antioxidant effect related to its role as a precursor of Glutathione which serves as an essential factor to overcome the harmful effects of internal and external toxic agents [14].

NAC is the drug of choice in acetaminophen overdose which is used frequently in self-poising [15]. Moreover, it is considered as mucus dissolving agent in the chronic obstructive pulmonary diseases such as bronchitis and cystic fibrosis [16].

NAC increases the resistance to influenza virus [17], it reduces the symptoms of schizophrenia, depression and bipolar disorder [18].

Additionally, NAC protects the body from toxic effects of alcohol and tobacco smoke. Recently, it has been used successfully to treat arsenic and mercury poisoning [19].

The aim of the present work is to evaluate the protective role of NAC against toxicity induced by 5-FU in the lungs of albino rats.

Methods

Eighteen adult healthy female and male Wistar albino rats of the same age group (2.5-3) months and weight (200-250 g) were obtained from the Animal House of the Experimental Research Unit, College of Medicine, and University of Mosul.

The animals were housed in a standard condition at a room temperature of about 25^{o}C and all animals were allowed for free access to laboratory pellet foods and tap water drink.

The experiment was conducted in the accordance of the ethical guidelines and internationally accepted principles for laboratory use and care in animal research. Lethal dose (LD_{50}), Pilot studies and related literature were taken into account and the accurate doses of 5-FU [20] and NAC [21] were calculated.

The body weight of each rat was recorded at the beginning of the experiment and recorded again at the end of the experiment just before killing of the animals.

The animals were randomly and equally divided into 3 groups of 6 animals each:

Group I: Each animal of this group was given2 ml/kg body weight/day of normal saline by

intraperitoneal injection for 7 consecutive days and served as a control group.

Group II: each animal of this group was given 5-FUin a dose of 20 mg in 2ml normal saline /kg /day by Intraperitoneal injection for 7 consecutive days.

Group III: first received NAC eats a dose of (200 mg/kg) by intraperitoneal injections and subsequently after 24 hour received 5-FU by intraperitoneal injection20 mg/kg/day for 7 consecutive days.

One day after the last injection of the three groups, all the animals were scarified and dissected under light ether to collect the two lungs from each animal, then the extracted lungs were fixed in 10% neutral buffered formalin solution for about 24 hours.

The histological sections were prepared according to Bancroft *et al* [22] in which small pieces of about 4-5 mm in thickness were cut from each lung and dehydrated in ascending grades of ethanol (70%, 90%, 100%). Clearing was done in xylene and embedded in paraffin wax. Serial ribbons of 4-7 sections of about (4-5) microns in thickness were collected from each paraffin block using Reichert's Rotatory Microtome.

The sections were spread in a hot water bath with 40-45^{o}C temperature then loaded on clean, labeled glass slides after making light scan of egg-albumin and put in oven at 60^{o}C for 30 minutes then left to dry at laboratory room temperature.

The sections were stained with Harris Haematoxylin and Eosin (H & E) stain and Masson's Trichrome stain according to Kim *et al* [23]. Then the stained sections were examined microscopically to detect any structural changes using (Olympus-BX51) light microscope using objective lenses X10, X40, X60 and eye piece lens X10. Micrographs from some sections were taken using Digital Camera (SONY-Cybershot 14.1 Mega Pixels) at X100, X400, X600 magnifications.

Morphometric Measurements

Morphometric estimation of the alveolar wall thickness was done by using a highly optimized microscope (Visopan projection microscope) at X400 magnification from 6 randomly chosen non-overlapping fields from the lung sections for each group then alveolar wall thickness was measured using perpendicular lines drawn across the section of the alveolar walls.

Statistical analysis

Statistical analysis for the animal's mean body weight and alveolar wall thickness was performed by SPSS version 20 for windows software. Data were presented as mean±SD and were analyzed using one-way Analysis of Variance followed by Bonferroni multiple comparisons for post-hoc analysis to compare the animals' mean body weight before and after injection of the drugs.

The cutoff point for statistical significance was set at 0.05. P value ≤ 0.05 were considered to be significant whereas P value > 0.05 were considered to be non significant.

Results

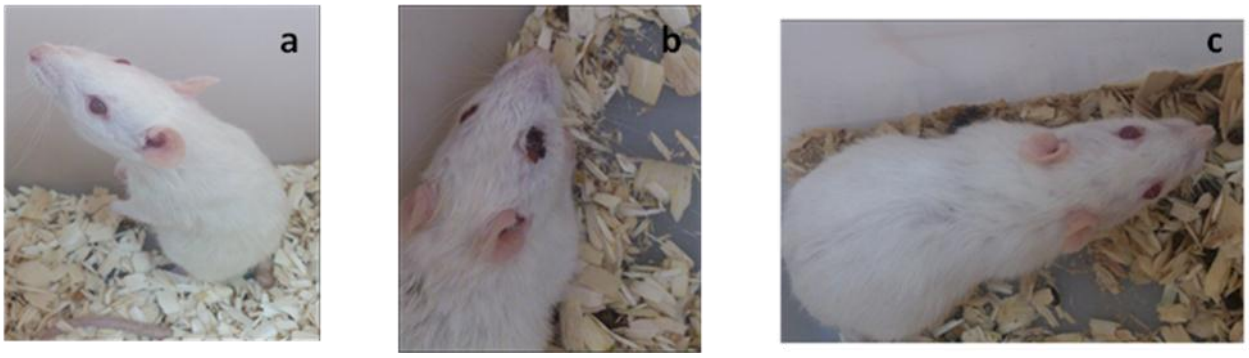
Physical Observations

The animals of the control group (group I) stayed alive till the end of the experiment. They were active, responded very quickly to stimuli, and they had a good appetite.

The sites of injections showed no swelling or inflammation **(Photo. 1a)** whereas the animals of group II became less active and gathered themselves at one corner of the cage on the 3rd day of the experiment and onward, their appetite was greatly reduced. Some rats had frequent diarrhea with ulcerations around the eyes and mouth and loss of furring of the skin which was recorded on the 7th day of the experiment **(Photo. 1b).** The animals of group III remained alert until the end of the experiment, their response to stimuli and food intake were normal **(Photo. 1c)**.

Histopathological results

(1) The lungs of the control group appeared pinkish, soft; with spongy like appearance, the left lung consisted of one large lobe while the right lung consisted of four lobes. The lung section of the control group showed:



Photograph showing normal appearance of rat from group I looks healthy and active (a); general appearance of rat from group II looks inactive with ulcerations around the eyes (b); general appearance of rat from group II showed return of activity (c).

1. Normal alveolar duct, alveolar spaces and terminal bronchioles with regular size and shape of the alveoli (Fig. 1).

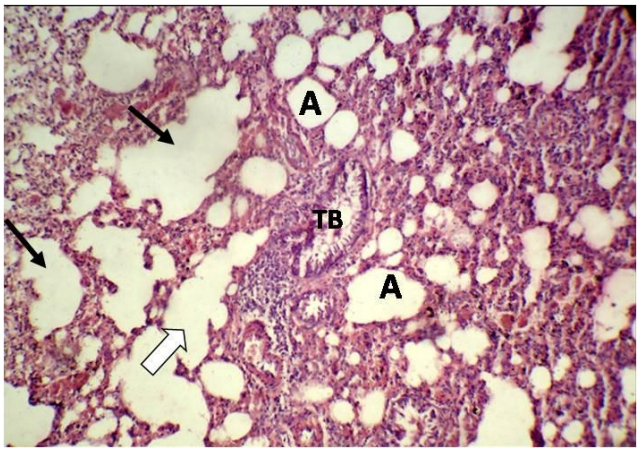


Fig. 1. Rat's lung of group I showing alveoli (A), alveolar duct (white arrow) alveolar sacs (black arrows) and terminal bronchiole (TB) (H&E X100).

2. Alveolar walls are of normal thickness and are lined by spindle shaped pneumocytes type I and rounded shaped pneumocytes type II (Fig. 2).
3. Few collagen fibers in the alveolar walls, in the wall of the pulmonary vessel and in the wall of the terminal bronchiole (Fig. 3).

(2) The lung tissue of group II showed focal areas of congestion and hemorrhage while the lung sections showed:

1. Abnormal alveolar spaces with destruction of the alveolar walls and fusion of some adjacent alveoli causing emphysematous dilatation with hyperplasia of bronchus associated lymphatic tissue (BALT) forming aggregates of lymphoid follicles (Fig. 4).

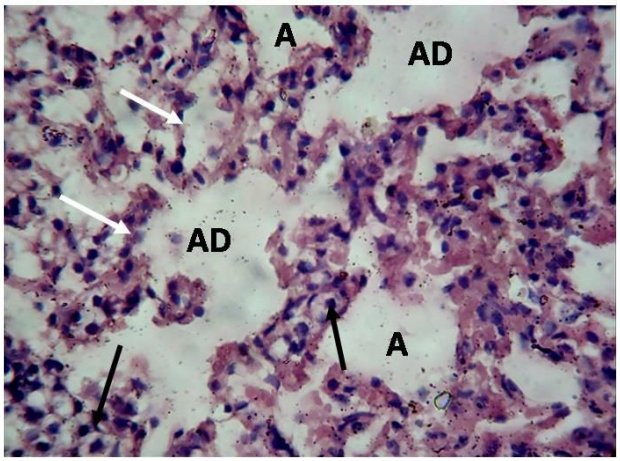


Fig. 2. Rat's lung of group I showing alveolar duct (AD), alveoli (A) with normal thickness of their wallslined by pneumocyte type I (white arrows) and pneumocyte type II (black arrows) (H&E X 400).

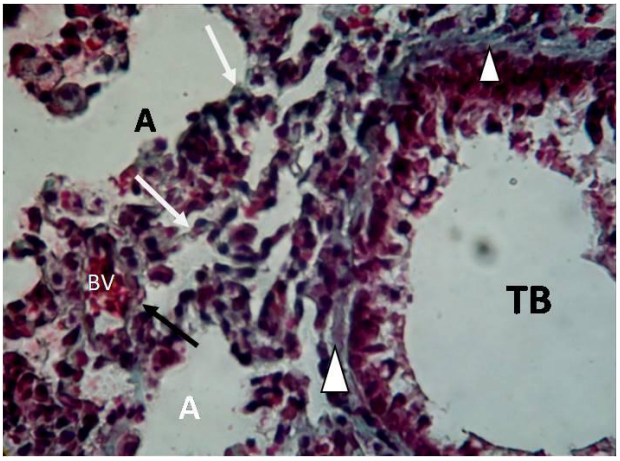


Fig. 3. Rat's lung of group I showing few collagen fibers stained with green color (white arrows) in the walls of the alveoli (A), in the wall of the blood vessel (BV)(black arrow) and in the wall of terminal bronchiole(TB)(arrow heads)(Masson's trichrome X 400).

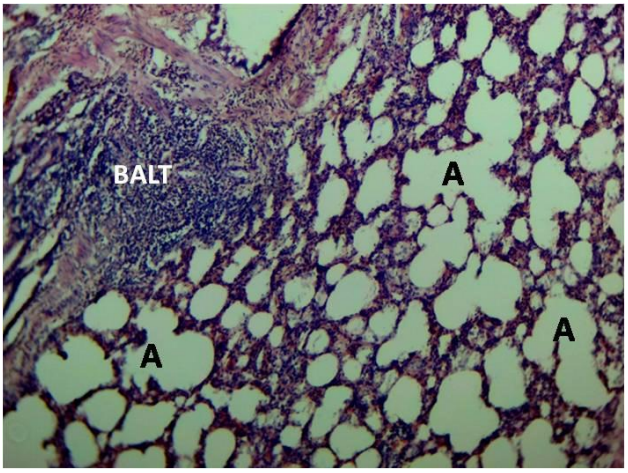


Fig. 4. Rat's lung of group II showing emphysematous dilatation of the alveoli (A) with hyperplasia of bronchus associated lymphatic tissue (BALT) (H&E X 100).

1. Less emphysematous dilatation of alveoli than that observed in group II with normal epithelial lining of the terminal bronchiole (Fig. 9).
2. Normal thickness of alveolar walls with no congestion of capillary bed (Fig. 10).
3. Thickening of alveolar walls with hemorrhage and congestion of the capillary bed and mononuclear inflammatory cells infiltration in the wall of the alveoli (Fig. 5).
4. Deposition of collagen fibers in the alveolar walls (interstitial fibrosis) (Fig.6).
5. Pulmonary vascular congestion and perivascular fibrosis with mononuclear inflammatory cells infiltration and adipocytes in the perivascular tissue (Fig. 7) and around the terminal bronchioles (Fig.8).

(3) The lung of group III appeared soft, pinkish, with few congested areas while the lung sections of group III showed:

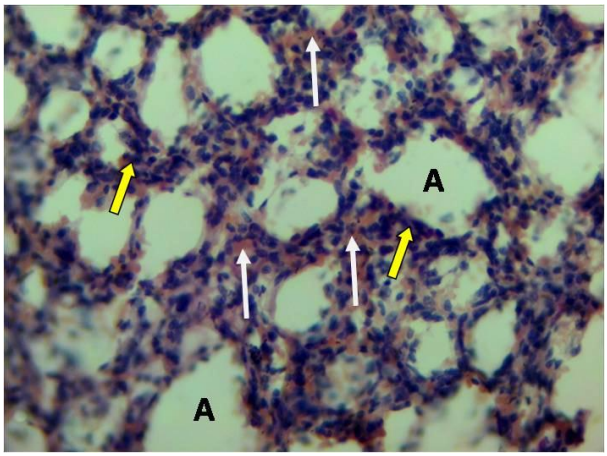


Fig. 5. Rat's lung of group II showing thickening in the walls of alveoli (A) with hemorrhage and congestion of the capillary bed (white arrows) and mononuclear inflammatory cells infiltration (yellow arrows) (H&E X 400).

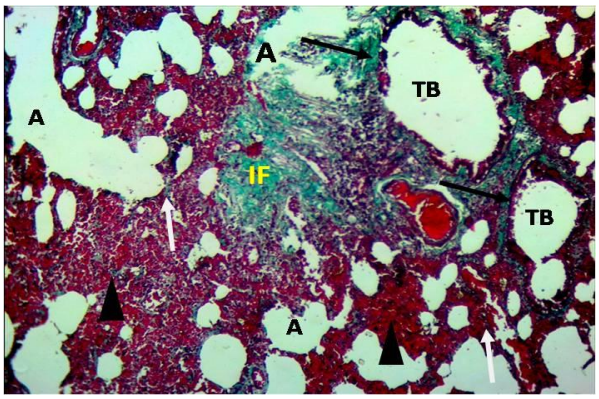


Fig. 6. Rat's lung of group II showing deposition of collagen fibers (interstitial fibrosis) (IF) between the alveoli (A) and around the terminal bronchioles (TB) (black arrows), thickening of alveolar walls with capillary congestion and fibrosis stained green (arrow heads) (Masson's trichrome X 100).

6. Few collagen fibers around the pulmonary vessels and around the terminal bronchiole (Fig.11) as well as in the alveolar walls (Fig.12).

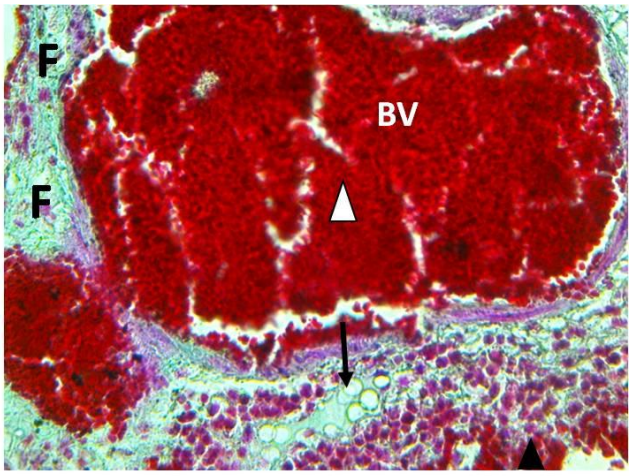


Fig. 7. Rat's lung of group II showing pulmonary vascular congestion (white arrow head) and perivascular fibrosis green in color (F) with mononuclear inflammatory cells infiltration (black arrow head) and adipocytes (black arrow) in the perivascular area (Masson's trichrome X 600).

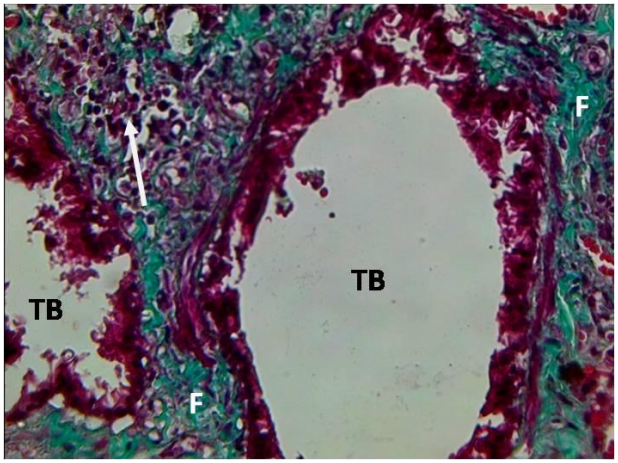


Fig. 8. Rat's lung of group II showing damage to the epithelial lining of the terminal bronchiole (TB) and fibrosis green in color (F) with mononuclear inflammatory cells infiltration (white arrow) around the terminal bronchioles (TB) (Masson's trichrome stain X 400).

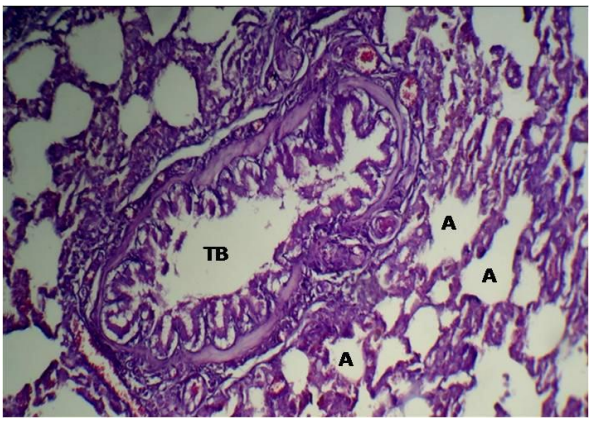


Fig. 9. Rat's lung of group III showing less emphysematous dilatation of the alveoli (A) with normal epithelial lining of the terminal bronchiole (TB) (H&E stain X 100).

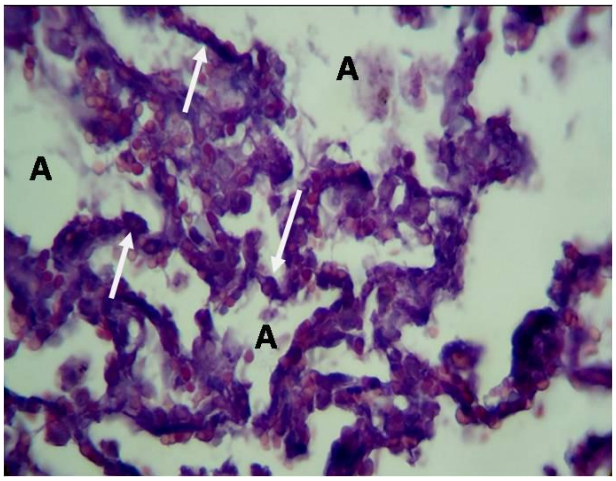


Fig. 10. Rat's lung of group III showing normal thickness of the alveolar walls (white arrows) between the alveoli (A) and mild emphysematous changes with no congestion of capillary bed (H&E stain X 400).

Body weight results

Results were expressed as mean±SD, very high significant reduction (P = 0.001) of the animals' mean body weight was observed in group II compared with the control group. Furthermore, group III showed no significant differences in the body weight compared to the control group (P = 0.1) but showed significant differences in their body weight compared to group II (P = 0.02) (Table 1).

Morphometric results

Results were expressed as mean±SD, very high significant increase (*P* = 0.001) in the alveolar wall thickness (14 ± 5.1) was observed in group II compared with the control group (4.3 ± 2.2).

Furthermore, group III showed no significant differences in the alveolar wall thickness (5.2 ± 2) compared to the control group (*P* = 0.2) but showed significant differences when compared with group II (*P* = 0.01) (Table 2).

Discussion

5-FU is a widely used chemotherapeutic drug which acts by blocking DNA synthesis. However, the clinical use of 5-FU is limited by its toxicity which interfere with its therapeutic efficacy [24] and several studies had been performed to prove the protective effects of certain agents against 5-FU induced toxicity [25]. However, there is very limited information on the protective role of NAC against 5-FU induced pulmonary toxicity.

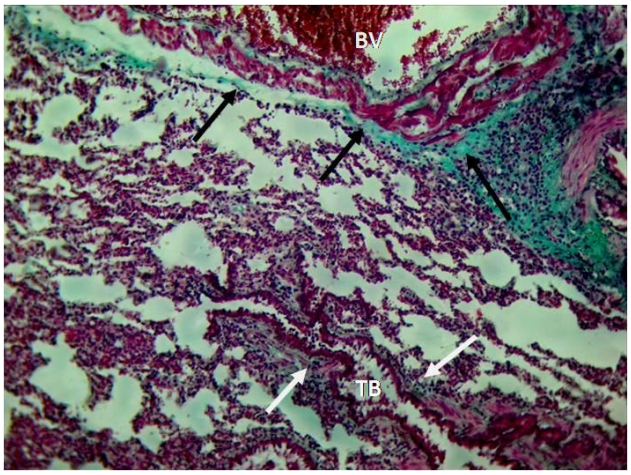


Fig. 11. Rat's lung of group III showing few collagen fibers around the pulmonary vessels green color (black arrows) and around the terminal bronchiole (TB) (white arrows)(Masson's trichrome stain X 100).

In the present study, the animals were injected by intraperitoneal 5-FU since this approach allows very high concentrations of 5-FU to deliver into the peritoneal cavity without increasing the risk of systemic toxicity [26]. Normal saline solution (0.9% sodium chloride) was used as a carrier for 5-FU in order to get a quicker absorption from the peritoneum and to achieve a higher level of toxicity [27].

The animals of group II showed very high significant reduction in their body weight .These results are in agreement with those reported by Cheah et al [28] who mentioned that 5-FU induced weight loss might be due to oral mucositis which is a painful condition associated with inflammation and ulceration affecting the mucosa of the mouth and causing a difficulty in eating and drinking and reduced food intake. The animals of group III showed no significant differences in their body weight compared to the control group. This observation was attributed to the ameliorative effect of NAC on the 5-FU induced oral mucositis.

In this study, the structural changes in the lung tissue induced by 5-FUinclude emphysema, mononuclear inflammatory cells infiltration and interstitial fibrosis are in agreement with that reported by Zidan [29] who stated that prolonged administration of amiodarone (an effective anti-arrhythmic drug indicated for cardiac arrhythmia) in rats may induce severe pulmonary changes such as.

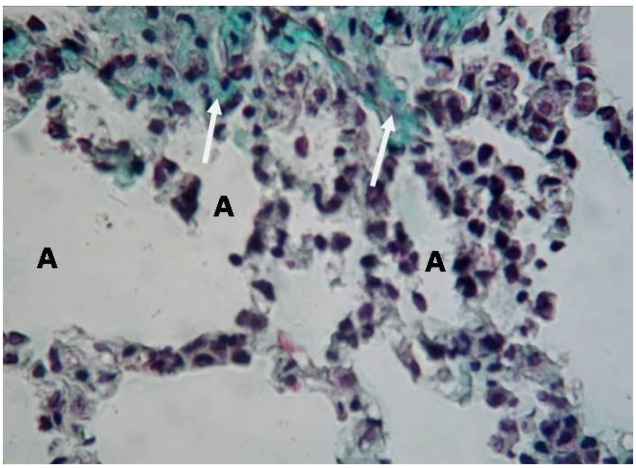


Fig. 12. Rat's lung of group III showing normal amount of collagen fibers (green color) (white arrows) in the alveolar walls (Masson's trichrome stain X 400).

Moreover, the results of the present work are nearly similar to what has been noted by Ahmed [30] who investigated the effects of Rifampicin and Isoniazide on the rat's lung tissue and found that combined rifampicin and isoniazid administration showed more intense emphysema and

inflammatory cells infiltration than when each drug was given separately.

The alveolar emphysematous changes observed in the rats treated with5-FU might be attributed to inadequate production of surfactant by pneumocytes type II due to direct cellular damage caused by the drug, thus most of the alveolar walls are destructed and the alveolar spaces are communicated with each other leading to emphysema.

Table 1. Body weight of the control group and experimental rats

Group	No.	Body weight	
		Before injection (Mean ± SD)	After injection (Mean ± SD)
Group I (control)	6	155 ± 16.43	158.5 ± 17.78**
Group II (5-FU)	6	160.5 ± 69.72	106.6 ± 39.39
Group III (5-FU+NAC)	6	185 ± 21.67	159.2 ± 28.17*

**: $P = 0.001$ (I versus II), $P = 0.1$ (I versus III), *: $P = 0.02$ (II versus III)

Similar changes were previously described by Ganesan *et al* [31] who referred these finding to the oxidative stress created by some oxidizing substances released from the alveolar macrophages and neutrophiles. In addition, some proteolytic enzymes such as metalloproteinases released by macrophages might cause alveolar collapse and emphysema thus alveolar macrophages play an important role in the pathogenesis of emphysema [32].

Table 2. Morphometric measurements of the alveolar wall thickness for the control group and experimental rats

Group	No.	Alveolar wall thickness (µm)
Group I (control)	6	4.3 ± 2.2**
Group II (5-FU)	6	14 ± 5.1
Group III (5-FU+NAC)	6	5.2 ± 2*

**: $P = 0.001$ (I versus II), $P = 0.2$ (I versus III), *: $P = 0.01$ (II versus III)

The presence of adipocytes in the perivascular tissue of pulmonary vessels in the present study was observed by previous workers [33,34] and may be explained by abnormal metabolism of phospholipids promoting accumulation of large quantities of adipocytes particularly in the perivascular areas.

This study showed thickening of alveolar walls due to congestion of capillaries, marked mononuclear inflammatory cell infiltration and edema.

Congested capillaries resulted from direct toxic effect of 5-FU on the wall of capillaries causing ischemia and necrosis followed by vasodilatation and escape of blood through their necrotic wall to the interalveolar septa and lumen of alveoli. This modifications in the vascular bed resulted in inflammatory cells infiltration and edema which is regarded as a defense mechanism against the toxic effects because the infiltrated cells assist in the rapid removal of tissue debris and red blood cells to facilitate regeneration [35]. The present finding agrees with previous studies which concluded that body defense reaction takes place against invading pathogenic bacteria or irritating agents due to the activity of alveolar macrophages which might release many mediators that augment the inflammatory response of the alveoli [36]. In addition, lymphoid hyperplasia of bronchus associated lymphatic tissue (BALT) might be provoked by some inflammatory chemotactic mediators released by the bronchial epithelium which stimulate lymphocytic proliferation around the terminal bronchioles. Similar activation of BALT had been previously observed in the rat's lung [37].

The pulmonary vascular congestion noticed in group II could be due to release of some vasodilator substances into the blood stream then the stagnant blood in the dilated vessels

will cause tissue hypoxia of the lung resulting in more pulmonary congestion. Similar finding had been noticed by [38] who observed congested pulmonary vessels in the rat's lung after exposure to aluminum chloride.

The lungs of group II rats showed interstitial fibrosis and deposition of collagen fibers in the perivascular and peribronchial area. This finding might be due to destruction of the lung tissue induced by the drug with subsequent inflammatory reaction thus, more fibroblasts might be brought to the irritated area leading to more collagen fiber deposition [39]. In addition, some previous studies revealed that in normal lung, pneumocytes type II are able to secrete prostaglandin E2, which acts to suppress fibroblast activity and proliferation and 5-FU might reduce prostaglandin E2 secretion which in turn leads to overproduction of fibroblasts with consequent interstitial fibrosis[40].

Danic *et al* [41] reported that one of the main events in the pathogenesis of broncho-pulmonary dysplasia and lung fibrosis following the administration of 5-FU is the formation of ROS. Pulmonary toxicity of 5-FU mediated by reactive oxygen species are nearly similar to the alterations observed in the chronic obstructive airway diseases such as desquamation of alveolar epithelium with increased vascular permeability, stimulation of mucous secretion, activation of fibroblast and mast cells with increased elastic and collagen fibers synthesis. All these changes can be attenuated by NAC which is a safe drug and easy to use in clinical practice [42].

The lung tissue of group III showed few congested areas with near normal appearance and this might reflect the improvement in the histological changes induced by 5-FU. Concomitant administration of NAC with 5-FU showed a considerable protection of the lung tissue thus the pulmonary architecture was preserved due to restoration of the oxidative imbalance by NAC directly by free radical scavenging and indirectly by glutathione synthesis thus, it increase pulmonary defense mechanisms. Supporting this finding, previous studies on a rat model revealed that administration of NAC together with cigarette smoke through the trachea might increase pulmonary glutathione, prevent thickening of the alveolar walls and improved phagocytic activity of the alveolar macrophages.

The lungs of group III rats showed less emphysematous dilatation of the alveoli with less fibrosis than that observed in group II and no congestion of the capillary beds. Such protective antioxidant role of NAC was previously demonstrated by Zhang *et al* [43] who reported that NAC can reduce ROS content, inhibit the mitochondrial apoptotic pathway and thus it can alleviate pulmonary fibrosis in rats exposed to intrapulmonary injection of silica suspension. Mononuclear inflammatory cells infiltration was alleviated by pretreatment with NAC due to reduction in the cytokins released from mast cells and alveolar macrophages [44].

Such protective antioxidant role of NAC was previously demonstrated in the liver tissue by Wang*et al* [45] who reported that acute liver damage induced by ethanol in mice was markedly alleviated by intraperitoneal injection of NAC. Additionally, Kilciksiz *et al* [46] noticed that the prophylactic use of NAC effectively reduce tissue damage caused by oxidative stress induced by radiation and give a clue about the probability of radioprotective effect of NAC.

In conclusion, the use of 5-FU for the treatment of some tumors seriously affects the structure of the lung causing emphysema and lung fibrosis. Using NAC before the injection of 5-FU protects the lung tissue against the toxic effects by increasing pulmonary defense mechanisms through its direct antioxidant action and its indirect role as a precursor in the glutathione synthesis. So that NAC may be considered as a useful dietary supplement for patients taking antineoplastic drugs like 5-FU.

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Author Contribution

The injection of the animals, histological preparation and staining of the sections, microscopical examination and photographing were done by Al-Hamdany. The reading, evaluation and writing of the article were done by Al-Hubaity.

Conflict of Interest

The authors declare of interest.

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Guided Percutaneous Drainage for Intra-abdominal Collections: The First Choice in Modern Surgical Practice.

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Abstract

Background Over the past 20 years, percutaneous drainage of intra-abdominal collections has evolved from revolutionary to routine, replacing open surgical abscess drainage in all but the most difficult or inaccessible cases.

Objective To evaluate the practical safety and efficacy of image-guided perctaneous drainage for different intra-abdominal collection.

Method Patients with intra-abdominal collections underwent percutaneous drainage under ultrasound guide were studied prospectively in Al-Imamian Al-Kadhymian Medical City over the period of 20 month from February 2011 to September 2012. The procedure done under local anesthesia and aseptic technique under ultrasound or CT scan guidance.

Results There were 50 patients (32 females and 18 males), Age ranging 12-58 years. Forty seven patients had a previous operative procedure and three patients had no such history. The post-operative collections in majority of patients 34 (72%) were single while in 13 patients (28%) were multiple. The type of content was as following: pus in 32 patients (64%), bile in 13 patients (26%), infected pancreatic/gastric secretions in 3 patients (6%) and infected urine in 2 patients (4%)., Percutaneous drainage was successful in 42 cases (84%), while 8 cases (16%) needed further surgical intervention to cure the collection. No major complications were recorded only minor complications like minor bleeding and wound infections. Mortality was in one case and it is not directly related to percutaneous drainage procedure rather to underlying problem and sepsis after second exploration.

Conclusion Guided percutaneous drainage is a safe and effective procedure for treating an intra-abdominal collections and it can be the first line treatment in severely ill patients.

Key Words Guided percutaneous drainage, abdominal collections.

List of abbreviation: GPD = Guided percutaneous drainage, PD = Percutaneous drainage, IAA = Intra-abdominal abscess, IAC = Intra-abdominal collections, US = Ultrasonography, CT = Computerized tomography (CT-scan).

Introduction

Intra-abdominal abscess (IAA), also known as intraperitoneal abscess, is an intra-abdominal collection of pus or infected material and is usually due to a localized infection inside the peritoneal cavity. It can involve any intra-abdominal organ or be located in between bowel loops. IAA is almost always secondary to a pre-existing or other disease process [(1)].

In more than 80% of cases, the collections derive from intra-abdominal organs and, in many cases, they develop after operative procedures. It is estimated that about 70% of cases are post-surgical [(2)]. The classic treatment of IAA and collections has been operative drainage. However, in recent years, improvement in ultrasonography (US) and computed tomography (CT) provides accurate noninvasive

recognition of fluid masses in the abdominal cavity and facilitates needle aspiration and catheter drainage. Since 1976, the authors have routinely used percutaneous drainage (PD) in the treatment of abscesses at this facility [3].

Percutaneous drainage is a successful modality in most cases for simple abscesses that are not associated with suspected malignancy or large anastomotic leaks. PD, if feasible, could be the first-line therapy and can be performed using guidance from either ultrasound or CT scan [4].

The aim of the study is to evaluate the practical safety and efficacy of GPD for different intra-abdominal collections.

Methods

The study is a prospective interventional one, done in the Department of General Surgery, Al-Imamain Al-kadhymain Medical City, Baghdad, in conjunction with department of radiology over a period of 20 months from February 2011 to September 2012. Patients with simple plus complex abscesses and collections as multiple, recurrent or secondary were included. Abscesses with obvious external fistula and tubo-ovarian and splenic abscesses were excluded. Patients who did not complete treatment or follow-up were also excluded. Ultrasound was done by a trained radiologist. Patients received the general treatment including appropriate antimicrobials as usual.

Technique:

The collection was precisely delineated and a safe route from skin to the cavity was identified by ultrasound prior to the catheter introduction, a diagnostic needle aspiration was done. The catheter was introduced into the abscess cavity, either directly using a trocar catheter (as used for chest intubation (Protex 10-I 6F)) or by modified Seldinger's technique using a guide-wire. The former was used when a direct route from skin to the abscess cavity was available and the latter when the abscess was deep with likelihood of inadvertent injury to the nearby viscera. Maneuvering of the trocar or guide-wire within the abdominal cavity was done strictly under ultrasound surveillance. Once in position, the catheter was secured and attached to a drainage bag. Drainage was recorded daily and the response to the treatment was assessed by clinical parameters and also by serial ultrasound. Normal saline irrigation of the cavity was used to enhance clearance of thick debris and prevent catheter blockage.

The procedure was considered successful if the patient was cured without the need for surgical drainage. After catheter removal, patients were followed up for three months. The results were compared with historical records.

Result

Fifty patient with intra-abdominal collections, 42 patients (84%) sustained percutaneous drainage procedure under ultrasonic guide and the remaining 8 patients (16%) did the procedure under CT scan guidance, there were 32 females (64 %) and 18 males (36%) , the age of the entire group ranging from 12- 58 years with a mean age of 36 year±2.

The collections diagnosed basically on US and/or CT scan. Ultrasound and CT-scanning was needed in 16 patients (32 %).

These intra-abdominal collections were 47 cases followed a previous operative procedure and 3 occurred spontaneously.

The post-operative cases were as follow:13 patients (28%) after cholecystectomy, 10(21%) for acute abdomen, 9(19%) for abdominal trauma, 5(10%) for gynecological problem, 3(6.3%) after colonic surgery, 2(4.2%) for Hydatid cyst, 2(4.2%), 2(4.2%)cases after renal surgery, 2 patients (4.2%) followed gastro-duodenal surgery and one (2.1%) after whipple operation (Table 1).

Three pt had spontaneous abscesses (6% from total number): one infected pseudocyst of pancreas, one liver abscess and last one was psoas abscess. The post-operative collections in majority of pt 34 (72%) were single while in 13 patients (28%) were multiple (like pelvic and subhepatic) for whom multiple catheters were used for their drainage. The sites of a single and multiple collections are shown in table 2 and 3.

Table 1. Distribution of post-operative collections according to primary operation.

Primary operation	No.	%
Cholecystectomy	13	28
Surgery for acute abdomen	10	21
Surgery for abdominal trauma	9	19
Gynecological surgery	5	10
Colonic surgery	3	6.3
Liver hydatid cyst surgery	2	4.2
Gastro-doudenal surgery	2	4.2
Renal surgery	2	4.2
Whipple operation	1	2.1
Total	47	100

IAA associated with underlying fistulae are called complex abscesses, in our study 15 pt (30%) had a complex abscesses, the fistulae and their number were as the followings (biliary = 10, intestinal= 3, pancreatic= 1, duodenal =1, urinary= 1). complex abscesses represented 77% of multiple site abscesses (10 patients).

Table 2. Different locations of single intra-abdominal collections.

The site of collection	No.	%
Pelvic	14	41
Rt. subhepatic	13	38
Rt. subphrenic	2	5.8
Lt. subhepatic	2	5.8
paracolic	1	2.9
Retroperitoneal (renal bed)	2	5.8
Total	34	100

Table 3. Different locations of multiple intra-abdominal collections

The site of collection	No.	%
Pelvic/ Rt. subhepatic	6	46
Rt. Subphrenic/ subhepatic	3	23
Rt.Subhepatic/Lt.subhepatic	2	15.5
Lt.subhepatic/Lt. paracolic	2	15.5
Total	13	100

The amount of material drained after the initial catheter placement was 25-1800 ml. nearly all patients showed signs of improvement after initial catheter placement. The fever subsided within a few days. Irrigation and drainage was continued for an average length of 10 days (range 4-35 days).

Table 4. Types of collection

Type of content	No.	%
Pus	32	64
bile	13	26
Infected pancr./gastr. secretions	3	6
Infected urine	2	4
Total	50	100

Thirteen (26%) patients needed repeated percutaneous drainage (re-insertion of drainage catheters) due to continuous leak in 8 of them (the leak was bile due to complicated cholecystectomy, intestinal secretions in patient with whipple surgery) or due to thick pus that needed larger catheter gage for drainage in 3 patients and last 2 patients were due improper placement of catheter. Out of 13, six patients (12%) underwent surgical interventions to tackle the primary pathology (those patients not cure from PD and conservative treatment and surgical corrections were necessary to deal with underlying intestinal, biliary and urinary fistulae) and one of them unfortunately died after second surgery due to sepsis and multi-organ dysfunction.

Table 5. End result and complications of percutaneous drainage procedure.

Result		infected	Non-infected	Total
No. of collection		35 (70)	15 (30)	50 (100)
Successful drainage		31 (62)	11 (22)	42 (84)
Complications	Major	0	0	0
	Minor	4	3	7

Number between brackets represents the percentage

In general GPD was successful in 42 (84%) cases, while 8 (16%) cases needed further surgical intervention to cure the collection. For simple abscesses PD were 100% successful, while in complex type it was successful in only 8 (50%) patients.

The type of content was as following: pus in 32 (64%) patients, bile in 13 (26%) patients, infected pancreatic/gastric secretions in 3 (6%) patients and infected urine in 2 (4%) patients (Table 4).

No major complications were recorded only minor complications like minor bleeding and wound infections as stated in table 5. Mortality was in one case and it is not directly related to percutaneous drainage procedure rather to underlying problem and sepsis after second exploration.

Discussion

In recent years the indications for percutaneous methods has expanded significantly. The results of percutaneous procedures have been so good and so widely accepted that the indications and applications have continued to expand. Ultrasound-guided and computed tomography-guided puncture or catheter drainage is an easy, gentle, and relatively atraumatic procedure with few complications. General anesthesia is unnecessary. The patients are mobile immediately, and the risk of pulmonary infections and thromboembolism is minimized [5,6].

CT and US are excellent at identifying potential abscess areas [2,7], in current study 84% of cases achieved under US guidance as it is available, cheap and simple and can be portable and the CT guidance reserved for difficult cases in whom localization and accessibility of collections were awkward under US as in some cases of subhepatic, subphrenic and pelvic collections, in current study 8 (16%) patients needed CT guidance, CT after ultrasound has fast emerged to provide radiological guidance more for its specificity than its sensitivity. Haggas and Weinstein [8] prefer CT over ultrasound. Gerzof et al [9] consider CT and ultrasound complementary rather than competitive, the former better for localization of abscess and route planning (as bowel gas or bone does not hamper it) and the latter for catheter placement (as imaging and sector plane flexibility are achieved simultaneously). In one of our pt where ultrasound was unclear, CT helped in outlining the abscess and ultrasound was again resorted for introduction of catheter.

We conclude from our series that most common cause of intra-abdominal collection is an iatrogenic reasons, I mean post-operative as in 47 (94%) patients and this is in agreement with Talib et al [10] and other series [11,12].

We considered percutaneous drainage successful if the pt was cured without undergoing surgery. With these criteria, our overall success rate was 84%, and failed in 16% as 8 patients needed surgical exploration to tackle with underlying pathology and it is nearly similar to other series like Mueller et al [13], Lameris et al [14] and Haage et al [15].

The results are quite satisfactory and approaching 100% in simple than in complex abscesses (50%). The latter is understandable as conventional open surgical drainage also shows such difference, indicating interplay of other variables in addition to the drainage technique in the outcome i.e., the natural underlying pathology (fistulae) render clearance of abscess cavity difficult or impossible. It also explains why earlier series [3,8,9] on percutaneous drainage showed better results as these included only simple abscesses and had excluded complex one. In fact the initial success in percutaneous drainage of simple abscess encouraged its use in complex abscesses, like multiple and multilocular abscesses[16], infected pseudocysts [9,17,18], splenic abscess [16,19,20] and abscesses with fistula [9,21]. Thus compared with the initial 40%, up to 90% abdominal abscesses can now be subjected to percutaneous drainage [22].

Percutaneous abscess drainage can help stabilize critically ill patients by reducing the systemic toxic impact and perhaps, improving the outcome of necessary surgical procedures. Second, it can improve patient management by

changing a 2-step surgical procedure into a 1-step procedure [15]. Percutaneous collection drainage is now a commonly used staging method for the resolution of intra-abdominal sepsis prior to corrective operation [23], So that the successful treatment of abscesses with percutaneous drainage either obviated surgery altogether or facilitated surgery by providing a clean operative field [24].

Therefore, we consider percutaneous drainage worth trying as even if it fails to prevent surgical intervention, it can be a useful temporizing measure [25]. Further in moribund pt, percutaneous drainage would be the only option available[18].

We had catheter related problems in some patients; as a narrow caliber catheter is used, its blockade is common. To avoid this many workers recommend routine saline irrigation of the catheter and it may be the secret of success in most of cases as it not only flush the catheter, but also enhance dissolution of necrotic tissue and dilute thickened collection thereby enhancing drainage and hasten the collapse of cavity.

Complications noted in our series were mostly minor. Major complications reported are bowel and vascular injury [12,26] in addition to recurrent and secondary abscesses. Bowel injury may go unrecognized at the time of procedure to appear later as enterocutaneous fistula. It often closes spontaneously[16]. Vascular injury can lead to visceral hematoma or bleeding in the parieties. Serious bowel and vascular injury can be avoided by proper technique and careful planning prior diagnostic needle aspiration is an additional safe guard [8]. Bowel or vascular injuries were notably absent in our series.

In conclusion, GPD is an efficient and safe procedure for treating IAA and IAC as a definite or temporizing method; the results are very good in patients with simple abscesses and fair in those with complex abscesses; the outcome of GPD is comparable to that of conventional open drainage and has the merit of simplicity and feasibility to be performed under local anesthesia and with very minimal trauma.

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Author contribution

The author is responsible for preparing for all steps of this case report.

Declaration of interest

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Using Atorvastatin and L-Carnitine in Prevention of Pilocarpine-Induced Seizures: Animal Model Study

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Abstract

Background Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures.

Objective To investigate the possible antiepileptic effect of both atorvastatin and L-carnitine on seizure induced by pilocarpine.

Methods Fifty male albino mice weighing between 30-35 gm were equally allocated into five groups (each group contained 10 mice) and were given one of the following; control group; distal water group (0.1 ml), diazepam group (1mg/kg), atorvastatin group (5 mg/kg) and L-carnitine group (300 mg/kg). All animals (except normal group) were injected with Pilocarpine hydrochloride (350 mg/kg) to induce generalized tonic-clonic seizures 30 minutes after the tested drugs had been administrated. The mean onset of seizure were determined as well as the mean serum concentration of electrolytes, glutathione (GSH) and malondialdehyde (MDA) were measured after seizure had been induced.

Results Pilocarpine induced seizure at approximately 7 minutes after injection, while both atorvastatin and L-carnitine produced highly significant increase in mean onset of seizure 14 ± 0.471 and 14.5 ± 0.909 respectively as compared to that of D.W. group, also both drugs produced highly significant changes in mean serum concentration of electrolytes, GSH and MDA.

Conclusion Atorvastatin and L-carnitine had antiepileptic effects against seizures induced by pilocarpine when used at applied doses.

Key words Epileptic seizure, Atorvastatin and L-carnitine.

List of Abbreviation: eNOS = Endothelial nitric oxide synthase, FTI = Franeyltransferase inhibitor, GABA = Gamma-aminobutyric acid, GSH = Reduced glutathione, GTPase = Guanosine triphosphatase, HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A, H-Ras = Harvey rat sarcoma viral oncogene homolog, I.P = Intraperitoneal, MDA, Malondialdehyde, NMDA = N-methyl-D-aspartate, NO = Nitric oxide, PTZ = Pentylenetetrazole, RhoA = Ras homolog gene family, member A, SEM = Standard error of mean.

Introduction

Epilepsy is one of the major neurological diseases in humans and about one percent of the population is affected by some forms of epilepsy [(1)]. It is characterized by recurrent, unprovoked, paroxysmal episodes of brain dysfunction manifesting as a large number of clinical phenomena [(2)].

Epilepsy occurs due to many different cellular or biochemical changes such as alterations in ion channel function, neurotransmitter level (excitatory and inhibitory), neurotransmitter receptor function and energy metabolism, in addition to the body electrolytes, level of some trace elements, and membrane lipid peroxidation due to increase in free radicals or decrease in activities of antioxidant defense mechanisms all these may be causally involved in some forms of epilepsy and may increase the recurrence of seizures [(3)].

Pilocarpine provides a useful animal model for studying epilepsy. In this model, the seizures induced by pilocarpine show the involvement of the cholinergic system in seizures and status epilepticus [4]. The activation of muscarinic receptors is the first step for seizure activity, while GABAergic and glutamatergic systems appear to mediate seizure propagation and/or maintenance in rodent epilepsy models [5].

Atorvastatin is antihyperlipidemic agent that belongs to statins group and inhibits the first committed enzymatic step of cholesterol synthesis, in addition to its antioxidant activity [6]. While L-carnitine is a quaternary ammonium compound, it is biosynthesized from the amino acids lysine and methionine in both liver and kidney [7]. In living cells, it is required for the transport of free long-chain fatty acids from the cytosol into the mitochondria during the breakdown of lipids, in addition it is has antioxidant effect [8]. These effects of both drugs are useful in terminating epilepsy.

The current study was performed to investigate the possible antiepileptic effects of both atorvastatin and L-carnitine against seizures induced by pilocarpine.

Methods

This study was performed on fifty healthy male albino mice weighing between 30-35 gm, they were supplied by animal house of Al-Nahrain College of Medicine and were housed under good conditions and fed standard oxoid palate with water ad libitum, and they were equally allocated into five groups (10 mice in each group):

- ***Group 1: (Normal group):*** This group served as normal control and takes no drug used to detect the normal values of serum electrolytes, GSH and MDA.
- ***Group 2: (Distilled water group):*** They were injected 0.1 ml of distilled water (I.P.) 30 mint. before pilocarpine injection, to induce epileptic seizures.
- ***Group 3: (Diazepam group):*** They were injected 1mg/kg of diazepam (I.P.) 30 mint. before pilocarpine injection. This group served as positive control and was used to compare onset of seizure only with tested groups.
- ***Group 4: (Atorvastatin group):*** They were injected atorvastatin 5 mg/kg (I.P.) 30 mint. before pilocarpine injection.
- ***Group 5: (L-Carnitine group):*** They were injected L-carnitine 300 mg/kg (I.P.) 30 mint. before pilocarpine injection.

After giving the pilocarpine, each mouse was carefully evaluated by detecting the following parameters, which include the onset of the first seizure recorded by naked eyes. At the end of observations the blood samples were collected from survival mice for measuring other parameters which are the serum concentrations Na^+, K^+ ,Ca^{2+}, GSH and MDA.

Statistical analysis was performed with the SPSS 19.0 statistical package for social sciences, data were expressed as mean ± Standard error of mean (SEM), unpaired t-test at ($P \leq 0.01$) and ($P \leq 0.05$) for independent data was used [9].

Results

All the mice exhibited generalized limbic seizures after pilocarpine administration, at a latency of (7 ± 0.394) minutes; besides, it caused highly significant reduction in mean serum Na^+, Ca^{2+} and GSH concentration, while it caused highly significant increase in mean serum concentration of K^+ and MDA when compared to that of normal group.

Diazepam caused highly significant increase in mean onset of seizure; also diazepam had no effect on other parameters when compared with D.W group.

Both atorvastatin and L-carnitine resulted in highly significant increase and highly significant reduction in mean onset of seizure when compared to that of both D.W. and diazepam groups respectively, also both drugs resulted in a highly significant increase in mean serum concentration of Na^+, Ca^{2+} and GSH with highly significant reduction and non-significant change in mean serum concentration of MDA and K^+ respectively when compared to that of D.W group (Table 1).

Table 1. Effect of Atorvastatin and L-Carnitine on Onset of Seizure and Serum Na^+, K^+, Ca^{2+}, GSH and MDA Concentrations in Group I, II, and III in Pilocarpine-induced Seizure in Mice

Group	Seizure Onset (minute) M ± SEM	Concentration				
		Sodium mmol/l M ± SEM	Potassium mmol/l M ± SEM	Calcium mg/dl M ± SEM	GSH mmol/l M ± SEM	MDA mmol/l M ± SEM
I	0	144.8 ± 2.958	5.47 ± 0.212	8.5 ± 0.306	1.13 ± 0.063	5.02 ± 0.103
II	7 ± 0.394	133.2 ± 1.781a**	8.11 ± 0.502a**	7.03 ± 0.157a**	0.8 ± 0.052a**	10.66 ± 0.265a**
III	18.6 ± 0.858b**	134.5 ± 1.45	7.78 ± 0.54	7.35 ± 0.34	0.85 ± 0.05	10.5 ± 0.28
IV	14 ± 0.471b**,c**	157.8 ± 2.439a**,b**	7.9 ± 0.436a**	9.37 ± 0.379b**	1.5 ± 0.158a*, b**	7.52 ± 0.442a**, b**
V	14.5 ± 0.909b**,c**	148.3 ± 3.72b**	7.71 ± 0.281 a**	11.32 ± 0.198a**, b**	1.2 ± 0.076b**	5.09 ± 0.211b**

* P = < ≤0.05, ** P = ≤0.01, a = as compared to group I, b= as compared to group II, c= as compared to group III, n = (10/group)

Discussion

Epilepsy is one of the most common neurological problems all over the world, being associated with paroxysmal discharge of cerebral neurons and is characterized by several symptoms including alterations of behaviors and consciousness sustained alteration in brain function [10].

Statins are competitive HMG-CoA reductase inhibitors, the later are the rate limiting enzyme for synthesis of cholesterol and isoprenoids. Indeed, the inhibitory role of statins in cholesterol biosynthesis previously reported to inhibit glutamate receptor (NMDA) function in induction of status epilepticus and excitotoxicity [11,12], this provide that statins possess NMDA antagonist-like effects, which would partially explain the anti-seizure and neuroprotective activity [13].

Statins are also expected to exert their anti-seizure and anti-excitotoxic activities through inhibition of isoprenoid synthesis and interfering with small GTPase signaling. Indeed, recent reports have demonstrated that inhibition of H-Ras farnesylation by treatment with franeyltransferase inhibitor (FTI) can inhibit NMDA-mediated excitotoxicity in the rat brain [14]. These reports support the suggestion that statins may modulate Kainic acid mediated seizure activity and excitotoxicity by down-regulation of H-Ras isoprenylation.

The neuroprotective efficacies of statins also mediated by their anti-inflammatory activity through inhibition of isoprenylation of small GTPase (Ras and RhoA) [15]. A similar inhibitory role of statins in inflammatory reactions was observed in Kainic acid treated rats.

In addition statins act as anticonvulsant substance by upregulation eNOS, may be pivotal in enhancing cerebral arterial vasodilator responses and decreasing the firing threshold [16].

The neuroprotective and anticonvulsive actions of L-carnitine can be mediated by a reduction in lipid peroxidation levels and nitrite content levels. This reduction is possibly due to the modulatory activity of L-carnitine in the antioxidant enzymes (superoxide dismutase and catalase) in the hippocampus of adult rats [8].

The neuroprotective effect of acetyl-L-carnitine may be due to at least three modes of action. First, acetyl-L-carnitine has been shown to maintain cellular membrane stability, it can also act as an antioxidant, scavenging harmful superoxide radicals. Second, preserve normal levels of nerve growth factor in brain tissue during aging. Third, acetyl-L-carnitine increases cerebral blood flow [17].

L-carnitine has anticonvulsive effects through the suppression of c-fos gene expression in the brain of mice after single administration of PTZ which play an important role in the development of seizures [18,19].

L-carnitine has neuroprotictive and antioxidant effect by preventing glutamate neurotoxicity and neuronal death mediated by activation of NMDA receptor, L-carnitine decrease the affinity of glutamate for the NMDA receptor [20], L-carnitine also prevent the formation of NO radical due to over activation of NMDA receptor,

since NO reduces the activity of antioxidant enzymes, leading to increased formation of superoxides and oxidative stress [21]. In addition L-carnitine elevates nigral levels of glutathione and GABA which plays an important role in it is anticonvulsant effect [22].

All the tested drugs had valid preventive effect against seizure induced by pilocarpine in mice when compared to that of diazepam.

Finally the aim of present study to find a new drugs or combination of drugs which are more potent with less adverse effects to be used in epilepsy after confirmation with clinical trials.

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Author contribution

The authors designed the experiments, interpreted the results and drafted the manuscript. Uday did the technique of this work and conducted the writing of manuscript. Dr. Faruk supervised scientifically reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Relationship between the Expression of CD34, CD123 and Myeloperoxidase Markers by Flow Cytometry and Response to Induction Therapy in Acute Myeloid Leukemia

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Abstract

Background Different immunophenotypic markers were found to be related to the prognosis of acute myeloid leukemia. Among them are CD34, CD123 and myeloperoxidase.

Objective To evaluate the relationship between the expression of CD34, CD123 and myeloperoxidase markers by flow cytometry; and the initial response to induction therapy in acute myeloid leukemia patients.

Method A cohort of forty one patients with newly diagnosed *de novo* acute myeloid leukemia were prospectively tested for the expression of CD34, CD123 and myeloperoxidase using multicolor flow cytometry and re-evaluated for the response to a 7+3 induction therapy regimen.

Results It was found that 64.29% of $CD123^-$ patients achieved complete remission while 70.37% of $CD123^+$ patients not ($P = 0.035$). For CD34, 55.56% of $CD34^-$ patients achieved complete remission while 63.64% of $CD34^+$ cases not. The induction failure in $CD34^+$M3 cases was 100% ($P = 0.045$). Regarding myeloperoxidase, 61.54% of patients who had >20% myeloperoxidase expression achieved complete remission while 70.37% (myeloperoxidase expression in <20% of cells) failed to achieve complete remission ($P = 0.05$).

Conclusion Expression of CD34 and CD123 and weak expression of myeloperoxidase (<20% of blast cells) are associated with poor response to induction therapy in acute myeloid leukemia patients.

Keywords Flow cytometry, CD34, CD123, myeloperoxidase, acute myeloid leukemia

List of Abbreviation: AML = Acute myeloid leukemia, HSCs = hematopoietic stem cells, MPO = Myeloperoxidase, PMNs = polymorphonuclear cells, ALL = acute lymphoblastic leukemia, SBB = Sudan Black B, WBC = white blood cell count.

Introduction

Acute myeloid leukemia (AML) is a clonal, malignant disease of hematopoietic tissues that is characterized by accumulation of leukemic blast cells, principally in the marrow, that impair the production of normal blood cells leading to neutropenia, anemia and thrombocytopenia [(1)]. Iraq is among the world countries with both high incidence and low survival rate of AML [(2-4)].

The clinical outcome of acute myeloid leukemia (AML) is extremely variable, ranging from survival of a few days to cure. Different clinical and biological features at diagnosis have been reported as useful for the prediction of clinical outcome [(5)].

CD34 is a member of the CD34 family of cell-surface transmembrane proteins [(6)]. It's expressed on the most immature hemopoietic stem cells. It may play a role in the attachment of stem cells to the bone marrow extracellular matrix or to stromal cells [(7)]. In addition, it has a signal transducing capacity, causing actin

polymerization [8] and it's widely used for the identification and isolation of hematopoietic stem cells (HSCs) and progenitors [9].

High expression of CD34 has been linked to poor prognosis in acute myeloid leukemia patients [10].

Interleukin-3 receptor subunit alpha or CD123 is a single-pass type I membrane protein which belongs to the type I cytokine receptor family and type 5 subfamily. CD123 is strongly expressed in various leukemic blasts and leukemic stem cells although it is not expressed by normal hemopoietic stem cells.

CD123 seems to be an excellent target for the therapy of leukemia [11,12]. Its ligand is interleukin-3 which is a multipotent cytokine that promotes the development of hemopoietic progenitors into cells of the erythroid, myeloid and lymphoid lineages [12], and induces blast cells formation [13].

Myeloperoxidase (MPO) is a heme-containing peroxidase highly expressed by polymorphonuclear neutrophils. Its major function is generation of hypochlorous acid as part of the neutrophil's antimicrobial armory [14].

It is unique to neutrophils and monocytes. However, monocytes contain only one third of the MPO found in polymorphonuclear cells (PMNs) [14].

The demonstration of peroxidase in at least 3% by cytochemistry or 10% by flow cytometry of bone marrow blasts defines an acute leukemia as AML [15].

Anti-MPO antibodies had shown consistent negative results in acute lymphoblastic leukemia (ALL) cases [15, 16].

Anti-MPO has an important role in distinguishing minimally differentiated AML (M0) and biphenotypic acute leukemia from acute unclassified leukemia (AUL) and ALL even when CD13 and CD33 are negative [17].

The objective of the current study is to clarify the relation between the expression of CD34, CD123 and MPO measured by multiparametric flow cytometry and the response to induction therapy in acute myeloid leukemia in Iraqi patients.

Methods

This prospective cohort study was conducted on 41 patients older than 15 years with newly diagnosed *de novo* AML. Patients were taken from the National Center of Hematology and Baghdad Teaching Hospital, between February and July 2013. This research was approved by the Ethical Committee at the College of Medicine, Al-Nahrain University. Signed informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

Patients suspected to have acute myeloid leukemia were subjected to further investigations. About 2.5 ml of peripheral blood and about 0.5 ml of bone marrow aspirate was collected in K2-EDTA tube from each patient. The hematological parameters were obtained by automated hematology analyzer (CELLDYN Ruby). Peripheral blood and bone marrow films were stained with Leishman and Sudan Black B (SBB) stains. After morphological diagnosis of AML in the hematology laboratory of the national center of hematology and the teaching laboratories in the medical city, samples were sent for flow cytometry.

After the morphological diagnosis was established in the hematology laboratory, the specimen was transferred within 24 hours to a private laboratory where the flow cytometric markers specific to this study were tested.

Multi-color immunophenotyping by Partec Cyflow Cube 6 flow cytometer and interpretation of markers by FACS Express 4 software was done at the private lab. Anti-CD45 and Anti-CD34 reagents from Partec Company; and anti-CD123 and anti-MPO from BD Company were used. Staining for the surface anti-CD123, anti-CD45 and anti-CD34 was accomplished in one tube, while staining for the cytoplasmic anti-MPO was done in a separate tube.

Blasts and leukemia cells were gated according to the expression of CD45 and side scatter. Cell population with low side scatter and negative or dim expression of CD45 were regarded as the blast cells or leukemia cells. From this population of cells, the expression of CD123 and

CD34 was calculated in comparison to the isocontrol. On the other hand; the gating for MPO was done according to the physical properties (forward scatter FSC/side scatter SSC) in comparison with the normal lymphocytes in the same blood or bone marrow sample (internal control) [18-20].

Patients received 7+3 regimen containing cytarabine, given continuously for seven days through an intravenous line. Daunorubicin, was given in a single IV dose for the first three days of treatment. Patients with French American British (FAB) M3 received, in addition to the drugs mentioned, All-transretinoic acid 45 mg/m^2 divided into two doses and given orally. Older patients (> 60 years old) received cytarabine at 100 mg/m^2 for 5 days [21].

All patients were followed up for assessment of response to the first course of induction therapy. The same procedure of peripheral blood and bone marrow collection and processing mentioned above was done after approximately 4 weeks after the start of therapy.

Patients were classified into complete remission (Bone marrow blasts < 5%; absence of blasts with Auer rods; absolute neutrophil count > 1.0 X10^9/L; and platelet count > 100 X10^9/L), resistant disease (persistent leukemia by blood and/or bone marrow examination) and death during induction according to the Cheson criteria of response [21]. For statistical purposes, the last two groups were gathered into one group, the non-remission group.

All statistical operations were done by Microsoft excel 2010 and SPSS programs. The *P* value for significance was calculated from Chi square test and Fisher Exact test for 2X2 contingency tables when the Chi square test was not applicable.

A 20% cut-off value was put to indicate the positivity of surface markers CD34 and CD123; while the cut-off value for the cytoplasmic marker myeloperoxidase was 10%. A cut-off value (20%) for MPO was assessed for prognostic significance [20].

Results

Forty one patients were enrolled in the study; the mean age was 38.7±17.23 years (mean±SD), the range was 16-78 years. Highest incidence was reported at the age group 15-24 years while lowest at > 65 years. The mean WBC count at presentation was 51.3 X10^9/l, and ranged from 1.1-230 (X10^9/L). Seven patients (17.07%) were leukopenic, four patients (9.75%) had normal WBC count, while the majority of cases (30 patients, 73.17%) had leukocytosis (table 1 and figure 1).

FAB M3 formed the major portion of AML subtypes (26.83%), followed by M2 and M5 (19.51% for each) then M1 and M4 (17.07% for each). M0, M6 and M7 subtypes were not represented in the current study (Table 1).Because of a technical error; one sample was not tested for MPO marker. Thirty six of 40 patients who were tested for MPO showed positive results (90%). All the other four negative cases were M5 subtype. Regarding CD34 marker, 33 cases (80.49%) were positive; while CD123 was positive in 27 patients (65.85%) as shown in table 2.

The peak of CD34 expression was detected in M4 subtype (100%), while its expression was poor in M3 subtype (45.45 %). CD123 was mostly expressed in M1 (85.72 %) and least expressed in M3 (54.54%) subtypes.

At the end of induction therapy; twelve patients (29.26%) died during the first 4 weeks of induction therapy, twelve patients (29.26%) did not achieve the morphological criteria of complete remission, and seventeen patients (41%) achieved complete remission by examination of their bone marrow after about 4 weeks of treatment.

Out of the 27 patients who were CD123$^+$, only 8 (29.63%) achieved complete remission, in contrast to the rest 19 (70.37%). On the other hand, 9 (64.28%) of the 14 patients who were CD123- achieved CR, while the rest 5 (35.72%) failed to achieve response (Table 3 and fig. 1).

Table 1. Distribution of AML patients according to gender, WBC count and FAB subtype

Variable		Number (%)	Range	Mean±SD
Gender	Male Female	24 (58.54) 17 (41.46)	N/A	N/A
WBC count ($X10^9$/l)	< 4 4-11 > 11	7 (17.07) 4 (9.76) 30 (73.17)	1.1-230	51.3±55.5
Hemoglobin (g/dl)	Low* Normal	40 (97.56) 1 (2.44)	3.48-12.7	8.48±2.19
Platelet count ($X10^9$/l)	Low** Normal	39 (95.12) 2 (4.88)	12-396 $X10^9$/l	63.07±74.03
FAB Subtype	M0 M1 M2 M3 M4 M5 M6 M7	0 (0) 7 (17.07) 8 (19.51) 11 (26.83) 7 (17.07) 8 (19.51) 0 (0) 0 (0)	N/A	N/A

*Low hemoglobin if less than 13 g/dl in men and 12 g/dl in women, **Low Platelet if less than 150$X10^9$/l (men and women)

Table 2 Flow cytometric expression of the studied markers

Parameter	+ve	%	-ve	%	Median	Mean±SD	Total
CD123	27	65.85	14	34.15	27.59	35.31±22.62	41
CD34	33	80.49	8	19.51	29.26	29.46±17.99	41
MPO	36	90	4	10	18.07	22.37±17.88	40

Thirty two patients (80.49%) were CD34+, of them 12 (39.39%) achieved complete remission and 20 (60.61%) did not respond to induction therapy. On the other side 9 patients (19.51) were CD34-; 5 (55.56%) of them achieved complete remission, while 4 (44.44%) did not (Table 3).

Table 3. The Remission status of patients distributed according to the flow cytometric expression of markers

Maker	Remission		Total	*P* value
	Complete No (%)	Not No (%)		
$CD123^+$	8 (29.63%)	19 (70.37%)	27	0.035
$CD123^-$	9 (64.29%)	5 (35.71%)	14	
$CD34^+$	12 (36.36)	20 (63.64)	33	0.27
$CD34^-$	5 (55.56)	4 (44.44)	9	
MPO <20%	8 (29.63%)	19 (70.37%)	27	0.057
MPO ≥20%	8 (61.54%)	5 (38.64%)	13	

Regarding myeloperoxidase; a 20% cut-off value was used to separate two prognostic groups. 7 (58.33%) of 12 patients who had MPO expression of more than 20% achieved complete remission and 5 (41.67) did not achieve complete remission, while 10 (34.48%) of patients who expressed MPO in less than 20% of cases achieved complete remission and 19 (65.52%) did not.

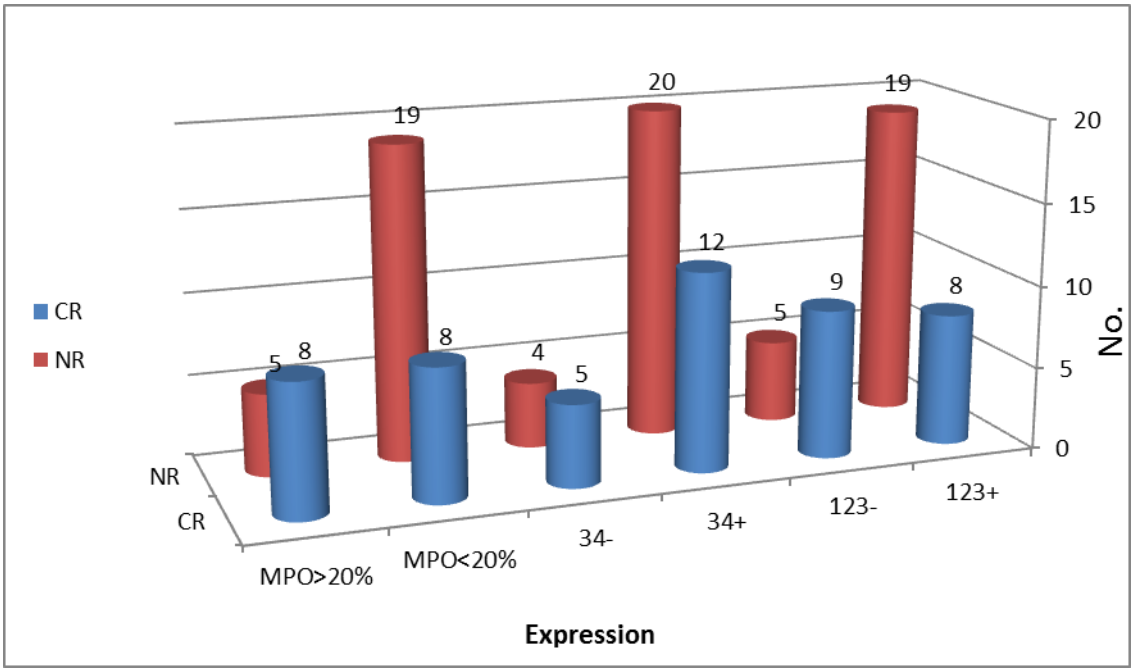

Fig. 1. Remission status of the studied patients according to the expression profile (MPO=myeloperoxidase, CR=complete remission, NR=Non-remission).

Discussion

The mean age of AML patients was 38.73 years and the peak incidence of AML was in the age group 15-24 years, while the least incidence was found in the age group >64 years. Those findings were comparable with the results of survey made by Hussein *et al* [4], Al-Husseiny [22] and Dhahir *et al* [23]. The mean age of patients was markedly lower than that observed in other developed countries where the majority of cases were above 55 years of age [24]. This may be attributed to sample size.

The male to female ratio was 1:1.41 which was higher than the average found by previous Iraqis studies [4,23].

The mean WBC count, hemoglobin (Hb) concentration and platelet count were comparable to previous Iraqi studies. Leukocytosis (> 11X 10^9/L) was found in 73% of AML patients which is more than that reported by Al-Husseiny [22] whereas leukopenia and normal leukocyte count on presentation were less observed findings than in the other studies [4,23].

Regarding FAB subtyping, the findings of the current study were comparable to previous Iraqi studies. FAB M3 was the most common subtype, which was the same reported by most local previous studies. However, Dhahir *et al* [23] and Alwan *et al* [27] reported that M3 was the third most common factor. This variation may be attributed to differences in sample sizes.

The percentage of $CD34^+$ cases was comparable to that found by Oyan *et al* in 2005 [25], but more than that found by Petrovici *et al* [18] in 2010, whereas CD123 expression was less expressed compared to the study of Muñoz *et al* [26] in 2001. The overall positivity of MPO in the current study was comparable to that found by Buccheri *et al* [16] and Leong *et al* [27].

In the present study, the rate of induction failure was 58.53% which was high compared to

previous studies from Iraq [28]. A significant relationship between expression of CD123 and induction failure was found. This finding is comparable to that found by Testa [29] in 2002 who stated that elevated expression of CD123 (IL-3Rα) in AML is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. CD123 was related to the leukemic nature of stem cells and experiments showed that $CD123^+$ cells were competent to establish and maintain leukemic populations *in vivo* [28].

The rate of induction failure in $CD34^+$ group was higher than that observed in $CD34^-$ group. This finding is comparable to the results of previous studies [19,31] and proves that CD34 expression predicts for poor prognosis in AML patients.

The results of this study were comparable with the results of Matsuo *et al* [20] who stated that the percentage of myeloperoxidase-positive blast cells is a strong independent prognostic factor in acute myeloid leukemia, even in the patients with normal karyotype.

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Author Contribution

Research proposal, analysis of results by statistics, collection of samples, patient interview, sample analysis, patients follow up and final printout of article were done by Dr. Faez and the co-author assistant professor Dr. Subh S. Al-Mudallal.

Conflict of Interest

There's no financial or personal relationship with other people that could inappropriately influence this work.

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Surgical Outcome of 65 Cases of Congenital Esophageal Atresia with Tracheoesophageal Fistula: Experience of 5 Years in Two Institutes

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Abstract

Background Congenital esophageal atresia and tracheoesophageal fistula are well-known congenital anomalies which affect 1 in 2400 – 4500 live births. The survival rate has dramatically improved in the last decades due to the advances in the neonatal intensive care, anesthetic management, ventilatory support and the surgical techniques.

Objective To evaluate the surgical outcomes and postoperative complications in patient with esophageal atresia and tracheoesophageal fistula who were admitted to our hospital.

Methods A retrospective study of 65 cases of esophageal atresia with distal tracheoesophageal fistula was studied over a period of 5 years from January 2008 to January 2013. Patients demographic, frequency of associated anomalies, postoperative complications and surgical outcomes were studied.

Results There were 38 male and 27 female with frequency of forty-seven (72%) infants were full term and 18 (28%) were preterm. Survival rate was (66%) for newborn who underwent surgery within the first 24 hours which was higher than those newborn with age above two days. Congenital heart disease was the commonest associated congenital anomalies and accounted for 28% of all our patients. Our patients developed many complications including respiratory tract infection (26%) and anastomatic leak (20%) as the most common complications.

Conclusion The survival rate of the patients with EA/TEF is influenced mainly by associated life -threatening congenital anomalies, prematurity of newborn and the age at the time of surgical repair.

Keywords Esophageal atresia, tracheoesophageal fistula, congenital anomalies.

List of abbreviations: EA = Esophageal atresia, TEF = TracheoEsophageal fisula

Introduction

Esophageal atresia (EA) with distal fistula is the most common subtype of EA, accounting for about 85% of this anomaly. The much-dilated proximal esophagus has a thickened wall and descends into the superior mediastanium. The distal esophagus is slender and has a thin wall. It enters the trachea posteriorly either at the level the level of the carina or 1 to 2 cm above. The survival of infants born with EA, tracheoesophageal fistula (TEF), or both has improved dramatically since Cameron Haight's first successful repair in 1941 [(1,2)]. Since then, the management of EA and TEF has evolved considerably over the years. At present, an overall survival rate of 85% -90% and survival rate of over 95% in those without major anomalies have been reported from developed countries. This is not the same in developing countries, where many preoperative, postoperative and socioeconomic factors continue to contribute to the persisting high mortality [(3)].

The incidence of the various forms of EA in the general population is approximately 1 in 4000 live births. Although nearly all esophageal atresia variants seem to be sporadic, familial esophageal atresia has been reported. The incidence of recognizable congenital defects associate with EA is about 55%. The presence of cardiac malformation is particularly important and is often the major determinant of mortality [3,4].

The infant with EA is unable to swallow, drools saliva and spits up indigestive formula. As liquid pooling in the blind proximal esophageal pouch spills into the airway, the infant may cough or choke.

The diagnosis of EA can be made by chest radiography after placement of a soft nasogastric tube as far as possible in the esophagus. The chest radiograph shows the tube coiled in the upper mediatanium and the presence of gas filled intestinal loops establishes the presence of distal TEF [4,5]. The ideal management of AE With TEF is division of fistula and primary esophageal repair performed in single operation during the newborn period of life. This approach is successful in most patients born with EA and distal TEF, today. The premature infants with significant respiratory distress syndrome or newborn with associated congenital anomalies, specifically cardiac lesions, for whom it is difficult to provide effective support with mechanical ventilation, may not tolerate the lung retraction or operative time necessary for complete repair during single setting. Early surgical repair is done for those babies with adequate arterial blood gases, adequate weight and no significant associated anomalies and delayed repair (gastrostomy first) is used for all other patients.

The objectives of this study was to evaluate the surgical outcomes and postoperative complications in patient with esophageal atresia and tracheoesophageal fistula who were admitted to our hospital.

Methods

This is a retrospective study of 65 EA with distal TEF cases divided into 38 boys (58%) and 27 girls (41%) who were admitted to the pediatric surgery center at Central Teaching Hospital of Pediatrics and Al-Kadhymia Pediatric Hospital (Baghdad) over a period of 5 years from January 2008 to January 2013. Their medical records were reviewed for the age, sex, maturity, birth weight, associated congenital anomalies, operative technique, morbidity and mortality.

The diagnosis was depending on clinical presentation (frothy secretions due to excessive salivation, tachypnea, cyanosis …) and on plain x-ray of the chest with insertion of 10 Fr radio-opaque rubber catheters to the upper blind esophageal pouch. The diagnosis of the associated congenital anomalies was made on the basis of echocardiography (for congenital heart diseases), abdominal ultrasound for renal anomalies and careful systemic examination.

A standard surgical approach for the repair of EA with distal TEF was through right posterolateral thoracotomy, retorpleural approach with ligation of azygus vein directed toward primary repair with insertion of nasogastric transanastomatic tube for 7 days. Regular follow up of the patients for any late complications especially for anastomatic stricture and for exclusion of any other associated congenital anomalies.

Results

Forty-seven (72%) infants were full term and 18 (28%) were preterm. The average birth weight was 2500±500 grams. Early repair of EA with distal TEF within first 24 hours of life was performed in 15 (23%) newborn with survival rate (66%) which is high in comparison to late presentation and late repair for newborn with age more than 24 hours. Survival rate for newborns who operated at age between 1-3 day were (46%) while for those more than three days were (31%) as shown in table 1.

All our patients underwent surgical repair through the right posterolateral thoracotomy via retorpleural approach whenever possible. A

single layer end to end esophageal anastomosis was performed by using interrupted 5-0 silk with ligation of fistula by 3-0 silk.

Table 1. Demographic details and their effect on survival rate

Variables		Cases		Survival rate	
		No.	%	No.	%
Age at the time of admission	< 1day	15	23	10	67
	1-3 day	37	57	17	46
	>3 day	13	20	4	31
Maturity	Full term	47	72	37	79
	Preterm	18	28	4	22
Sex	Male	38	58	25	65
	Female	27	41	16	59

Associated congenital anomalies occurred in 37 patients (57%) and the congenital heart diseases were the most common associated anomalies and occurred in 18 patients (28%). Other associated congenital anomalies were less common like multiple congenital anomalies (12%), genitourinary anomalies in 6 patients (9%) and imperforate anus In 2 patients to whom were divided descending colostomy done in added for thoracotomy.

Table 2. Associated congenital anomalies with esophageal atresia

Type of congenital anomaly	No.	%
Congenital heart disease	18	28
Multiple congenital anomalies	8	12
Genitourinary	6	9
Imperforate anus	2	3
Musculoskeletal	2	3
Chromosomal (Down's syndrome)	1	1.5
Total	37	57

Table 2 shows associated congenital anomalies with esophageal atresia. The mortality rate was 37% (24 patients) and the most common cause for death was due to associated congenital anomalies and pneumonia, while the survival rate was 63 % (41 patients) which occurred mostly in those patients with early presentation within the first day of life as shown in table 1.

The most common postoperative complication was pneumonia (17 patients -26%) which is a result of preoperative aspiration while anastomatic leak occurred in (13 patients -20%), only three of them needed re-thoracotomy and reanastomosis (two of them died postoperatively) while other 10 patients resolved spontaneously on conservative treatment. Anastomatic stricture occurred in 7 patients (11%) all of them had initially anastomatic leak. We referred all of those patients to cardiothoracic department for esophageal dilatation (Table 3).

Table 3. Postoperative complications

Complication	No.	%
Postoperative RTI	17	26
Anastomatic leak	13	20
Anastomatic stricture	7	11
Recurrent tracheoesophageal fistula	2	3

RTI = respiratory tract infection

During long follow up, two of our patients developed recurrent respiratory tract infection with chocking on feeding and failure to thrive. Ba-swallow was done for them and revealed esophageal stricture. Dilatation done for them by thoracic surgeon for many times but without benefit so we did for them a new thoracotomy one of them at 3 year and another one at 4 year of their age and recurrent tracheoesophageal

fistula recognized so surgical repair was done successfully. According to waterstone classification, the survival rate in group A (birth weight more than 2500 gm and otherwise healthy) was 87% while in group B (birth weight 2000-2500 gm with moderate pneumonia and congenital anomaly) was 64% and in group C (birth weight less than 2000 gm with severe pneumonia and congenital anomaly) was 21% (Table 4).

Table 4. Survival rate in patients with esophageal atresia based on Waterston classification

Group	No. of cases	Survival Rate	
		No.	%
A	23	20	87
B	18	18	100
C	14	3	21

Discussion

Esophageal atresia with or without tracheoesophageal fistula is one of commonest gastrointestinal malformations, second only to anorectal malformations. The exact incidence of EA with or without TEF is not known but incidence of 1 in 3000 to 1 in 4500 live births have been reported [7].

The Waterston *et al* devised one of the first classification systems for EA - TEF. They compared the results based on preoperative stratification of cases by severity and expected outcome. Risk factors that Waterston included were pneumonia, birth weight, and associated congenital anomalies [8]. In 1962, Waterstone proposed the risk classification of patients with EA and he reported that survival rate was 100% for group A, 86% for group B and 73% for group C which is higher in comparison to our study (group A: 86.9%, group B: 46.2% and group c: 21.4%) this low survival rate in our hospital is attributed to bad post-operative respiratory care in compare to well-developed countries.

In our study, the survival rate was 66% for newborn who underwent to the surgery within the first 24 hours of life which is high in compare to other newborn older than this age because in early presentation there is low risk for aspiration pneumonia. The prematurity is considered as risk factor for survival for our patient not due to the surgical technique but due to lack of respiratory support preoperative and postoperative (intensive respiratory care unit) and also due to physiological problems in premature neonates. The overall survival rate was 63% which similar to the Maj Daud et al study which was done in Bangladesh [9] but it is lower than Chia-Feng Yang *et al* [10] study where survival rate (83%).

In our study 57% of patients had associated congenital and the commonest being congenital heart diseases, which occurred in 28%. Maj Daud *et al* reported 42.85% associated anomalies with congenital heart disease 29%. Spitz et al [11] reported 47% and Rokitansky *et al* [12] reported 52.4% associated congenital anomalies. Thirty two patients (49.2%) developed different complications with postoperative respiratory tract infection due to aspiration was the commonest complication (26%) followed by anastomatic leak (20%). similar postoperative complications are reported by Hassab *et al* [13] and Okada *et al* [14].

In conclusion, the surgical outcome in a neonate with EA and TEF influenced by many factors including the age associated congenital anomalies and maturity of neonate. The survival rate in our study was 66% which was low as compared with developed countries due to lack of advanced respiratory care and late presentation.

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Author Contribution

The authors are responsible for preparing for all steps of this article.

Declaration of Interest

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Effect of Dialysate Temperature on Hemodynamic Stability among Hemodialysis Patients

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Abstract

Background Hypotension is one of the complications of hemodialysis treatment. It increases morbidity and mortality and can compromise the dialysis efficacy. Cooling the dialysate below 36.5°C is an important factor that contributes to hemodynamic stability in patients during hemodialysis.

Objective To assess the effect of dialysate temperature on hemodynamic stability during hemodialysis sessions, post dialysis fatigue and the adequacy of dialysis.

Methods A total of 40 patients were assessed during six dialysis sessions; in three sessions, the dialysate temperature was (37 °C) and in three other sessions, the dialysate temperature was (35 °C). Specific scale questionnaires were used in each dialysis session, to evaluate the symptoms during the dialysis procedure as well as post-dialysis fatigue, and respective scores were noted. Blood pressure, heart rate, temperature were recorded. Also dialysis efficacy using Kt/v, urea reduction ratio were measured.

Results The results showed that usage of low dialysate temperature was associated with the following : higher post dialysis systolic blood pressure ($P < 0.05$) and lower post dialysis heart rate ($P < 0.05$), better intra-dialysis symptoms score and post-dialysis fatigue scores ($P < 0.05$ and $P < 0.05$, respectively), shorter post-dialysis fatigue period ($P < 0.05$) as well as Similar urea removal and Kt/V .

Conclusion Cool dialysis is an important factor in hemodynamic stability during hemodialysis. Also it improves symptoms during and after hemodialysis. Cool dialysis has no effect on adequacy of dialysis.

Key words Hemodialysis, Cool dialysate, Hypotension, Hemodynamic

List of abbreviations: BP = blood pressure, Bu = blood urea, BUN = blood urea nitrogen, ESRD = end stage renal disease, HD = hemodialysis, URR = urea reduction ratio.

Introduction

Cooling of dialysate fluid below 36.5 °C has been proposed as a factor contributing to hemodynamic stability in patients during hemodialysis (HD) [(1)]. Cool dialysate improves cardiovascular tolerance in HD and reduces hypotension episodes without compromising the efficacy of HD [(2)]. Hypotension during HD is a source of considerable morbidity and mortality, as many as 20% to 50% of HD treatments are complicated by this problem [(3)]. Elderly patients and those with diabetes, as well as those with autonomic insufficiency and structural heart disease, are particularly affected [(4)].

The patients often suffer from variable combinations of nausea, vomiting, cramps, dizziness, and frank syncope, seizure like episodes, weakness, and fatigue both during and after dialysis sessions [(5)]. They may discontinue their sessions prematurely, resulting chronic underdialysis and fluid overload, also the patients may suffer from cerebrovascular insults and, myocardial ischemia [(5)].

During standard dialysis, the combination of low blood volume and loss of peripheral vascular

resistance causes hypotension [6]. Loss of vascular resistance is multi factorial in cause, but uremic autonomic insufficiency, vasodilation from thermal amplification, and paradoxical withdrawal of sympathetic activity are believed to have the most important roles [6,7].

The improvement in blood pressure by using cool dialysate may be due to increased total peripheral resistance and increased venous tone. Cool temperature dialysate also improves left ventricular contractility independently of pre load and after load [8]. Post-dialysis fatigue is a frequent complication that limits activity and quality of life among patients in the period immediately following the HD session, cool dialysis is a presumed mechanism for improving this fatigue [8].

The aim of this study was to assess the impact of dialysate temperature on hemodynamic stability, efficacy of HD and on post-dialysis fatigue syndrome scoring assessment in patients on maintenance HD.

Methods

The cross sectional study is carried out on samples of patients in the dialysis unit of Al-Imamain Al-Kadhimain Medical City, Baghdad, for the period from April to May 2010, and compared the response at two dialysate temperatures: 37 °C as the usual temperature, and 35 °C as the low temperature. We used empiric fixed reduction of dialyaste temperature not isothermic dialysis.

Patients' selection

Forty patients were selected randomly, 23 males and 17 females, with ages ranging between 28 and 73 years. The mean and standard deviation were (48±13 years). The etiology of renal failure in study patients was shown in table 1. The vascular access used was an arteriovenous fistula in 35 patients, and a dual lumen catheter in subclavian vein in 5 patients. Twenty two were hypertensive and told to omit antihypertensive drugs at the day of HD and not to eat during the session. They continue on regular medications for end stage renal disease (ESRD).

The patients were assessed during six dialysis sessions; in three sessions, the dialysate temperature was normal (37 °C) and in three other sessions, the dialysate temperature was low (35 °C). Patients had dialysis two to three times per week, in 3-4 hour sessions.

Blood flow rate was in average of 200-250 mL/min, and dialysate flow rate equal to 500 mL/min. Dialyzer machine was GAMBRO Ak95S and all patients used hollow fiber dialyzers (GAMBRO) with synthetic membrane; polyflux 17 L, surface area = 1.7 m^2. The dialysate fluid consisted of the following constituents: sodium 140 mmol/L, potassium 2.0 mmol/L, calcium 1.5 mmol/L, magnesium 0.5 mmol/L, chloride 111.0 mmol/L, bicarbonate 32.0 mmol/L and acetate 3 mmol/L , osmolality 290 mmol/L.

The dialysis technique was conventional HD on all patients; no patient was on hemodiafiltration. Fluid removal was calculated as the difference between the patients' weight before and after a dialysis session. Blood pressure (BP) was determined with a mercury sphygmomanometer with the patient in sitting position, and axillary temperature was measured with a mercury thermometer. In patients having an arteriovenous fistula, the contralateral arm was used for BP measurements.

Body weight, blood pressure, pulse rate and axillary temperature were measured before dialysis. The BP, pulse rate, arterial line pressure, venous line pressure, blood flow rate were all checked half hourly during the session and the mean of these readings of each parameter for each patient was calculated and considered intradialytic reading. Weight, BP, pulse rate and temperature were recorded post-dialysis. Blood flow during dialysis was slowed to 100 mL/min before collecting post-dialysis blood samples for urea. The urea reduction ratio (URR) was calculated using the formula:

Urea pre - urea post/urea pre × 100 % [3].

Dialysis efficacy was measured by equilibrated Kt/V (Kt/ Veq). Kt/V is defined

as the dialyzer as the dialyzer clearance of urea (K, obtained from the manufacturer in mL/min, and periodically measured and verified by the dialysis team) multiplied by the duration of the dialysis treatment (t, in minutes) divided by the volume of distribution of urea in the body (V, in mL), which is approximately equal to the total body water [9]. Kt/V values below 1.0 indicating under-dialysis and above 1.30 indicating adequate dialysis [9].

The single pool Kt/V (Kt/Vsp) was determined from the Daugirdas second generation formula [3].

$Kt/Veq = (1- 0.47 / t) \times Kt/Vsp + 0.02$

$Kt/V\ sp = -\ln (R - 0.03) + [(4 - 3.5R) \times (UF \div W)]$

UF is the ultrafiltration volume in liters, W is the postdialysis weight in kg, and R is the ratio of the postdialysis to predialysis BUN, t is treatment time in hours. We measured blood urea (BU) and converted to blood urea nitrogen (BUN) by this relation (BU = 2.141 BUN mg / dl) [10].

The number of hypotensive events, symptoms and complications were registered. A hypotensive event was defined according to the criteria established by Dialysis Outcomes Quality Initiative (DOQI) guidelines [11] which refer to:

1. Systolic BP below 100 mmHg, or
2. Decrease in systolic BP of 20 mmHg associated with symptoms such as nausea, vomiting, muscle cramps, dizziness or fainting, or
3. Decrease in systolic pressure more than 25 % [11].

Special questionnaire was administered during each session to assess hypotensive symptoms and to assess postdialysis fatigue syndrome, it contained the following questions:

Have you had any discomfort during the dialysis session?

Which one?

What level of discomfort have you noticed?

If the patient recovered rapidly, the discomfort was considered as being mild and scored 1 if it persisted for longer than half an hour it was considered moderate and scored 2, and if it persisted throughout the whole session, it was considered as severe and scored 3, if no discomfort noticed then scored 0 [12]. The grades were added to produce a total score. Patients were asked whether they felt cold at any time during or at the end of each HD treatment and considered one of the discomforts.

To assess postdialysis fatigue syndrome, before each dialysis session, the patient was asked the following questions:

How long did it take to recover from the last dialysis session?

What was the main complaint he/she had?

What level of discomfort did he/she experience?

The discomfort was considered mild if it did not prevent the patient from doing his/her usual activity and scored 1, moderate if his/her activity was limited but he/she did not have to take bed-rest and scored 2, severe if he/she had to take bed-rest to recover and scored 3; if no discomfort then it scored 0 [12]. Also grades were added to produce a total score. The periods of fatigue in hours were used as index.

Statistical analysis

Statistical analysis was performed using SPSS 14.0. The T test used to elicit the statistical significance concerning the comparison of multi characteristics or variables in two different set of temperatures. *P* values < 0.05 were considered as statistically significant

Results

The most common cause of renal failure in the study group was diabetic nephropathy, which constitutes about 45% of the patients (Table1).

Table 1. The main possible causes of renal failure

Etiology of renal failure	No.	%
Diabetic nephropathy	18	45
Hypertension	7	17.5
Clinical based glomerulonephritis	6	15
Pyelonephritis	6	15
Unknown cause	3	7.5

The changes noted in clinical parameters by decreasing the dialysate temperature are shown in table 2. Systolic BP decreased during and after dialysis in both dialysate 35°C and 37 °C. While this decrease was statistically significant in standard dialysis, it was not in cool dialysis. There is significant difference between cool and standard dialysis in postdialytic systolic pressure (P = 0.0104) but not in intradialytic systolic blood pressure (P = 0.1893). Diastolic blood pressure decreased in both dialyste temperatures not significantly, and there was not significant difference in diastolic blood pressure between the two conditions (P = 0.4395). There is a significant decrease in the number of hypotensive events when using cold dialysate (P = 0.0008).

Axillary temperature decreased after cool dialysis, and increased after standard dialysis with significant difference in postdialytic axillary temperature between the two dialyste temperatures (P < 0.05).

The heart rate increased with bath temperature at 37 °C and decreased with bath temperature at 35 °C with significant difference between the two baths in postdialysis, but not during the dialysis.

There is a significant difference in the scoring of symptomatology index during hemodialysis between the two baths (P < 0.001) with improvement by cool dialysate. In assessing postdialysis fatigue there was also improvement in symptomatology index with cool dialysis (P < 0.0134). Postdialysis fatigue period decreased in cool dialysis (P = 0.0064) as shown in table 2.

Table 2. Changes in clinical parameters in using two types of dialysate fluid

Clinical parameter	Dialysate Temperature		P value
	37ºC	35ºC	
Pre-dialysis temperature (ºC)	36.4 ± 0.3	36.3 ± 0.4	0.2097
Post-dialysis temperature (ºC)	36.8 ± 0.3	36.0 ± 0.3	< 0.001
Pre-dialysis systolic BP (mmHg)	145 ± 28	143 ± 29	0.7545
Intra-dialytic systolic BP(mmHg)	134 ± 26	142 ± 28	0.1893
Post-dialysis systolic BP (mmHg)	126±24	141 ± 27	0.0104
Pre-dialysis diastolic BP (mmHg)	91 ± 13	92 ± 11	0.7114
Intra-dialytic diastolic BP(mmHg)	90 ± 11	91 ± 10	0.6717
Post-dialysis diastolic blood pressure (mmHg)	88 ± 11	90 ± 12	0.4395
Pre-dialysis heart rate (beats/ minute)	83±10	82 ± 11	0.6717
Intra-dialytic heart rate (beats/ minute)	85 ± 12	81 ± 10	0.1034
Post-dialysis heart rate (beats/ minute)	85± 11	79 ± 9	0.0156
Hypotensive events	1.7± 0.9	1.1 ± 0.6	0.0008
Symptomatology index during hemodialysis	0.8 ± 0.2	0.5 ± 0.1	< 0.001
Post-hemodialysis fatigue symptomatology index	1.3 ± 0.4	1.1 ± 0.3	0.0134
Post-dialysis fatigue period (hours)	4.4 ± 1.3	3.7 ± 0.9	0.0064

The difference in Kt/Veq values with the use of cool dialysate (35 °C) and normal dialysate (37 °C) was statistically not significant. Also, there was non-significant difference in the URR between the two dialysate temperatures (Table 3).

Table 3. Changes in adequacy of dialysis by using two types dialysate fluids

Parameter	Dialysate temperature		P value
	37°C	35°C	
URR%	49.71 ± 2.21	50.21 ± 1.8	0.2706
Kt/Veq	0.901 ± 0.095	0.897±0.105	0.8587

Usage of cool dialysate resulted in maintaining hemodynamic stability with nearly similar ultrafiltration compared with normal dialysate (37 °C). Ultrfiltration at 37°C was 1.362 ± 0.273 L, and at 35°C was 1.397 ± 0.308 L. There was no significant difference between the two types of dialysis (*P* = 0.592).

Discussion

From the results of the study we found that reduction of the dialysate temperature from 37 °C to 35 °C, increases hemodynamic stability, decreased subjective symptomatology index during dialysis, and improves post dialysis fatigue syndrome. By decreasing dialysate temperature, patients complete the dialysis session with higher systolic blood pressure and lower heart rate, with nearly equal degree of ultrafiltration. Also reduction in dialysate temperature resulted in improvements in scoring system of post-hemodialysis fatigue symptomatology index. We choose 35 °C because several studies have shown that this degree of cooling produces the least variations in core body temperature [13,14].

The results of our study are in accordance with previous studies that showed improvement in haemodynamic stability when using cool dialysate [9,15,16]. Compensatory physiological mechanism may play a role Removal of body heat by cool dialysis helped the patients to sustain their peripheral vasoconstriction and cardiac filling. Cool dialysate increases left ventricular contractility in hemodialysis patients [17]. The stability of blood pressure during cool dialysis may at least in part be due to an increase in plasma norepinephrine concentrations, which is not observed during warm dialysis [18]. Similarly, cooling of the blood during hemodialysis may result in physiologic responses such as skin vasoconstriction and shivering to restore body temperature which is considered pathophysiological sign carries bad outcomes [8].

Previous studies such as the study of Fine and Penner, suggested that dialysate temperature should be reduced only in patients whose body temperature was low, since they represent the group of patients who are likely to improve with this measure [9]. Fine and Penner showed that dialysis patients with subnormal body temperature below 36°C dialyzed with 37 °C dialysate had the highest hypotesive episodes. Those patients who should most benefited from cool dialysate using 35 °C [9]. In this study we did not find such relation. Skin temperature does not help in identifying which patients who benefit from cool dialysate. In our data nearly 95 % of the patients had predialysis temperature above 36°C, and improved with cool dialysis.

There were not significant differences in the Kt/V eq values between dialysis with cool dialysate and standard dialysate. Therefore, cool dialysate had no effect on urea removal and equilibrated Kt/V. In one study done by Azar in Egypt in 2009, he showed that cool dialysate increase the efficacy of dialysis [8].

Since cool dialysis causes increase in peripheral vascular resistance, some investigaters have expressed a concern that this haemodynamic effect could cause urea compartmentalization in vasocostricted bed and thus decrease in the

efficiency of dialysis [3]. However, in this study efficacy of dialysis was not changed significantly by decreasing dialyste temperature. This may be due to the fact that vasoconstriction in cool dialysis involves mainly skin which contains only 10 % to 15 % of total body water and hence urea; so it has little impact on urea extraction [19].

From the results of this study it was that HD in dialysis unit is not adequate (Kt/V < 1). This may be attributed to many factors such as malnutrition, anemia, premature ending of dialysis session due to hypotension or other technical reasons, also non compliance of the patients, dialysate flow rate that is inappropriately low, dialyzer leaks. Inadequate blood flow from the vascular access, and blood clotting during dialysis, which reduces effective dialyzer surface area. This is may be the cause of non significant difference between cool and normal dialysate temperature regarding the adequacy of dialysis. In conclusion cool dialysis is an important factor in haemodynamic stability during HD. Also it improves scoring of symptoms during and after HD. Cool dialysis has no effect on adequacy of dialysis.

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Author contribution

Dr. Arif has designed the study and co-writes the manuscript, Dr. Tarik has collected and analyzed the data and write the manuscript.

Declaration of interest

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Effects of Verapamil and Olanzapine in Terminating Pilocarpine-Induced Epileptic Seizures in Mice.

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Abstract

Background Epilepsy is one of the oldest known neurological conditions characterized by recurrent seizures.

Objective To explore the possible antiepileptic effect of both Verapamil and Olanzapine in pilocarpine epileptic model in mice.

Methods Fifty healthy male albino mice weighing between 30-35 gm were equally allocated into five groups (10 mice in each group) and were distributed into: normal group (without drug); distilled water group (0.1 ml); diazepam group (1mg/kg); verapamil group (20 mg/kg) and olanzapine group (10 mg/kg). All animals (except normal group) were injected with pilocarpine hydrochloride (350 mg/kg) to induce generalized tonic-clonic seizure 30 minutes after the tested drugs had been administrated. The mean onset of seizure were determined as well as the mean serum concentration of electrolytes, glutathione and malondialdehyde were measured after seizure had been induced.

Results Pilocarpine-induced seizure at approximately 7 minutes after injection. While both verapamil and olanzapine produced highly significant increase in mean onset of seizure 16±1.549 and 13.1±1.566 respectively as compared to D.W. group, also both drugs produced highly significant changes in mean serum concentration of electrolytes, glutathione and malondialdehyde.

Conclusion Verapamil and olanzapine had anticonvulsant activity when used at applied doses in the pilocarpine model of seizures in mice.

Key words Epilepsy, seizure, verapamil and olanzapine.

List of Abbreviation: 5-HT = 5-hydroxytryptamine, Bax = Bcl-2-associated X protein, Bcl-2 = B-cell lymphoma 2, Bcl-XL = B-cell lymphoma-extra-large, BDNF =brain-derived neurotrophic factor, GABA = gamma-aminobutyric acid, GSH = reduced glutathione, I.P = intraperitoneal, MDA = malondialdehyde, GSH = glutathione, SOD= superoxide dismutase.

Introduction

Epilepsy is one of the major neurological diseases [(1)], it is characterized by recurrent, unprovoked, paroxysmal episodes of brain dysfunction manifesting as a large number of clinical phenomena [(2)]. Epilepsy occurs due to many different cellular or biochemical changes such as alterations in ion channel function, neurotransmitter level (excitatory and inhibitory), neurotransmitter receptor function and energy metabolism, in addition to the body electrolytes, level of some trace elements, and membrane lipid peroxidation due to increase in free radicals or decrease in activities of antioxidant defense mechanisms all these may be causally involved in some forms of epilepsy and may increase the recurrence of seizures [(3)].

The pilocarpine provides a useful animal model for studying epilepsy. Generalized tonic-clonic convulsion induced by pilocarpine shows the

involvement of the cholinergic system in seizures and status epilepticus [4]. The activation of muscarinic receptors is the first step for seizure activity, while GABAergic and glutamatergic systems appear to mediate seizure propagation and/or maintenance in rodent epilepsy models [5].

Verapamil is an L-type calcium channel blocker of the phenylalkylamine class which has cardiac effects more than vascular smooth muscle effects. While olanzapine is an atypical antipsychotic, antimanic and mood stabilizing agent belongs to the thienobenzodiazepine class that demonstrates a broad pharmacological profile across a number of receptor systems [6], such as anticholinergic and antioxidant effects [7]. These effects of both drugs are useful in terminating of epilepsy. The current study was carried out to explore the possible antiepileptic effects of Verapamil and Olanzapine on pilocarpine induced seizures in mice.

Methods

This study was carried out on fifty healthy male albino mice weighing between 30-35 gm, they were supplied by animal house of Al-Nahrain College of Medicine and were housed under good conditions and fed standard oxoid palate with water ad libitum, and they were equally allocated into five groups (10 mice in each group):

- ***Group 1: (Normal group):*** This group served as normal control and takes no drug used to detect the normal values of serum electrolytes, glutathione (GSH) and malondialdehyde (MDA).
- ***Group 2: (Distilled water group):*** They were injected 0.1 ml of distilled water (I.P.) 30 mint. before pilocarpine injection, to induce epileptic seizures.
- ***Group 3: (Diazepam group):*** They were injected 1mg/kg of diazepam (I.P.) 30 mint. before pilocarpine injection. This group served as positive control and was used to compare onset of seizure only with tested groups.
- ***Group 4: (Verapamil group):*** They were injected verapamil 20 mg/kg (I.P.) 30 mint. before pilocarpine injection.
- ***Group 5: (Olanzapine group):*** They were injected olanzapine 10 mg/kg (I.P.) 30 mint. before pilocarpine injection.

After giving the pilocarpine, each mouse was carefully evaluated by detecting the parameters which includes the onset of the first seizure recorded by naked eyes. At the end of observations the blood samples were collected from survival mice for measuring other parameters which are the serum concentrations of Na^+, K^+ ,Ca^{2+}, GSH and MDA.

Statistical analysis was performed with the SPSS 19.0 statistical package for social sciences, data were expressed as mean ± Standard error of mean (S.E.M.), unpaired t-test at ($p \leq 0.01$) and ($p \leq 0.05$) for independent data was used [8].

Results

All the mice exhibited generalized limbic seizures after pilocarpine administration, at a latency of (7±0.394) minutes; besides, it caused highly significant reduction in mean serum Na^+, Ca^{2+} and GSH concentration, while it caused highly significant increase in mean serum concentration of K^+ and MDA when compared to that of normal group.

Diazepam caused highly significant increase in mean onset of seizure; also diazepam had no effect on other parameters when compared with D.W group.

Both verapamil and olanzapine showed highly significant increase, and highly significant reduction for olanzapine in mean onset of seizure when compared to that of both D.W. and diazepam groups respectively, this indicated that verapamil is more potent than olanzapine, also both drugs revealed highly significant increase in mean serum concentration of Na^+, Ca^{2+} and GSH with highly significant reduction and non-significant change in mean serum concentration of MDA and K^+ respectively when compared to that of D.W group(Table 1).

Table 1. Effect of Verapamil and Olanzapine on Onset of Seizure and Serum Na^+, K^+, Ca^{2+}, GSH and MDA Concentrations in Group I, II, and III in Pilocarpine-induced Seizure in Mice

Group	Seizure Onset (minute) M ± SEM	Concentration				
		Sodium mmol/l M ± SEM	Potassium mmol/l M ± SEM	Calcium mg/dl M ± SEM	GSH mmol/l M ± SEM	MDA mmol/l M ± SEM
I	0	144.8 ± 2.958	5.47 ± 0.212	8.5 ± 0.306	1.13 ± 0.063	5.02 ± 0.103
II	7 ± 0.394	133.2 ± 1.781a**	8.11 ± 0.502a**	7.03 ± 0.157a**	0.8 ± 0.052a**	10.66 ± 0.265a**
III	18.6 ± 0.858b**	134.5 ± 1.45	7.78 ± 0.54	7.35 ± 0.34	0.85 ± 0.05	10.5 ± 0.28
IV	16 ± 1.549b**	147.3 ± 3.464b**	8.38 ± 0.082 a**	10.12 ± 0.087a**, b**	1.605 ± 0.104a**,b**	5.8 ± 0.203a**, b**
V	13.1 ± 1.566b**,c**	151.9 ± 2.41b**	8.38 ± 0.44 a**	9 ± 0.522 b**	1.2 ± 0.051b**	8 ± 0.316a**, b**

*= $P \leq 0.05$, **= $P \leq 0.01$, a = as compared to group I, b= as compared to group II, c= as compared to group III), n = 10/group).

Discussion

Epilepsy is one of the most common neurological problems all over the world, being associated with paroxysmal discharge of cerebral neurons and is characterized by several symptoms including alterations of behaviors and consciousness sustained alteration in brain function [9].

The anticonvulsant activity of verapamil may be attributed to its ability in blocking the calcium channels and to prevent the increase in intracellular calcium which play important role in incidence of certain types of seizures [10,11].

In addition, calcium channel antagonist enhanced the anticonvulsive activity of carbamazepine, valproate and Phenobarbital against maximal electroshock-induced seizures in mice [12,13]. Verapamil is a known inhibitor of P-glycoprotein and may block P-glycoprotein–modulated efflux of antiepileptic drugs in the brain [14]. The antioxidant activity of verapamil may contribute to new neuroprotective activity by which verapamil inhibit free radical-induced damage to lipid constituents of the membrane, and by inhibition of cellular oxidative stress and reduction of MDA level and augmented the activity of antioxidant enzymes [15].

Furthermore, calcium channel antagonist have been found to inhibit the release of steroids and also block the inflow of sodium into detonated neuron as well as inhibit calcium-dependent glutamate channels, thereby inhibit the release of glutamate, an excitatory neurotransmitter which has been implicated in the pathogenesis of convulsive seizures from such neurons [16,17].

The increase in serum calcium and sodium concentration may be related to blocking calcium channel and antioxidant activity of verapamil.

Olanzapine has neuroprotective and cytoprotective effects mediated by the acceleration of metabolic processing of free radicals, since olanzapine increase SOD and decrease MDA level [7,18], also olanzapine increases the GABAergic neuroactive steroid allopregnanolone in rat cerebral cortex (which is a potent GABAA- receptor modulator with anxiolytic and anticonvulsant effects) [19].

Olanzapine attenuates cocaine neurotoxicity in a mouse model by blocking the dopamine, alpha adrenergic, 5HT-2 and 6 serotonin, muscarinic receptors and monoamine transporters [20].

The neuroprotective effects of olanzapine on the Okadaic acid (a selective and potent inhibitor of the serine/threonine phosphatases 1 and 2A) induced neurodegeneration and apoptosis in brain [21], which are mediated through the following mechanisms:

- Olanzapine upregulate the level of brain-derived neurotrophic factor (BDNF), an important neurotrophin mainly expressed and distributed in brain neurons that prevent cell degeneration [22].
- Olanzapine upregulate the level of Bcl-2 and modulate the Bcl-XL/Bax ratio in brain. Bcl-2, a neuroprotective protein that inhibit apoptosis [23].
- Olanzapine has antioxidant activity by increasing the gene expression of superoxide

dismutase (SOD1) in PC12 cells, and prevent cell death after serum withdrawal [18].

- Olanzapine can increase cell proliferation and neurogenesis in adult rat brain [24].

The increase in serum calcium and sodium concentration may be related to antioxidant, antimuscarinic activity and $Na^{+}K^{+}$ ATPase modulation of olanzapine. All the tested drugs had valid preventive effect against seizure induced by pilocarpine in mice when were compared to that of diazepam, verapamil had the most potent effect while olanzapine had the weakest effect, the latter can be used for schizophrenic patient with symptoms of epileptic seizures.

Finally the aim of present study to find a new drugs or combination of drugs which are more potent with less adverse effects to be used in epilepsy after confirmation with clinical trials.

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Author contributions

Uday is a researcher who has done the technique of this work and conducted the writing of manuscript. Dr. Faruk participated in supervision and in scientific review of the manuscript.

Conflict of interest

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A Giant Solitary Primary Retroperitoneal Hydatid Cyst in 5-Year Old Child: Case Report.

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Abstract

Hydatid disease is one of the commonest zoonotic diseases. It is caused by the larval cyst of Echinococcusgranulosus and result in the most severe form of cetodiases in man**.** The hydatid disease is endemic in some Mediterranean countries, the Middle East, the South America, and South Africa and Oceania. The combination of imaging and serology usually enables diagnosis**.** We report a case of single and giant hydatid cyst in an unusual site (retroperitoneal region) which is very rare and also in a five-year old child which is unlikely to happen at such age with this big size.
Keywords: Hydatid disease, retoperitoneal region.

List of abbreviations: HD = hydatid disease, HC = hydatid cyst, CT = computed tomography, MRI = magnetic resonance imaging study

Introduction

Hydatid disease (HD) is prevalent in most sheep-raising Mediterranean countries. It is often manifested by a slowly growing cystic mass.

In 85%-95% of the cases, the liver and / or lung are involved and only 5%-15% of the cyst occurs in other sites. Retroperitoneal HD is usually the result of spontaneous, traumatic, or surgical rupture of hepatic cyst.

Primary retroperitoneal HD without any other organ involvement is very rare [1,2].

Retroperitoneal location of hydatid cyst (HC) is encountered rarely and only occasional cases were reported since Lackart and Spinza in 1958 [3].

HD is seen more frequently at ages 20 to 40 years. Infestation usually occurs in childhood. The HC grow slowly (about 1-3 cm per year) that is to say the organism may take up to 20 years to reach considerable size.

The cyst grows and increases by means of daughter cysts that they produce. The natural course of infection varies; some cysts spontaneously collapse or calcify.

The clinical manifestation is related to compression of the involved organ. Routine blood tests are generally normal but eosinophilia occurs in 25% of the cases. Ultrasound, computed tomography (CT) scan, magnetic resonance imaging study (MRI) and Casoni skin test or complement fixation and hemagglutination inhibition serological test may help in the diagnosis [4].

Total cystectomy is the best technique to get rid of the parasite, but when the cyst can't be removed completely, partial cystectomy is recommended.

Laproscpic approach also described and encouraging results have been achieved in some series. Spillage of the cysts must be avoided and scolicidal agents must be used (such as hydrogen peroxide and 10% povidone-iodone).

Medical treatment with albendazole or praziquantel is indicated for inoperable or disseminated cases. Percutaneous aspiration, injection and re-aspiration (PIAR technique) is also another non-surgical option [5,6].

Case report

A five-year old girl was admitted to Al-Imamian Al-Kadhymian Medical City with a swelling in the right hypochondrium which grew very rapidly. Abdominal examination revealed a big mass of 15 cm in diameter in right hypochondriac region; the mass was immobile and not tender.

Her vital signs were within normal limits. Laboratory findings included: hemoglobin 10.5 gm/dl, white blood cell count 10.000/mm^3, blood urea = 15 mg/dl and serum creatinine = 0.8 mg/dl. Other biochemical results were within normal limits.

Chest x-ray revealed no pathological signs and abdominal plain x-ray showed increased soft tissue density in right hypochondriac region. Intravenous urography has shown that both kidneys were functioning normally.

Abdominal ultrasound and CT scan showed a 11 cm x 16 cm retroperitoneal HC causing a downward displacement of right kidney as illustrated in fig. 1.

The patient was started to take 15 mg/kg/day albendazole for 2 weeks before doing surgery.

Surgical procedures included abdominal exploration through right upper transverse incision to explore the retroperitoneal cyst [5].

The cyst was identified grossly as HC and was subjected to aspiration of its fluid contents, whereby 10cc of the fluid was aspirated using a 10 ml syringe with a 22 gauge needle. The fluid was found to be clear and did not contain pus or bile colored.

A scolicidal agent (10% povidone iodine) was injected in a volume of 15 ml [5,6]. The germenative membrane and cystic contents were evacuated through a cystotomy and partial cystectomy was done as illustrated in fig. 2 A and B. Two tube drains were inserted one in cystic cavity which was removed at the third day postoperatively and the other one within the pelvic cavity which was removed at seventh day post operatively.

The child was kept on albendazole treatment in dose of 15 mg/kg/day in two divided doses for three months with monitoring of liver function [5,6].

The cyst contents were sent for histopathology which proved the diagnosis with presence of laminated membrane and germinal membrane in the examined section.

Discussion

Primary retroperitoneal HC is extremely rare even in endemic areas. The majority of abdominal and pelvic HC are considered to be secondary to prior hepatic involvement following spontaneous rupture or surgical inoculation [6].

HC in children is rare because the cyst needs several years to reach considerable size (1-3 cm/year), This patient was five-year old and had a large primary HC (11 cm x 16 cm) in an unusual site (retroperitoneal region).

Angulo *et al* [7] reviewed cases of this condition in endemic areas of central Spain and estimated that 1.1% of newly diagnosed cases were isolated retroperitoneal cysts.

Turkyilmaz *et al* [8] reported a case of nine year old female child with primary retroperitoneal HC of 8cm in diameter in turkey.

Hydatid disease in extrahepatic locations usually remains asymptomatic unless the cyst grows and produces pressure symptoms, rupture to pleural or peritoneal cavity, secondary infection, or an allergic reaction, this patient presented with abdominal distension, which was increasing over the last few months.

In conclusion, Hydatid disease should be kept in mind in the differential diagnosis of retroperitoneal masses in patient living in endemic areas; also we should expect HC even in large size and in unusual site in pediatric age group.

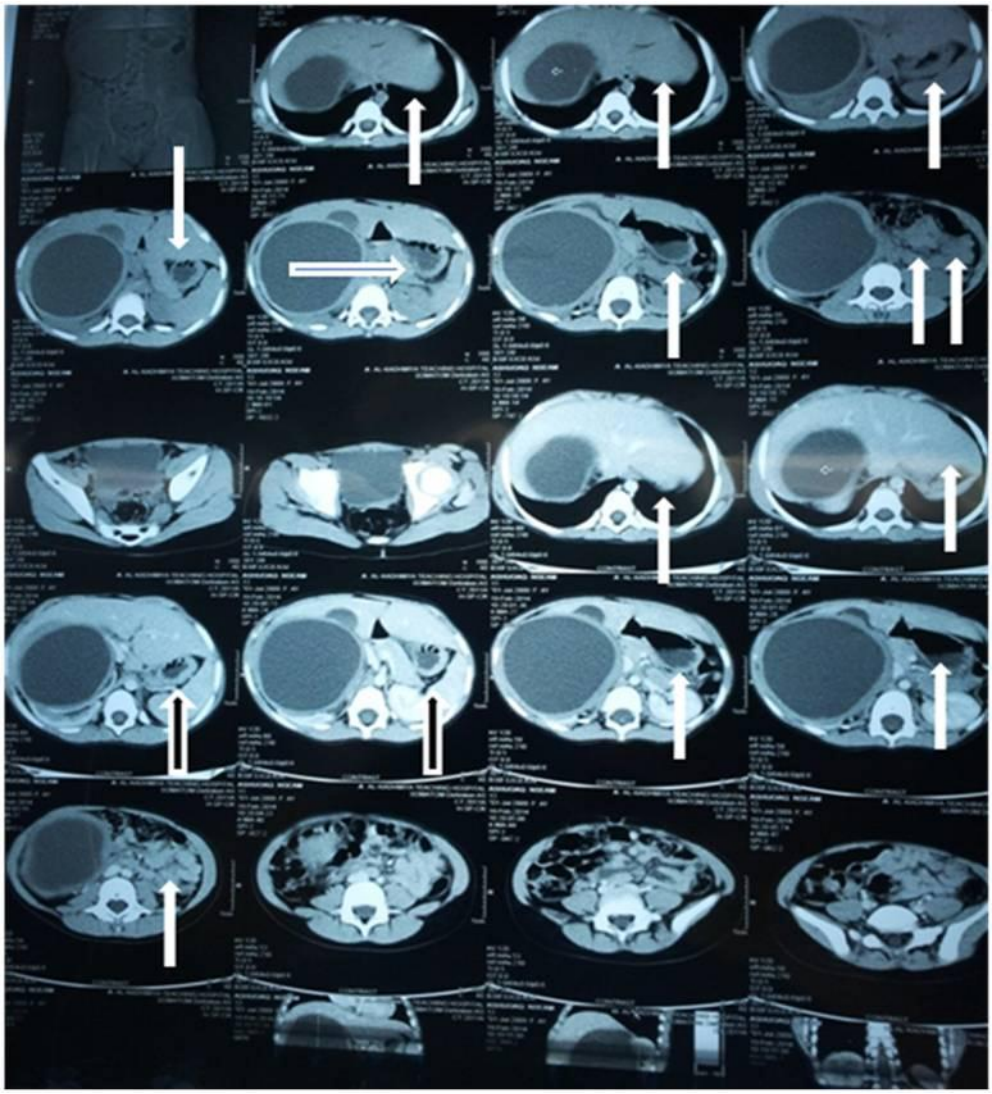

Fig. 1. CT-scan of abdomen showing a big retroperitoneal Hydatid cyst (arrows showing a huge Hydatid cyst)

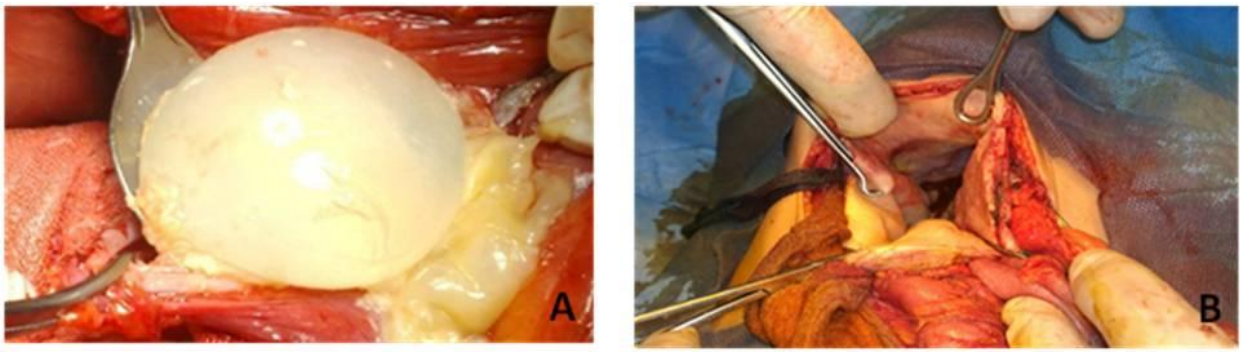

Fig. 2. Intraoperative retroperitoneal Hydatid cyst (Exploration of the cyst); A: Gross appearance of a whitish cyst measuring 11cmx 16 cm, B: cystotomy and partial cystectomy.

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