Measurement of Plasma Lipid per oxidation Marker (MDA) and Vitamin A in Sudanese Patients with Type2 Diabetes.

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Abstract

It has been well documented that there is a link between oxidative stress and secondary complications of diabetes. In the present study we measured and evaluated changes in levels of malondialdehyde (MDA) as marker of lipid per oxidation and antioxidant vitamin A in plasma of Sudanese patients with Type 2 Diabetes Mellitus. Total of 200 diabetic patients (90 male, 110 female) with mean age of 55.48±12.14 years were recruited into the study. Control group was composed of 100 healthy volunteers (47 male, 53 female) with mean age of 53.53±11.43 years. In addition to two mentioned parameters, levels of fasting blood glucose, percentage HbA1C levels were determined in diabetic patients and controls.

There was a significant increase in MDA level (test group) which is used as an indicator of metabolic stress, oxidative stress or lipid per oxidation marker. On the other hand; antioxidant vitamin A of the test group was reduced meaningfully. Reduction in vitamin A levels was probable due to antioxidant effect of this antioxidant vitamin. In conclusion supplementation of antioxidant vitamin (A) into the daily diets of diabetic patients will enhance power of non-enzymatic antioxidant defense systems.

Keywords—Diabetes Mellitus, Vitamin A, MDA.
Introduction

Diabetes Mellitus (DM) is a chronic disease characterized by the disorder of the glucose metabolism and associated with a reduced ability of the tissues to respond to insulin (insulin resistance). DM causes high morbidity and mortality derived by chronic micro- and macro-vascular complications\(^1\). Diabetes was reported to be the fifth leading cause of death in the United States\(^2\). DM is now one of the major health problems in the Sudan resulting in 10% of all hospital admissions and mortality. A small population based study in 1993 of a sample of 1284 adult men, showed a prevalence of 3.4% of type 2 diabetes\(^3\). A combination of genetic and environmental risk factors contributed to DM pathogenesis\(^4\).

Although there are several reports on complications of diabetes, pathophysiology of these complications are still needed to be deciphered\(^5\). Recent reports indicate that free radicals have important roles in pathogenesis of diabetes and a relationship between oxidative stress and secondary complications of diabetes exists\(^6\,^7\). It is well established that there is an increased production of damaging free radicals in Non-insulin dependent diabetes mellitus (NIDDM) patients which may be due to auto-oxidation of glucose and glycosylated proteins\(^8\,^9\,^10\,^11\). Subsequently, free radicals change lipid/protein ratio of membranes by affecting poly unsaturated fatty acids and lipid per oxidation, causes functional irregularities of several cellular organelles\(^12\,^13\). Lipid peroxides are disintegrated quickly and form reactive carbon compounds. Among these, MDA is an important reactive carbon compound which is used commonly as an indicator of lipid per oxidation\(^14\). Since free radical production is increased whereas capacity of antioxidant systems is reduced in diabetes, it has been proposed that diabetic patients may require more antioxidants compared to healthy individuals\(^13\,^14\). Since effects of free radicals in diabetes are now documented, it has been proposed to use antioxidant vitamins to block formation of free radicals and hence prevent development of diabetes\(^16\,^17\). Glutathione is a very important non-enzymatic antioxidant together with antioxidant vitamins. Vitamins A, E and C are among these important non enzymatic antioxidants\(^18\,^19\). It has been proposed that in diabetic patients several abnormalities related with absorption develop in the absence of antioxidant vitamins\(^20\). Vitamin A and glutathione are some of the major non-enzymatic antioxidants in the
body. Therefore, the idea of using antioxidant vitamin to prohibit development complications and/or to treat diabetic patients is getting more attention than ever \(^{16,22}\). Although there are studies reporting serum or plasma levels of antioxidant vitamins in diabetic patients, results from different groups are rather contradictory. Studies focusing on involvement of vitamin A in diabetic patients are rather limited.

Therefore, the present study was designed to measured and evaluate changes in level of antioxidant vitamin A and MDA in Sudanese patients with type 2 diabetes and healthy subjects. Furthermore, we examined possible relationship between HbA1c, vitamin A, and MDA.

**MATERIAL AND METHODS**

Total of 200 patients (90 male, 110 female) who were diagnosed with type 2 diabetes mellitus in Jabir Abulizz Diabetes Centre, Omdurman teaching hospital (Abdelmoniem referring center), and other private clinics for diabetic care in Khartoum state, Sudan. Mean age of diabetic patient was 54.8±11.4 years and who were free of clinical symptoms of neuropathy, retinopathy. Control group was consisted of 100 healthy volunteers (47 male, 53 female) whose mean age were 53.53±11.48 years. Venous blood samples were withdrawn after an overnight fasting from patients and controls. Fasting blood glucose levels were determined by a commercial kit by using enzymatic method (glucose oxidase / peroxidase) (Biosystem S.A Costa Brava 30, Barcelona- Spain by auto analyzer humalyzer 2000 human- German.)

Hb A1c percentage level was determined by method based on aboronate affinity chromatography by using NYCOCARD READER II –AXIS-SHIELD Po C AS NO-0504 Oslo, Norway, rapid in vitro test for the measurement of glycated hemoglobin (Hb A1c) % in human whole blood. The machine (NYCOCARD READE II) is traceable to the international federation of clinical chemistry (IFCC) reference method for measurement of Hb A1c, and it's measuring range 3- 18 % Hb A1c. MDA levels were determined by the method of Karataş et al. \(^{20}\) by HPLC utilizing a column (250 x 3.9 ID) 1.5 mL min-1 flow rate and 254 nm wavelength. Determination of vitamin A through the HPLC method a chromatographic
measurements were made using a Hewlett-Packard (Wald born, Germany) model 1050 pump system, water 717 plus Auto sampler (Milford, MA, USA), a.u.v. – vis detector, C18 (250 x 4.6 mm 1.D, 5μm particle size) protected with a guard cartridge (tracer, C18, 5μm). The frozen specimens preserved with metaphosphoric acid (5%) were thawed to around 22 ºC in water bath, protected from light, and then mixed. Statistical analysis was carried out using SPSS for Windows, SPD – 10 AV VP (Shimadzu Kyoto, Japan) and an HP- 3365 series II chemstation. The analytical column used was a tracer spherisorb OD52 Ver.10.5 (SPSS Inc. Chicago, IL, USA). The data obtained are expressed as mean values ± S.D. Student’s t-test and Pearson test was used to determine whether differences between the means were significant, with p<0.05 taken as the significance level.

**RESULT**

Demographic features of diabetic patients and controls are summarized in Table 1. MDA and vitamin E are given in Table 2. Fasting blood glucose and HbA1C% are given in Table 3.

Table 2. Shows a highly significant difference between the means of plasma MDA of the test group (n=200) and the control group (n=100). Mean± SD: (4.47±5.29) versus (1.93±0.41) n mol/l, respectively, (p=0.00).

And shows a significant difference between the means of plasma vitamin A of the test group (n=200) and the control group (n=100). Mean± SD: (46.33±12.68) versus (70.39±15.52) µg/dl, respectively, (p=0.00).

Table 3. Shows a significant difference between the means of plasma levels of fasting plasma glucose of the test group (n=200) and the control group (n=100). Mean± SD: (191.01±58.52) versus (94.74±10.81) mg/dl, (p=0.00), respectively, and shows a significant difference between the means of blood levels of hemoglobin HbA1c % of the test group (n=200) and the control group (n=100). Mean± SD: (9.18±2.19) versus (5.17±0.48) %, (p=0.03) respectively.

Figure (1) shows insignificant, very weak correlation between HbA1c percent and the plasma levels of Malondialdehyde of the test group (r=0.05, p= 0.5)

Figure (2) shows insignificant, very weak correlation between HbA1c percent and the plasma levels of vitamin A of the test group (r=0.05, p = 0.5).
Figure (3) shows significant difference, positive correlation between HbA1c percent and the fasting plasma glucose levels of the test group (r=0.81, p= 0.00).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test group N=200</th>
<th>Control group N =100</th>
<th>P -Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.48± 12.41 (23.00 – 86.00)</td>
<td>53.53± 11.43(22.00 – 78.00)</td>
<td>0.08</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.54± 9.35(132.00 -194.00)</td>
<td>173.24 ± 8.73(157.00 – 192.00)</td>
<td>0.09</td>
</tr>
<tr>
<td>Wight (Kg)</td>
<td>75.26± 9.85(53.00 – 125.00)</td>
<td>70.71± 9.42(52.00 – 104.00)</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>26.04± 3.18(18.1 – 41.4)</td>
<td>22.60 ± 2.41(19.30 – 31.00)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Variables</th>
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<tbody>
<tr>
<td>MDA</td>
<td>4.47±5.29 (0.62 – 34.98)</td>
<td>1.93± 0.41 (1.04 – 3.63)</td>
<td>0.00</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>46.33±12.68(13.26 – 95.50)</td>
<td>70.39±15.52(39.35- 131.70)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Variables</th>
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<th>Control group n=100</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>191.01 ± 58.52 (25.00 – 340.00)</td>
<td>94.75 ± 10.81 (68.00 – 124.00)</td>
<td>0.00</td>
</tr>
<tr>
<td>HbA1C %</td>
<td>9.18 ± 2.19(4.10 – 15.60)</td>
<td>5.17 ± 0.48(4.10 – 6.30)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table I
DEMOGRAPHICS FEATURES OF THE TEST GROUP AND THE CONTROL GROUP

Table II
COMPARISON OF THE MEANS OF PLASMA MDA AND VITAMIN A OF THE TEST GROUP AND CONTROL GROUP

Table III
COMPARISON OF THE MEANS OF PLASMA FASTING PLASMA GLUCOSE (MG/DL) AND HBA1C (%) OF THE TEST GROUP AND CONTROL GROUP

Values Are Means ± SD P<0. 05 When Compared To Control

Fig. (1) A scatter plot shows the relationship between HbA1C% and plasma MDA of the test group. (r = 0.05, P = 0.51).
Fig. (2) A scatter plot shows the relationship between HbA1C% and Vitamin A of the test group. \( r = 0.05, P = 0.50 \).

Fig. (3) A scatter plot shows the relationship between HbA1C% and fasting plasma glucoses of the test group. \( r = 0.81, P = 0.00 \).

Discussion

Diabetes is a serious public health problem throughout the world, in both type 1 and type 2 diabetes, increased oxidative stress and impaired antioxidant defense have been suggested as contributed factors for initiation and progression of late complications in diabetes. The hypothesis that hyperglycemia should be able to cause oxidation in diabetic patients is supported by several studies and particularly by evidence that several biochemical pathways activated during hyperglycemia can increase the production of reactive oxygen species (ROS)\(^{23}\).
also when diabetic complications are developed, an increase in oxidative damage and subsequently emaciation of antioxidant defense systems are observed 24. The current study shows a significant increase in the plasma levels of MDA of the test group when compared to the control group (Table:2), this agrees with studies done by (Noberasco, et al.1991)25, (Jiang, et al.1997)26, and (Ceriello, et al.1998)24 who reported a significant increase in the levels of plasma malondialdehyde of the diabetics when compared to healthy controls, this may be due to poor diabetic control which may enhance lipid per oxidation and diminishes the body’s antioxidant capacity, or may be due to mobilization of lipids for a further use as an energy sources rather than glucose. Also the present study shows insignificant, very weak correlation between HbA1c percent and the plasma levels of Malondialdehyde of the test group (Fig.1), this agrees with a study done by (Tan, et al.2000)27 and also agrees with a study done by (Hanachi P, et al. 2009)28 who reported no correlation between MDA and HbA1C %, this could be due to good glycemic control. The present a study shows a significant decrease of the plasma levels of vitamin A of the diabetic patients when compared to the healthy control (Table:2), this agrees with the studies done by (Sundaram, et al.1996)29, (Krempf, et al.1991)30, (Vatassery, et al.1983)31, and (Grando, et al.1998)32 who documented reduction in vitamin A levels of diabetic patients rather than increase when compared to healthy controls, this significant decrease most probably due to rapid depletion of this antioxidant (vitamin A) due to increased oxidative stress observed in type2 diabetic patients. The present study also shows insignificant, weak correlation between HbA1c percentage and the plasma levels of vitamin A (Fig.2) of the test group, this agrees with a study done by (Hermann, et al.1994)33, and (Loft, et al.1992)34 they demonstrated no correlation between the HbA1c and plasma levels of vitamin A. The current study shows a significant, positive correlation between HbA1c % and the fasting plasma glucose levels of the diabetics (Fig.3), this is in accordance with the study of (Kesavulu, et al .2001)35 and (Sundaram, et al.1996)29 who reported significant positive correlation between HbA1C % and FPG levels, this most probably due to poor glycemic control. From the results of this study it is concluded
that, in Sudanese patients with type 2 diabetes mellitus, the means of the plasma levels of malondialdehyde, fasting plasma glucose, and blood HbA1C are significantly raised when compared with healthy control subjects, where mean of plasma levels of antioxidant vitamins (A) is significantly decrease in Sudanese with type 2 diabetes mellitus when compared with healthy control subjects, also there is significant strong positive correlation between HbA1c percentage and fasting plasma glucose (FPG), where there is insignificant very weak correlation between Vitamin A, MDA and HbA1c of the test group. Therefore it is recommended that Diabetic patients should received supplements of antioxidant vitamins such as vitamin A, in order to enhance power of non-enzymatic antioxidant defense systems. Lipid peroxidation marker such as malondialdehyde (MDA) should be assessed regularly in diabetic patients, in order to minimize development of free radicals and oxidative stress. Good glycemic control in diabetic patients could be of vital importance in prevention or delay the development of complications in diabetic patients.

REFERENCES


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Management of abdominal trauma

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Abstract

Objective : This study prospectively evaluated inhospital patients who have got abdominal trauma . Methods : All patients with abdominal trauma admitted between January 2014 and June 2015 in Zawia teaching Hospital , Zawia , Libya , were assessed for different abdominal injuries using multiple diagnostic studies . Results : A total of 134 patients were included , the greatest number of patients were below the age of 40 years and the commonist cause of trauma were the penetrating gunshot injuries . A significant number of patients were shocked at presentation and the commonist organ involved in this study was the liver . There were associated extraabdominal injuries which involved other organs of the body .Conclusion : Organized systems of trauma care are required at the trauma site , in prehospital care and during hospital care . Prompt evaluation of the abdomen is necessary to minimize preventable morbidity and mortality .

Introduction

Trauma has been considered as the neglected disease of modern society 1 . Trauma is the leading cause for death and disability in the first four decades of life 1,2,3. Patients with severe abdominal injuries after blunt or penetrating trauma appear commonly in our society. The majority of preventable deaths after blunt trauma is attributed to unrecognized abdominal injuries 4. Approximately 25% of persons who die as a result of explosive or gunshot wounds have potentially survivable wounds if appropriate care can be initiated close to the time of injury 5. Significant abdominal trauma is present in 12-15 % of patients with blunt trauma and usually occurs in association with multisystem injuries 4 .Blunt abdominal injuries are not obvious and indication for operation is not clear as in penetrating abdominal trauma 6 . Penetrating trauma of the abdomen continues to be a major cause of trauma admission in USA 7, and in our society .Stab wounds have a lower mortality than gunshot because of the lower energy transmitted 7. In blast victims even a small penetrating skin wound may be accompanied by devastating underlying trauma .Rapid loss of 2000 cc blood will result In sever shock 5 , and massive haemorrhage as occurs in massive liver injuries 8 remains a potentially preventable cause of death 9 . Coagulopathy , acidosis and hypothermia
form a lethal triad 9. Splenic injuries account for 40%-55% of abdominal traumas in the emergency treatments. Spleen is the most vulnerable abdominal organ because it is superficial and fragile 10. Spleen is the most commonly injured abdominal organ following blunt abdominal trauma 11,12. Traumatic colorectal perforation leads to peritonitis and septic shock which is responsible for disseminated intravascular coagulation and organ failure 13. Diaphragmatic injuries occur in up to 20% of patients with penetrating thoracoabdominal injuries 14. Large diaphragmatic lacerations may cause intrathoracic herniation and visceral strangulation 7. Pancreatic injury is uncommon 15,16, the incidence in blunt trauma is 0.2% and in penetrating injuries ranging from 1% to 12% 16, pancreatic injuries associated with significant morbidity and mortality 15,16. Clinical signs suggestive of intraabdominal injury include diffuse and localized tenderness, hemodynamic instability, hematuria, haematemesis, blood on rectal exam and diminished or absent lower extremity pulses. Assessment of abdominal trauma initiated by clinical examination which may be inaccurate in presence of distracting injuries altered level of consciousness and nonspecific abdominal signs 17. Beside clinical abdominal examination 4,17,18,19, there are diagnostic tests which help in the diagnosis and management of intraabdominal injuries, among these evaluating modalities are CT scanning 1,4,7,17,19,20, X-ray of the chest and abdomen 17, focused abdominal sonography for trauma (FAST) 4,7,17,18,19,21, rigid sigmoidoscopy, intravenous pyelogram, contrast cystogram 17, and laparoscopy 4,7,17. The high incidence of abdominal trauma necessitates knowledge of the management of trauma and the complications associated with it 22. The aim of the initial assessment and management is to reduce the morbidity, the mortality, and improve recovery 23. Haemorrhage control is always an early priority in the management of injured person 11. The management of intraabdominal injuries changed in the last few decades from routine laparotomy to conservative nonoperative management in a haemodynamically stable patient 2,6,7,8,12,17,20,24,25. Almost all hollow visceral injuries require operation 6, the policy of mandatory colostomy was replaced by liberal primary repair in most cases in the 1990s 7.

Methods
This prospective study evaluating 134 patients with abdominal trauma who were admitted and for whom laparotomies had been done at Zawia Teaching Hospital from January 2014 to June 2015. After resuscitation, multiple diagnostic studies were used for diagnosis of abdominal injuries including X-rays, focused abdominal sonography for trauma (FAST), CT scanning, and diagnostic peritoneal lavage. All patients with significant abdominal trauma treated with laparotomies and definite procedure according to the type and organ involved.
Results

Demographic data revealed that the greatest number of patients (123 patients=91.7%) were below the age of 40 year and the majority of them are males (121 patients=90.2%) Table 1.

<table>
<thead>
<tr>
<th>Decade</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-&lt;10years</td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>5.2</td>
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<tr>
<td>10-&lt;20years</td>
<td>27</td>
<td>2</td>
<td>29</td>
<td>21.6</td>
</tr>
<tr>
<td>20-&lt;30years</td>
<td>55</td>
<td>6</td>
<td>61</td>
<td>45.5</td>
</tr>
<tr>
<td>30-&lt;40years</td>
<td>23</td>
<td>3</td>
<td>26</td>
<td>19.4</td>
</tr>
<tr>
<td>40-&lt;50years</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>5.9</td>
</tr>
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<td>0.7</td>
</tr>
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<td>0.7</td>
</tr>
<tr>
<td>70-&lt;80years</td>
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<td>1</td>
<td>0.7</td>
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<tr>
<td>Total</td>
<td>121</td>
<td>13</td>
<td>134</td>
<td>100</td>
</tr>
</tbody>
</table>

TABLE 1. Demographic data of injured patients

The type of trauma considered were both blunt and penetrating injuries. The majority of them were penetrating type (97 patients=72.3%) caused mainly by gun shot and explosion (89 patients=66.4%) the remaining penetrating trauma caused by stabbing injuries (8 patients=5.9%). While blunt trauma recorded in 37 patients (27.6%) which mainly caused by road traffic accident (RTA) (32 patients=23.8%) and the remaining blunt trauma caused by falldown (5 patients=7.3%) Table 2.

<table>
<thead>
<tr>
<th>Type of trauma</th>
<th>Number of patients</th>
<th>percentage</th>
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</thead>
<tbody>
<tr>
<td>R.T.A.</td>
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<td>23.8</td>
</tr>
<tr>
<td>Fall down</td>
<td>5</td>
<td>7.3</td>
</tr>
<tr>
<td>Gun shot &amp; explosion</td>
<td>89</td>
<td>66.4</td>
</tr>
<tr>
<td>Stabbing</td>
<td>8</td>
<td>5.9</td>
</tr>
</tbody>
</table>

TABLE 2. Types of trauma

During intial resuscitation there were 33 patients (24.6%) with sever shock and 40 patients (29.8%) received blood transfusion.

The involved intraabdominal organs with the operations which had been done are shown in Table 3.

<table>
<thead>
<tr>
<th>Injured organ</th>
<th>Number of patients</th>
<th>Percentage of patients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Blunt trauma</td>
<td>Penetrating trauma</td>
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<tr>
<td>Liver repair</td>
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<td>25</td>
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<tr>
<td>Splenectomy</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3</td>
<td>3</td>
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<tr>
<td></td>
<td>2</td>
<td>15</td>
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<tr>
<td>Large intestine</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

TABLE 3. Injured abdominal organs and their percentage.

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In this study the majority of intraabdominal organs received more damage by penetrating trauma as compared to blunt trauma except spleen which is involved more by blunt trauma (20 patients =14.9%) The commonest intraabdominal organ involved was the liver which injured by penetrating trauma in 25 patients (18.5%) as compared to blunt trauma which occur in 16 patients (11.9%). A large number of patients had penetrating trauma (35.8%) as compared to blunt trauma (1.4%) Simple repair for large bowel injury done for 32 patients (23.8%) resection and anastomosis performed for 8 patients (5.9%) and colostomy performed in 10 patients (7.4%) gastric injuries reported in 14 patients (10.4%) which treated by surgical repair Other operations performed include mesenteric repair in 8 patients (5.9%) and got small and large bowel injuries, mainly caused by penetrating trauma. Small bowel injury occurred in 42 patients of which 39 patients (29.1%) involved by penetrating injury and 3 patients (2.2%) involved by blunt trauma. In 27 patients (20.1%) of them simple intestinal repair done and in 15 patients (11.1%) resection and anastomosis required. Large bowel injury mainly caused by.

Gastric injuries reported in 21 patients (15.6%) which treated by repair in two layers. Retroperitoneal haematoma presented in 18 patients (13.4%) most of them caused by penetrating trauma. Diaphragmatic injuries reported in 14 patients (10.4%) which treated by surgical repair. Inferior vena cava injury which treated by suture repair occurred in one patient and portal vein injury also reported in one

<table>
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<tr>
<th>organ</th>
<th>n</th>
<th>n</th>
<th>n</th>
<th>n</th>
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<td>21</td>
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<tr>
<td>Retropertoneal hematoma</td>
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<td>13</td>
<td>18</td>
<td>3.7</td>
<td>9.7</td>
<td>13.4</td>
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<td>Diaphragmatic repair</td>
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<td>12</td>
<td>14</td>
<td>1.4</td>
<td>8.9</td>
<td>10.4</td>
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<tr>
<td>Mesenteric repair</td>
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<td>6</td>
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<td>1.4</td>
<td>4.4</td>
<td>5.9</td>
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<td>Cholecystectomy</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>2.9</td>
<td>2.9</td>
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<tr>
<td>G. Repare</td>
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<td>1</td>
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<td>0</td>
<td>0.7</td>
<td>0.7</td>
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<td>0</td>
<td>1</td>
<td>0.7</td>
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<td>0.7</td>
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<tr>
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<td>1</td>
<td>0.7</td>
<td>0</td>
<td>0.7</td>
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<tr>
<td>Nephrectomy</td>
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<td>16</td>
<td>3.7</td>
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<td>Kidney repair</td>
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<td>0</td>
<td>1</td>
<td>0.7</td>
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<td>0.7</td>
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<tr>
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<td>3</td>
<td>0</td>
<td>2.2</td>
<td>2.2</td>
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<tr>
<td>Orchidectomy</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.7</td>
<td>0.7</td>
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<tr>
<td>Caesarean section</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Negative laparotomy</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>2.9</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Table 3. The intraabdominal injuries and related operations performed
patient (0.7%) treated by suture repair. Significant urologic injuries reported which treated by nephrectomy in 16 patients (11.9%), kidney repair in one patient (0.7%), urinary bladder repair in 3 patients (2.2%) and orchidectomy in one patient (0.7%). One pregnant patient had got laparotomy and caesarean section at the same time. 4 negative laparotomies after penetrating trauma reported (2.9%).

There are associated injuries to the abdominal trauma Table 4 which involve chest in 32 patients (23.8%), head injury in 5 patients (3.7%), upper limb injury in 13 patients (9.7%), lower limb injury in 17 patients (12.6%), pelvic fracture in 6 patients (4.4%) and vertebral column injuries in 5 patients (3.7%).

<table>
<thead>
<tr>
<th>Associated injury</th>
<th>Number of patients</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>chest</td>
<td>haemopneumothorax</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Fractured ribs</td>
<td>7</td>
</tr>
<tr>
<td>Head injury</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Upper limb injury</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Lower limb injury</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Pelvic fracture</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Vertebral column injury</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4. Associated extraabdominal injuries

Duration of hospital stay varies from one day to more than four days. A significant number of patients discharged against medical advice DAMA (38 patients = 28.3%) to complete their management abroad Table 5.

<table>
<thead>
<tr>
<th>Duration of Hospital stay</th>
<th>D.A.M.A.</th>
<th>Normal discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients</td>
<td>Percentage of patients</td>
</tr>
<tr>
<td>1 day</td>
<td>38</td>
<td>28.3</td>
</tr>
<tr>
<td>2 days</td>
<td>18</td>
<td>13.4</td>
</tr>
<tr>
<td>3 days</td>
<td>5</td>
<td>3.7</td>
</tr>
<tr>
<td>4 days</td>
<td>8</td>
<td>5.9</td>
</tr>
<tr>
<td>&gt;4 days</td>
<td>6</td>
<td>4.4</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>55.9</td>
</tr>
</tbody>
</table>

Table 5. Duration of hospital stay

The mortality reported in 13 patients (9.7%), were seven patients (5.2%) died after gunshot injuries, six patients died (4.4%) after RTA and nine patients had sever shock at the time of presentation.

Discussion

Trauma is one of the most common cause of morbidity and mortality in our country. The pattern of trauma was changed in the last few years in our society. Nowadays
the major cause of trauma is gunshot injuries (66.4%) as compared to the major cause of trauma in the same city and hospital which was road traffic accident (68.3%) (Zarrouk et al) . In this study it is clear that there are multiple intraabdominal organ injuries especially in penetrating abdominal trauma . Table 3 . Blunt abdominal trauma does not occur in isolation and these patients often present with hypovolemic shock and is associated with significant extraabdominal injuries many of which will determine the overall outcome . During damage control surgery resuscitation involving early administration of plasma and platelets is associated with less mortality in patients with massive haemorrhage . Older patients and patients with lower blood pressure have high mortality rate . Nonoperative management of solid organ injury is one of the notable changes in the care of blunt abdominal trauma , also selective nonoperative management is used safely for penetrating abdominal trauma . Minimal invasive surgery can be used selectively in haemodynamically stable incidious and clinical suspicion must be high . The majority of renal injuries following blunt trauma does not require laparotomy which indicated if there is urinary extravasation that is persistant 48-72 hours . Among the renal injuries which may occur is the intraperitoneal bladder rupture which may not be present with clear clinical signs when it is diagnosed necessitating surgical repair .

Prophylactic and therapeutic antibiotics are required in trauma patients to reduce the incidence of infection and sepsis . It is clear that abdominal trauma is not a single organ injury and requires multidisplinary management according to the involved organ and according to the severity of the injury .

Conclusion

Abdominal trauma does occurs in association with other extraabdominal injuries many of which will determine the overall outcome . Organized systems of trauma care are required at the trauma site , in prehospital time , and during hospital care . It is important to doresuscitation according to advanced trauma life support systems .
ATLS including the primary, secondary and tertiary survey, appropriate radiologic diagnosis and documentation of abdominal findings. Prompt evaluation of the abdomen is necessary to minimize preventable morbidity and mortality. Education is still required for our injured patients regarding their health and management to increase their thrust to the managing doctors. There is a need for effective motor vehicle safety legislation. Better parental control will help to reduce the incidence of injuries from falls. Health care services need to be increased and improved for trauma care and the facilities for nonoperative management including fully equipped intensive care unit ICU, and angiography need to be established for our patients.

References


Simulation of electromagnetics absorption in human head for mobile telephone at 900 MHz and 1800 MHz

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Abstract
In this paper, Specific Absorption Rates (SAR) inside the human head and hand have been analyzed for a handheld mobile phone with Planar Inverted-F Antenna (PIFA) is used to radiate dual-band frequency of GSM 900/1800 MHz in the vicinity of the SAM head phantom. Simulations are performed using Three-dimensional Finite Integral technique (FIT) method via CST-Microwave studio has been used to simulate SAR induced in the head and mobile. Maximum peak 1-g and 10-g SARs of 0.5073 W/kg and 0.5479 W/kg are found at antenna resonance frequency of 900 MHz for 0.125 W applied input power respectively, while maximum peak 1-g and 10-g SARs of 0.762 W/kg and 0.9041 W/kg at 1800 MHz for 0.250 W applied input power respectively. All simulations are below the limits set by ANSI/IEEE and FCC.

1. Introduction
In recent years, interest has been paid to the potential health hazards resulting exposure to electromagnetic radiation (EM) and especially the head region. Absorption of Radio Frequency (RF) fields emitted from the mobile phone may change the proliferation rate of cells, enzyme activity and affect the genes in the DNA of cells and may form tumor in living tissues [1]. It has also been reported that the opening of the blood brain barrier due to low level EM radiation emitted from a mobile phone causes to release the dangerous chemicals into the brain, leak hemoglobin and building up of which can cause heart diseases and kidney stones [2]. The specific absorption rate (SAR) is a physical quantity that used to evaluate the power absorbed by biological tissue. SAR is used to quantify biological adverse effects and formulating safety guidelines or standards on exposure to RF fields [3-4]. The guideline that provides SAR exposure limit of 1.6 W/kg for any 1 g of tissue was approved by the IEEE in 1991 and was subsequently adopted by the American National Standards Institute (ANSI) in 1992 as a replacement for the previous (ANSI C95.1-1982 guideline). In April 1993, the FCC proposed using the ANSI/IEEE C95.1-1992 for evaluating environmental RF fields created by transmitters it licenses and authorizes [6,7]. IEEE C95.1-2005 the newly approved standard represents a complete revision...
and replaces IEEE Standard C95.1-1991[6]. The SAR limit specified in IEEE C95.1: 2005 has been updated to 2 W/kg over any 10-g of tissue. IEEE C95.3 is a recommended practice for measurements and computations of radio frequency electromagnetic fields with respect to human exposure to such fields, 100 kHz to 300 GHz. Direct measurement of SAR is very difficult inside a living human head or body parts using the experimental technique. Therefore, simulations using numerical techniques are used to calculate EM field components and SAR inside human head or body parts [8]. The work described in this paper is substantially extended from our previous work [9]. In this study, a 3D handset together with the SAM phantom model including hand was used to simulate the SAR distribution over the human head. A handset with a Planar Inverted-F Antenna (PIFA) was used. The 900 MHz and 1800 MHz frequencies were chosen for the simulations in this study. Finite integration in time-domain (FITD) method is used [10-11,13].

2. Physical Model.

In this study, a mobile phone with a Planar Inverted-F Antenna (PIFA) was used. The reference power of the phone was 0.125 W and 0.250 W, defined according to the Standard IEEE C.95.3. The mobile phone is located at the right side of a human head with a certain position. A near-field radiation source for human head model is considered. Fig. (1) shows the simulation model which includes the handset with PIFA type of antenna and the SAM phantom head provided by CST Microwave Studio [12]. Standard Anthropomorphic Model (SAM) head is a homogeneous model of the human head composed of two parts: fluid and shell as shown in Fig. 2.

![Fig. (1) Complete model used for simulation including handset and SAM phantom head with hand.](image1)

![Fig. (2) Component of homogeneous head model [13]](image2)
The simulated handset model is composed of the circuit board, LCD display, keypad, battery and housing. This handset is composed of a dual-band Planar Inverted-F Antenna (PIFA) operates at 900 MHZ and 1800 MHZ is used as the radiating antenna, which is shown in Fig. (3). The electrical properties of materials used for simulation are listed in Table (1).

![Fig. (3) The provided dual-band Planar Inverted-F Antenna](image)

<table>
<thead>
<tr>
<th>Phone Materials</th>
<th>$\varepsilon_r$</th>
<th>$\sigma$ (S/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circuit Board</td>
<td>4.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Housing Plastic</td>
<td>2.5</td>
<td>0.005</td>
</tr>
<tr>
<td>LCD Display</td>
<td>3.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Rubber</td>
<td>2.5</td>
<td>0.005</td>
</tr>
<tr>
<td>SAM Phantom Head</td>
<td>$\varepsilon_r$</td>
<td>$\sigma$ (S/m)</td>
</tr>
<tr>
<td>Shell</td>
<td>3.7</td>
<td>0.0016</td>
</tr>
<tr>
<td>Liquid @ 1.8 GHz</td>
<td>40</td>
<td>1.42</td>
</tr>
</tbody>
</table>

Fig. (3) The provided dual-band Planar Inverted-F Antenna

Table (1) Electrical properties of materials used for simulation

3. Numerical Method
The Finite Integral Time-Domain technique (FITD) proposed by Weiland in 1976 [14], CST Studio software package was used as the main simulation tool. Maxwell's equations and the related material equations are transformed from the continuous to the discrete space by allocating electric voltages on the edges of a grid and magnetic voltages on the edges of a dual grid [15-16]. Fig. (4) depicts the allocation of the electric grid voltages $e$ and magnetic facet fluxes $b$ on the primary grid $G$. In addition, the dielectric facet fluxes $d$ as well as the magnetic grid voltages $h$ are defined on the dual grid $\tilde{G}$ (indicated by the tilde):

![Fig. (4). Principle of FIT calculation](image)

$e_i$: electric voltage \hspace{1cm} h_i$: magnetic voltage

$b_i$: magnetic flux \hspace{1cm} d_i$: electric flux

Fig. (4). Principle of FIT calculation [17]

The spatial discretization of Maxwell’s equations is finally executed on these two orthogonal grid systems. The electric grid voltages ($e$), magnetic facet fluxes ($b$) are allocated on the primary grid ($G$). In addition, the dielectric facet fluxes ($d$) as well as the magnetic grid voltages ($h$) are defined on the dual grid (shown by the tilde). Applying this scheme to Ampere’s
law on the dual grid requires identification of a corresponding dual discrete curl operator (C tilde). Similarly, the discretization of the remaining divergence equations introduces discrete divergence operators (S and S tilde), belonging to the primary and dual grids, respectively. As previously indicated (Fig. 5), these discrete matrix operators consist of elements '0', '1' and '-1', representing merely topological information. Finally, the complete discretized set of Maxwell’s Grid Equations (MGEs) is set up (Fig. 6)

\[
\begin{align*}
\oint_{c_e} \mathbf{E} \cdot d\mathbf{l} &= -\frac{\partial}{\partial t} \int_{s} \mathbf{B} \cdot d\mathbf{S} \\
\therefore \quad C_\epsilon &= -\frac{\partial}{\partial t} \mathbf{b}
\end{align*}
\]

\[
\begin{array}{c}
\begin{pmatrix}
e_i \\
e_j \\
e_k \\
e_l
\end{pmatrix} = -\frac{\partial}{\partial t} \begin{pmatrix}b_n \\
\end{pmatrix}
\end{array}
\]

Fig. (5) Finite Integration Technique - geometric discretization method

<table>
<thead>
<tr>
<th>Maxwell’s equation</th>
<th>Maxwell’s grid equation (MGEs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[\oint_{c} \mathbf{H} \cdot d\mathbf{l} = \int_{s} \left( \mathbf{j} + \frac{\partial \mathbf{D}}{\partial t} \right) d\mathbf{S}]</td>
<td>[\mathbf{\check{c}h} = \frac{d}{dt} \mathbf{d} + \mathbf{j}]</td>
</tr>
<tr>
<td>[\oint_{c} \mathbf{E} \cdot d\mathbf{l} = -\int_{s} \frac{\partial \mathbf{B}}{\partial t} d\mathbf{S}]</td>
<td>[Ce = -\frac{d}{dt} \mathbf{b}]</td>
</tr>
<tr>
<td>[\oint_{c} \mathbf{B} \cdot d\mathbf{S} = -\int_{V} \rho dV]</td>
<td>[\mathbf{\check{S}d} = \mathbf{q}]</td>
</tr>
<tr>
<td>[\oint_{c} \mathbf{\check{B}} \cdot d\mathbf{S} = 0]</td>
<td>[\mathbf{Sb} = 0]</td>
</tr>
</tbody>
</table>

Fig.(6) Maxwell’s equations and algebraic matrixequations.

The main benefit of FIT is the possibility to have two different materials within one grid cell, whereas in FDTD only one material is allowed within one grid cell. Due to this advantage, the mesh can be significantly sparser, and hence, less memory is required in FIT simulations, especially in the objects with complex geometry [11]. Fig.(7) shows the mesh
view for two cut planes of the complete model indicating the area with denser meshing along the inhomogeneous boundaries.

![Mesh view for two cut phones of the complete model showing the non-uniform meshing scheme adopted for simulation.](image)

Fig. (7) Mesh view for two cut phones of the complete model showing the non-uniform meshing scheme adopted for simulation.

SAM phantom head was then included for SAR calculation using the standard definition as

\[
SAR = \frac{\sigma}{2\rho} E^2
\]

where \( E \) is the induced electric field (V/m); \( \rho \) is the density of the tissue (kg/m\(^3\)) and \( \sigma \) is the conductivity of the tissue (S/m). The corresponding SAR values averaged over 1 g and 10 g of tissue in the head were denoted as \( SAR_{1g} \) and \( SAR_{10g} \), respectively. These values were used as a benchmark to evaluate the effectiveness in peak SAR reduction.

4. Simulation results
4.1. PIFA antenna simulation
The numerical calculation of the phone with PIFA, were done as follows: \( f = 900 \) and 1800 MHz, \( P = 0.125 \text{ W} \), and \( 0.250 \text{ W} \) respectively, and \( Z = 50 \text{ Ω} \). The distance from the head (ear) to the edge of the mobile phone was 1 cm. The PIFA antenna is simulated CST SOFTWARE. The parameters evaluated were gain, beamwidth and return loss. Figure 5 shows the simulated \( S_{11} \) of the complete model including handset and the SAM phantom head. Figures (8-10) present simulation of 3D farfield radiation patterns at 900 and 1800 MHz. Fig. (8-9) simulate PIFA mobile antenna parameters \( S_{11} \) namely: return loss, radiation efficiency, total efficiency and directivity, the results obtained with the presence of the human head and at a frequency 900 and 1800 MHz. The return loss for 900 MHz shows a drops around -4 dB, while the 1800 MHz also shifts to the right but with less loss at -5.5 dB.
Fig. (8). Simulated S11 of the complete model including handset and SAM phantom head showing proper operation of the antenna.

The 3D farfield radiation patterns at 900 and 1800 MHz together with are included in Figs. 9-10.

![Simulated S11 of the complete model including handset and SAM phantom head showing proper operation of the antenna.](image)

**Figure 9.** Directivity for 900 and 19800 MHz resonance frequencies (3D directivity pattern has been magnified for better presentation).

Fig. (10) presents the polar plots at two corresponding cuts for the two frequencies.

![Simulated 2D radiation patterns of the PIFA antenna in the presence of head and hand on the XY, YZ and XZ planes at 900MHz (absolute gain).](image)

**Fig.10.** Simulated 2D radiation patterns of the PIFA antenna in the presence of head and hand on the XY, YZ and XZ planes at 900MHz (absolute gain).

### 4.2. SAR simulation

The electromagnetic field emitted by PIFA mobile phone antenna is simulated at two GSM frequencies (900 MHz and 1800 MHz) to see the 3D dimension effect of SAR penetrating the head phantom averaged over a mass of 10 gram and 1 gram cubic. Table (2) shows the results of
maximum SAR value in \(XY\)-plane (ear view) and cutplane-view of \(XZ\)-plane to evaluate at both axes the absorption direction into the head.

<table>
<thead>
<tr>
<th>Operating Frequency</th>
<th>900 MHz</th>
<th>1800 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAR 10 g ear view (xy plane)</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td>SAR 10 g cutplane view (xz plane)</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td>Max SAR :</td>
<td>0.5479 (W/Kg)</td>
<td>0.9041 (W/Kg)</td>
</tr>
<tr>
<td>SAR 1 g ear view (xy plane)</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td>SAR 1 g cutplane view (xz plane)</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td>Max SAR :</td>
<td>0.5073 (W/Kg)</td>
<td>0.762 (W/Kg)</td>
</tr>
</tbody>
</table>

Simulation results in tables (2) show that the 1-g and 10-g SAR values (cutplane view \(xz\) plane) are larger as frequency increases while penetration depth decreases with increasing frequency. Also, large SAR values are observed on the head-air interface at high frequencies (900 MHz and 1800 MHz) due to the immediate change of the refraction index from the value 1 in air to a much larger value in brain matter. Tables (2) also showed variations of maximum 1-g and 10-g SARs with varies distances along \(Y\)-axis in the mid-coronal plane at 900 MHz and 1800 MHz.
IV. CONCLUSION

In this work SAR distributions and peak SAR averaged over 1-g and 10-g mass of head phantom exposed to a mobile phone designed for two different frequencies (900-1800 MHZ) have been studied using FIT method. Calculation of SAR has been performed using commercially available software CST MWS. At 900 MHz, variation of peak 1-g and 10-g SARs with distance shows that both maximum 1-g and 10-g SAR value reach to maxima near the position of the mobile phone antenna and decreases gradually with increase of the distance from the mobile phone antenna. Results obtained by the simulation show that maximum peak of 10-g SAR obtained in head surface phantom at 900-1800 MHZ are 0.5479 (W/Kg) and 0.9041 (W/Kg) respectively whereas 0.5073 (W/Kg) and 0.762 (W/Kg) peak for 1-g. Variations of peak 1-g and 10-g SARs with varies distances along Y-axis in the mid-coronal plane at 900-1800 MHZ obtained using CST-Microwave studio are observed. Simulated peak 1-g and 10-g SARs for human head with hand held mobile is compared with measured SARs available in the literature and it is observed that obtained simulated and measured SAR values are close to each other and lower than the corresponding measured values within the ANSI/IEEE and FCC safety limits.

V. References


17. CST User Manual, CST AG, Darmstadt, Germany.
Isolated Polycystic Liver Disease: A Case Report with Review of Literature

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**) Sebha Medical Center, Sebha, Libya.

Abstract

Isolated polycystic liver disease is a rare condition that was not reported in Libya or North Africa as we found when reviewing literature available on it. In this paper we present a case of a 70-years-old Libyan male that attended Sebha Medical Center with a history of right hypochondrial swelling and pain of three months’ duration. On investigation, ultrasound showed numerous liver cysts with no renal involvement. The following is a review of literature pertaining cystic liver disease.

Introduction

Simple liver cysts are common findings on ultrasound examination of the abdomen1. When the patient has more than 20 liver cysts, he is diagnosed with polycystic liver disease (PLD). Since 1856, when PLD was first described in patients with autosomal dominant polycystic kidney disease (ADPKD)2, it was believed that it can only develop in association with ADPKD. This belief remained dominant until 2003, when it was found that there were many types of polycystic liver disease and some of them can occur alone3. Now it is known that PLD can develop in three forms: the first is isolated polycystic liver disease (PCLD), the second is in association with ADPKD and the third form is as a part of VonMeyenburg complexes4.

Studies have shown that Isolated polycystic liver disease (PCLD) is linked to mutations in PRKCSH and SEC63 genes. Both of these genes have an autosomal dominant mode of inheritance6,7. A study conducted in Netherland in 2010 by Van Keimpema et al. estimated the prevalence of PCLD to be around 1 per 158008, but Lantinga et al. in a review published in 2013, think that this
figure is an underestimation. For polycystic kidney disease (ADPKD), which is the most common inherited renal disorder \(^9\), was estimated by various studies to be between 1/400 and 1/1,000\(^{10,8}\). Their belief is based on the fact that the Dutch study was conducted on 137 patients from five tertiary referral centers, and that the patients who present to these centers are the symptomatic patients \(^{11}\) who constitute about 20% of PCLD case, as this disease is commonly asymptomatic\(^{12}\).

When we reviewed the literature available in online medical databases on this condition, we found no reported cases from North Africa including Libya. Our belief is that this might be due to under reporting of this condition, and the goal of this article is to report the case we encountered, and provide a brief review of the literature regarding PCLD.

Case presentation

A 70-years-old Libyan male, came to Sebha Medical Center complaining of right hypochondrial swelling and pain for the last three months. On further questioning, he admitted to have weight loss, shortness of breath on exertion, generalized fatigability and fever. On examination he was conscious and oriented but had severe pallor. His blood pressure was normal. On cardiac auscultation, he had normal first and second heart sound with to a systolic murmur. His breath sounds were decreased bilaterally and he had bilateral crepitations which were more on the left side. Examination of the abdomen revealed soft lax abdomen with hepatomegaly. No abnormalities were detected on neurological examination.

Routine investigations were ordered in addition to ultrasound examination for abdomen and pelvis. CBC showed normocytic normochromic anemia with HB=7.1g/l, MCV= 94fl, MCH=26.4pg. GUE showed urinary tract infection with 5-6 pus cells in HPF while urobilinogen was +, Bilirubin in urine was ++. ESR value was above 100mm in the first hour. Blood glucose, renal functions tests and electrolytes were all normal and virology examination for HIV, HBsAg and HCV was negative. Liver functions tests showed abnormal results in the form of total bilirubin value of 1.4 mg/dl, direct bilirubin was 1 mg/dl, while GPT was 127 U/L, GOT = 153 U/L, ALP = 630 U/L.
Echocardiography showed signs of ischemic heart disease, mild LV enlargement and moderate TR. Ultrasound examination of the abdomen revealed hugely enlarged liver that could not be measured, with numerous cysts of different sizes in both lobes of liver. The largest cyst measured about 9.9cm x 8.2 cm (Fig.1), and the smallest one was about 4.2 cm x 4.1 cm. One of the large cysts contained thick fluid which was consistent with hemorrhage (Fig.2). The patient was evaluated with CT scan which confirmed these findings (Fig.3). The relative took the patient to Tripoli Medical Center after the diagnosis was made.

Fig. 1: Ultrasound image showing the patient’s liver with some of its multiple cysts, with the measurements of the largest one.

Fig. 2: Ultrasound image showing hemorrhage in one of the liver cysts.
Fig. 3: Axial section from non-contrast enhanced CT scan of the patient abdomen showing his enlarged liver that contains multiple cysts.

Discussion

PCLD is a rare disease worldwide, and our search of literature showed that there are no previous reported cases from Libya or North Africa. This disease does not cause any symptoms in more than 80% of its patients. In the remaining 20%, the uncomplicated cysts can lead to hepatomegaly which causes abdominal distension and discomfort in some patients. The complications of liver cysts can add more symptoms to the patient presentation. Patients having hemorrhage in the cysts can experience acute pain, while cyst infection leads to fever. The liver cysts can also compress the liver vessels and inferior vena cava leading to portal hypertension, and in some cases they exert pressure on the surrounding structures leading to early satiety, nausea and vomiting. Obstructive jaundice is a rare complication of PCLD.

When complications of PCLD were studied, it was found that about 10% of untreated patients developed complications. The most common one was cyst bleeding, which was found in 8% of the untreated. The second was portal hypertension, which was found in 3% of the patients. Each of ascites and inferior vena cava compression was found in 2% of the untreated cases. The case we encountered fits the classical presentation of
the condition and its most common complication, as it presented with symptoms of liver enlargement and had hemorrhage in one of the cysts on ultrasound. The imaging findings of our case were classical and agreed with the described literature with the presence of the cysts and their effect on the liver size and the investigations showed the abnormality in liver functions that can result from this condition. Symptomatic patients with PCD have slight increase in Alkaline phosphatase (ALP) and total bilirubin levels [12], and elevated levels of gamma-glutamyltransferase (GGT) was found in 60-70% of symptomatic cases [15], but these abnormal values are commonly found in the symptomatic patients only, and asymptomatic patients usually have normal liver functions [16]. Elevation of serum levels of the gastrointestinal tract tumor marker CA19-9 was found in about 45% of patients with PCLD. On further evaluation, the level of this marker was found to be high in cyst fluid of simple and polycystic liver disease, with no evidence of tumors [17]. Treatment is given to cases with severe symptoms or with complication. [18]. There are both medical and surgical options for managing patients with PCLD. The medical approaches depend on using analogs for cimetidine and somatostatin to reduce fluid secretion inside the cysts [15,19,20]. The surgical options are aspiration and sclerotherapy, fenestration, liver Resection and liver transplantation [14].

Conclusion

Isolated polycystic Liver disease (PCLD) is a rare genetic disorder that is usually asymptomatic and presents with consequences of hepatomegaly and complications of the cyst in symptomatic patients. Its prevalence is estimated to be around 1 per 158000 in Netherlands [8], but there are no reported cases or major studies about its prevalence or features in the literature we reviewed from Libya and North Africa. Our case report can be the beginning of efforts to fill this gap in literature.
References


Knowledge, Attitude and Reasons for Nonparticipation in Cervical Cancer Screening Programme Among Female Nurses Medical Staff in Zawia Teaching Hospital, Libya

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Zawia Teaching hospital, Zawia, Libya

Abstract:
Cervical cancer is a malignant neoplasm arising from cells originating in the cervix. One of the most common symptoms of cervical cancer is abnormal vaginal bleeding, but in some cases there may be no obvious symptoms until the cancer has progressed to an advanced (GLOBOCAN, 2008). Among all malignant tumours, cervical cancer is the one which can be most effectively controlled by organized screening programmes (Arbyan M, 2009). The aim of the present study is to examine women’s awareness of cervical cancer, to investigate women’s perception of screening programmes, Finally To determine factors influencing utilization of services. This is a descriptive and cross sectional study was conducted in Zawia teaching hospital, from August 2015 to September 2015 with total of 200 respondents. With self-administered questionnaire was used to obtained information on the socio-demographic characteristics of the respondents, Knowledge, perception about cervical cancer screening, as well as barriers against screening services. Fifty-three point five percent (107) of the women were aged 30–39 years and 63 (31.5%) were aged 40–49 years. Ten percent (20) of the respondents were aged 50 years and above. This means that the majority of the respondents were still within the reproductive age group 90% of the respondents were heard of cervical cancer and 51% heard of cervical cancer screening. 91(45.5%) and 88(44%) of staff heard about cervical cancer from a physician and TV/Radio respectively. While 89(44.5%) and 56(28%) of staff heard about cervical cancer screening from TV/Radio and physician respectively.

Key words: Knowledge, attitude, barriers, cervical cancer, screening, Libya

INTRODUCTION
Cervical cancer is well recognized as the third most leading diagnosed in overall women’s cancer disease in the world. Most cases were detected in the developing countries in comparison to the developed countries within estimated 529,409 new cases and 274,883 deaths in 2008. About 86% of the cases occur in developing countries, representing 13% of female cancers (WHO/ICO 2010).
A literature search identified studies that examine factors influencing women's in screening program, their psychological reaction to the receipt of an abnormal cervical smear result, and experiences of colposcopy (Oboma YI and Onyije FM, 2012). Reasons given for nonparticipation included administrative failures, inconvenient clinic times, unavailability of a female screener, lack of awareness of the test's indications and benefits, considering oneself not to be at risk of developing cervical cancer, and fear of embarrassment, pain, or the detection of cancer. The receipt of an abnormal result and referral for colposcopy cause high levels of distress owing to limited, many women believe the test aims to detect existing cervical cancer (Al Sairafi and Mohamed, 2009). Inadequate knowledge and lack of awareness can become a barrier to cervical cancer prevention (Al-Naggar, 2012). Many participants in previous screening studies revealed that they have little knowledge of cervical cancer (Oon et al, 2011) and early screening using the Pap test can save their lives. Respondents also reported that they perceive that cervical cancer ultimately leads to death and can never be cured (Saslow D et al., 2012; Virtanen Aet al., 2010). Some respondents also feel that insufficient information is made available about the centers providing the screening facilities (Abotchie and Shokar, 2009; Al-Naggar et al., 2010; and Anttila A, 2010).

Other respondents expressed concern that they would lose their virginity if they undertook the cervical screening test. This may relate in part to lack of knowledge regarding Pap smear screening process and test also associated with the socio-demographic background of their families (Arbyn M, Anttila A et al., 2010).

Materials and Methods

Study design:

This is a descriptive and cross sectional study was conducted in Zawia teaching hospital, the study viewpoint was conducted over a 9 month period from November 2014 to August 2015 with total of 200 respondents, aimed at assessing and documenting the perception and utilization of cervical cancer screening services among female medical staff. It sought to understand the perception of this population about cervical cancer, its risk factors, severity and prevention. With self-administered questionnaire will be designed to assess the view, knowledge, level of perception and the attitude of female medical staff towards cervical cancer screening based upon similar studies conducted elsewhere and literature review. The questionnaire was used to obtained information on the socio-demographic characteristics of the respondents, Knowledge, perception about
cervical cancer screening, as well as barriers against screening services. The questionnaire included 19 questions and was divided into three sections: awareness about cervical cancer screening and risk factors for cervical cancer; reasons for non-participation in the national cervical cancer screening programme; A face to face interview technique according to a form translated into simple Arabic language to ensure its comprehensibility. Respondents were given a free hand in response to questions and were only guided in their responses when they voluntarily called for assistance. They were also assured that the information provided would be kept confidential.

**Study Setting:**
The data will be collected from Service departments in researcher area are grouped into clinical and non-clinical departments. The clinical department consisted of 8 departments (Anaesthesia, Obstetrics & Gynaecology, Dialysis, Radiology, General Surgery, Medicine, Theatre, Paediatrics) and the non-clinical comprised 3 departments (Pharmacy, Nursing Records, and Administrative/Finance Departments).

**Sampling Procedure:**
Stratified, proportionate and simple random sampling techniques will be adopted for the selection of participated from all department.

**Data Collection Process**
Each interview started with an introduction and overview of the research including the objectives of the study. The respondents will told not to write any name on the self-administered questionnaire. Respondents were encouraged to ask questions on what they do not understand in the questionnaire. Explanations were given to respondents as required to aid their understanding of unfamiliar terms. The questionnaires were retrieved back from each respondent immediately after completion and they were reviewed for completeness.

**Statistical Analysis:**
Data entry and analysis were performed with using statistical package for social sciences (SPSS) version 14. Demographic data were summarized using descriptive statistic. Descriptive and inferential statistics such as percentages, Chi-square test, and factor analysis were used to determine the nature of the problem. The test of significance was considered when p < 0.05.

**RESULTS**
The study was successfully conducted in the selected hospital. With a good response rate. Table 1 illustrates the background characteristics of the respondents. A total of 200 female nurses were recruited for this study. Females aged 20–60 years participated in the study. Fifty-three point five percent (107) of the women were aged 30–39 years and 63 (31.5%) were aged 40–49 years. Ten percent (20) of the respondents were aged 50 years and above. This means that the majority of the respondents were still within the reproductive age group. In terms of their marital status, 53% of the respondents were single, 44% married, 1% widowed, and 2% divorced. Most of the respondents resided at rural area within 49% and 64% of respondents were Libyan nationality and 36% were non Libyan, most of them were...
Filipino (30%), 2% from Bangladesh, 1.5% from Indian and Sudan and about 1% were from Egyptian. Fifty one percent of population study had 3 children.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>5</td>
<td>2.5%</td>
</tr>
<tr>
<td>30-39</td>
<td>107</td>
<td>53.5%</td>
</tr>
<tr>
<td>40-49</td>
<td>63</td>
<td>31.5%</td>
</tr>
<tr>
<td>50-59</td>
<td>20</td>
<td>10%</td>
</tr>
<tr>
<td>&gt;=60</td>
<td>5</td>
<td>2.5%</td>
</tr>
<tr>
<td><strong>Marital Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>106</td>
<td>53%</td>
</tr>
<tr>
<td>Married</td>
<td>88</td>
<td>44%</td>
</tr>
<tr>
<td>Widowed</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>Divorced</td>
<td>4</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Nationality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Libyan</td>
<td>128</td>
<td>64%</td>
</tr>
<tr>
<td>Non Libyan (Filipino, Indian, Bangladesh, Sudan, Egyptian)</td>
<td>72</td>
<td>36%</td>
</tr>
<tr>
<td>(Filipino)</td>
<td>60</td>
<td>30%</td>
</tr>
<tr>
<td>Indian</td>
<td>3</td>
<td>1.5%</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>4</td>
<td>2%</td>
</tr>
<tr>
<td>Sudan</td>
<td>3</td>
<td>1.5%</td>
</tr>
<tr>
<td>Egyptian)</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td><strong>Place of residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Big town</td>
<td>70</td>
<td>35%</td>
</tr>
<tr>
<td>Small town</td>
<td>32</td>
<td>16%</td>
</tr>
<tr>
<td>Country side</td>
<td>98</td>
<td>49%</td>
</tr>
<tr>
<td><strong>Number of children</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>69</td>
<td>34.5%</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>5%</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>4%</td>
</tr>
<tr>
<td>3</td>
<td>102</td>
<td>51%</td>
</tr>
<tr>
<td>&gt;=4</td>
<td>11</td>
<td>5.5%</td>
</tr>
</tbody>
</table>

Table 1 Socio demographic data of respondents (n=200)

**Table 2** shows the awareness of cervical cancer and cervical cancer screening. 90% of the respondents were heard of cervical cancer and 51% heard of cervical cancer screening programme. 91(45.5%) and 88(44%) of staff heard about cervical cancer from a physician and TV/Radio respectively. While 89(44.5%) and 56(28%) of staff heard about cervical cancer screening programme from TV/Radio and physician respectively.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEARD OF CERVICAL CANCER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>180</td>
<td>90%</td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>10%</td>
</tr>
<tr>
<td>HEARD OF CERVICAL CANCER screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>102</td>
<td>51%</td>
</tr>
<tr>
<td>No</td>
<td>98</td>
<td>49%</td>
</tr>
<tr>
<td>Source of information for Cervical Cancer (For those that demonstrated awareness)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician/Health worker</td>
<td>91</td>
<td>45.5%</td>
</tr>
<tr>
<td>Family/ Friends</td>
<td>10</td>
<td>5%</td>
</tr>
<tr>
<td>Newspaper</td>
<td>6</td>
<td>3%</td>
</tr>
<tr>
<td>TV/Radio</td>
<td>88</td>
<td>44%</td>
</tr>
<tr>
<td>Internet</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>No response</td>
<td>3</td>
<td>1.5%</td>
</tr>
<tr>
<td>Source of information for Cervical Cancer screening (For those that demonstrated awareness)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician/Health worker</td>
<td>56</td>
<td>28%</td>
</tr>
<tr>
<td>Family/ Friends</td>
<td>8</td>
<td>4%</td>
</tr>
<tr>
<td>Newspaper</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TV/Radio</td>
<td>89</td>
<td>44.5%</td>
</tr>
<tr>
<td>Internet</td>
<td>12</td>
<td>6%</td>
</tr>
<tr>
<td>No response</td>
<td>35</td>
<td>17.5%</td>
</tr>
</tbody>
</table>

Table 2: Awareness of cervical cancer and cervical cancer screening
In the adjusted model screening awareness depended on nationality - Non Libyan-speaking women were better aware of the programme than the others 95% CI: 6.72-7.78 and $P$ value 0.03 those were explained in table 3 below.

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>Categories</th>
<th>(CI 95%)</th>
<th>Test statistic $P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group, yrs</td>
<td>20-29</td>
<td>1.43-1.57</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>6.48-7.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>5.82-8.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>3.95-8.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\geq$60</td>
<td>2.52-7.48</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>Single</td>
<td>6.90-8.75</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Married</td>
<td>6.11-7.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Divorced</td>
<td>6.61-8.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Widowed</td>
<td>5.48-8.52</td>
<td></td>
</tr>
<tr>
<td>Nationality</td>
<td>Libyan</td>
<td>4.81-6.99</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Non Libyan</td>
<td>6.72-7.78</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(Filipino</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indian</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bangladesh</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sudan</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Egyptian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Place of residence</td>
<td>Big town</td>
<td>6.12-7.78</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Small town</td>
<td>6.61-8.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Country side</td>
<td>5.08-8.04</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: demographic characteristics of women awareness of cervical cancer screening programme

In the questionnaire all the cervical cancer risk factors were given without stating this and women were asked whether they think that these are risk factors or not. About 93% of the respondents had no knowledge of the common cause of this disease. In general Women did not have a good overview about cervical cancer risk factors, for example, Respondents did not have any idea about the impact of smoking as a cause of cervical cancer and HPV was better known as a risk factor.

86.6% from the non Libyan women and 75.7% of the Libyan women responded that they were planning to participate in a cervical cancer screening programme. The overwhelming majority (97.7%) of the respondents had never heard about the Pap smear test. On the other hand, eight (2%) of the respondents had a correct understanding of Pap smears. Of the respondents (non Libyan) who had undergone the Pap smear test in the study, only three (0.8%) had been screened. The
three respondents who had had a Pap smear test reported that they were referred by their health care providers.

The main barriers identified by respondents for not seeking Pap smear tests were institutional and personal, as shown in Table 4. These were lack of screening sites, screening sites being too far away, limited information on cervical cancer, and absence of health education programs. The personal factors were lack of knowledge about the Pap smear test and the facilities where it can be carried out. This is because these two barriers had accounted for 16.25% and 12.73% of the total variation in the data, respectively. This means that these are the most devastating barriers. Specifically, items such as “the screening sites are too far from where I live” and “there is limited information on cervical cancer in the community” were dominant in the institutional barriers to the Pap smear test among the respondents. With regard to the personal barriers, the respondents lacked adequate knowledge about the test and where it could be done. Similarly, negative beliefs, and negative misconception barriers collectively explained about less than 47% of the total variation. The negative beliefs identified by the majority of the respondents were that the Pap smear test was embarrassing and painful. The negative misconceptions identified were that women did not feel at risk and therefore felt no need for Pap screening.

<table>
<thead>
<tr>
<th>Scale</th>
<th>Loadings(MBS)*</th>
<th>Barrier factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>There are no screening sites in the community</td>
<td>0.849</td>
<td>Institutional barriers</td>
</tr>
<tr>
<td>There is limited information on cervical cancer in the community</td>
<td>0.873</td>
<td>Institutional barriers</td>
</tr>
<tr>
<td>The screening sites are too far from where I live</td>
<td>0.939</td>
<td>Institutional barriers</td>
</tr>
<tr>
<td>There are no health education programs to promote screening</td>
<td></td>
<td>Institutional barriers</td>
</tr>
<tr>
<td>I do not know what the test is all about</td>
<td>0.376</td>
<td>Personal barriers</td>
</tr>
<tr>
<td>I do not know of any screening sites</td>
<td>0.944</td>
<td>Personal barriers</td>
</tr>
<tr>
<td>Pap smear test is painful</td>
<td>0.948</td>
<td>Negative belief barriers</td>
</tr>
<tr>
<td>Recent visit to gynaecologist</td>
<td>0.885</td>
<td>Negative belief barriers</td>
</tr>
<tr>
<td>Appointment times not suitable</td>
<td>0.188</td>
<td>Negative misconceptions</td>
</tr>
<tr>
<td>It is not necessary for me</td>
<td>0.580</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Barriers for non-attendance in the cervical cancer screening programme

*(MBS)= Mean barrier score calculated by totalling the subject responses for each question in order to get the mean barrier score. Higher scores indicate a greater perceived barrier scale.
The study revealed that not having Pap smear tests had significant associations with all seven barriers at the 5% significance level as shown in Table 5. This confirmed the negative implications of these barriers on respondents’ decision not to undergo a Pap smear test for cervical cancer. Confirming the results from the factor analysis, the Chi-square test also revealed that institutional barriers were the main barriers to seeking a Pap smear test, followed by personal barriers, since they had the highest Chi-square values of $28.965 (df=4; P=0.000)$ and $26.055 (df=5; P=0.000)$, respectively.

<table>
<thead>
<tr>
<th>Barriers</th>
<th>Chi-square values</th>
<th>$Df$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal barriers</td>
<td>26.055</td>
<td>5</td>
<td>0.000</td>
</tr>
<tr>
<td>Institutional barriers</td>
<td>28.965</td>
<td>4</td>
<td>0.000</td>
</tr>
<tr>
<td>Negative belief barriers</td>
<td>21.915</td>
<td>4</td>
<td>0.000</td>
</tr>
<tr>
<td>Negative misconception barriers</td>
<td>20.965</td>
<td>4</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 5 : Association between Pap smear test and the barriers to Pap smear test

**Discussion**

Libya has a population of 2.21 million women ages 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 241 women are diagnosed with cervical cancer and 95 die from the disease. Cervical cancer ranks as the 3rd most frequent cancer among women in Libya and the 7th most frequent cancer among women between 15 and 44 years of age. Data is not yet available on the HPV burden in the general population of Libya. However, in Northern Africa, the region Libya belongs to, about 3.0% of women in the general population are estimated to harbor cervical HPV-16/18 infection at a given time and 81.2% of invasive cervical cancers are attributed to HPVs16 or 18 (ICO - HPV Cancer Libya 2014). This study had three principal aims. First, to estimate which socio-economic characteristics are associated with female nursing awareness about cervical cancer. Secondly, to investigate women’s perception of
screening programmes and risk factors for cervical cancer. And finally, to study reasons why so many Libyan women do not participate in the cervical cancer screening programme. An important outcome of my study was that approximately a half of the respondents were not at all or were only partially aware of cervical cancer screening. According to Libya Human Papilloma virus and proportion (49%) of the sampled population had never heard of cervical cancer screening. This finding is consistent with other research, which reported a lack of knowledge about cervical cancer among women in neighbourhood countries (Getahun F, 2013). Education on cervical screening through the mass media and health talks in delivering health care are imperative to informing women about cervical cancer and the facilities available for them. Opportunistic screening in health facilities could be encouraged to improve screening uptake, especially in women in rural area. It is evident that information about cervical cancer needs to be made available to women through mass campaigns about the disease, especially specific preventive measures and the screening facilities available. According to the WHO, cervical cancer is caused by HPV, which is a sexually transmitted infection and mostly affects sexually active men and women. However, in the current Related Cancers, Fact Sheet 2014 (Dec 15, 2014). No data available on Cervical screening practices and recommendations. The study results revealed that there is a strong need to improve women’s knowledge about cervical cancer risk factors. Knowledge of women on cervical cancer and the Pap smear test are critical in cervical cancer prevention strategies. The results of this study shows that a great study, only few respondent (non Libyan) knew that cancer of the cervix could be transmitted sexually. This implies that a greater proportion of sexually active women might acquire HPV through sex without them knowing the source of the infection. Awareness of the Pap smear test was low of the respondents had never heard about the Pap smear test. This is consistent with the findings of previous studies, in underdeveloped countries. In the present study, only 2% had correct understanding of Pap smears, ie, they could describe the test and identify facilities where one can obtain such services. This affirmed the findings of Kumar V (2011), in which a significant proportion (49%) of those who had been screened had inadequate knowledge about Pap smears. In Libya and other developing countries, there is poor institutional framework to promote screening. This is different from industrialized nations, which have largely succeeded in implementing
successful programs. A possible explanation is that women will engage in cervical screening if they are well-informed about it and the enabling factors are present to facilitate effective screening uptake. In the present study, institutional and personal factors were the main barriers regarding seeking Pap smear tests in Zawia, Libya. These barriers could impact the health-seeking behaviors of women at the community level. Evidence suggests that inadequate information on cervical cancer most important of this study was the response rate, probably concerning especially women who are not planning to participate in screening. This is a common problem in studies among non-attenders in a population with a very low attendance rate in screening. In the early stage of planning the study, I had to consider the fact that women who are most likely to respond to the questionnaire are the ones who wish to participate in the screening.

Conclusion

Cervical cancer is a problem of global health concern. Cervical cancer screening services such as the Pap smear test might be effective in detecting early precancerous lesions. A greater proportion of the staff respondents had little or no knowledge of cervical cancer screening. There is a need for the authorities of tertiary educational institutions and particularly those of Zawia teaching hospital to incorporate regular cervical cancer screening into the health care of their staff. Adoption of alternative screening techniques, such as visual inspection with acetic acid (VIA) may be necessary to widen patients’ coverage. Pap smears should be accorded priority like other Maternal and Child Health Programs.
state government needs to put in place a policy on screening for cervical cancer with appropriate screening guidelines.
REFERENCES


a. at http://www.who.int/hpvcentre website.

ICO HPV Information Centre Institut Català d’Oncologia Avda. Gran Via de l’Hospitalet, 199-203 08908 L’Hospitalet de Llobregat (Barcelona, Spain)

14. e-mail: info@hpvcentre.net internet adress: www.hpvcentre.net
Histo morphology of Helicobacter positive gastric biopsies - Libyan study of 607 cases

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Abstract

Helicobacter pylori (H. pylori), a microaerophilic, flagellated, curved or spiral, gram-negative bacterium, selectively colonizes the human stomach. Its infection affected more than half of the world’s population. It is assumed that the sequence of events in gastric cancer is as follows: chronic gastritis, atrophy, intestinal metaplasia (IM), dysplasia, and carcinoma. It is also known that H. pylori can be involved in the chain of these chronic phenomena.

Aim: To know the local incidence and histomorphological pattern of Helicobacter gastritis, especially its association with intestinal metaplasia, dysplasia and malignancy in gastric biopsies.

Methods: Gastric biopsy cases were selected from database of Tripoli medical center from 2002 to 2008. The biopsies were assessed for the parameters as per revised Sydney System. The age, gender and the microscopic findings in the gastric biopsies were tabulated and analysed.

Conclusion: Based on these results, H. pylori is a known common associated agent for chronic gastritis with the progression of atrophy, metaplasia and dysplasia and the high bacterial load possibly results in lymphoid proliferation.

Key words: H. pylori, chronic gastritis, gastric carcinoma

Introduction

Gastric cancer is the second commonest fatal malignancy in the world with a high incidence in China. H. pylori infection is an important factor in the pathogenesis of many gastrointestinal diseases including gastric cancer and even in some extraintestinal disorders. The clinical outcome of this disease is dependent on many variables, including H. pylori genotype, innate host physiology, genetic predisposition and environmental factors [1,2]. The studies showed that eradication of H. pylori infection, especially at the early stage, is effective in preventing H.pylori-related gastric carcinogenesis and this strategy is more useful in patients without atrophic gastritis or intestinal metaplasia [3]. The infection is widespread throughout the world, and is present in about 50% of the global human population; with 80% in developing countries and 20% in industrialized countries[4]. It is the major cause of chronic gastritis that plays a key role in the etiology of peptic ulcer, but there are controversial reports regarding its pathogenesis in intestinal metaplasia, dysplasia and malignancy [5, 6, 7]. The prevalence of Helicobacter gastritis in Libya remains unknown. The current study is aimed to know the local incidence and histomorphological pattern of Helicobacter gastritis, especially its association with intestinal metaplasia, dysplasia and malignancy in gastric biopsies.
Methods

We selected all gastric biopsy cases from database of department of anatomical pathology, Tripoli Medical Centre referred during January 2002 to December 2008. This retrospective review consists of only those patients demonstrating the H. pylori bacteria in their gastric endoscopic biopsies, who presented with clinical features of gastritis. Gastric biopsies were paraffin embedded, sectioned at 4 μm and stained with haematoxylin and eosin. The bacteria was identified with its spiral/ curved morphology either in H&E stained sections or in modified Giemsa sections. These biopsies were scored semiquantitatively according to the updated Sydney classification [8]. The following histological features were examined on each slide: type of gastritis, density of inflammation, density of H pylori infection, eosinophil count, lymphoid aggregates, intestinal metaplasia and dysplasia. The age, gender, presence, site of ulcer and the microscopic findings in the gastric biopsies were tabulated and analysed.

Sydney classification:

<table>
<thead>
<tr>
<th>Feature</th>
<th>Definition</th>
<th>Grading guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic inflammation</td>
<td>Increased lymphocytes &amp; plasma cells in lamina propria</td>
<td>Mild, moderate, or severe increase in density</td>
</tr>
<tr>
<td>H pylori density</td>
<td>H pylori density</td>
<td>Mild colonization: Scattered organisms covering less than 1/3 of the surface. Severe: large clusters or a continuous layer over 2/3 of surface. Intermediate numbers: moderate colonization</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>Intestinal metaplasia of surface epithelium</td>
<td>Mild: less than 1/3 of mucosa involved. Moderate: 1/3 to 2/3. Severe: more than 2/3</td>
</tr>
</tbody>
</table>

Results

H. pylori were detected histologically in a total of 607 patient’s gastric biopsies, out of 1256 patients who underwent endoscopic gastric biopsy, during this seven years period, constituting 48.3%.
The age & sex distribution

There were 275 male (45.3%) & 332 female (54.7) cases. In male, the maximum cases were seen in 7th decade (18.2%) and in female the maximum cases were seen in 3rd decade (23.2%). Minimum age was a boy with 10 years old and the maximum age was a female with 88 years old (Chart 1).

Chart 1: The age & sex distribution in helicobacter associated gastritis.

The presence and the site of ulcer

There were 137 cases (22.6%) of helicobacter gastritis with ulcer and 470 cases (77.4%) without ulcer. The ulcer was in gastric corpus in 5 cases (3.6%), 31 cases (22.6%) in antrum and in 101 cases (73.7%), the information about site was not mentioned. There were no cases with perforation of ulcer. The ulcer size was varied from 0.5-2.0 cm in maximum dimension, majority (71%) less than 1.2 cm.

The type of gastritis

The majority of cases are of non-atrophic gastritis, which was noted in 529 (87.4%) cases. Atrophic gastritis was found in 78 cases (12.6%).

The density of inflammation
There were 85 cases (14%) of mild inflammation, 220 cases (36.4%) of moderate inflammation and 300 cases (49.6%) of severe inflammation (Chart 2). The inflammatory cells were mainly lymphocytes, plasma cells and polymorphonuclear infiltrate in active inflammation (Figure 1). Two cases did not show significant inflammatory cell infiltrate.

**Chart 2:** The density of inflammation in helicobacter associated gastritis.

**Figure 1:** Photomicrograph reveals chronic active gastritis with dense inflammatory cells (x5 & x10 power) (H & E stain).
The density of helicobacter infection

There were 170 cases (28%) with mild density, 268 cases (44.2%) with moderate and 169 cases (27.8%) of severe density (Chart 3). The bacteria were seen as spiral or curved shaped mainly on the surface epithelium and in some cases in gastric pits (Figure 1).

Chart 3: The density of helicobacter infection in helicobacter associated gastritis

Figure 2: Photomicrograph reveals H. pylori spiral organism (arrow) (x40 power) (H & E stain).
Distribution of H. pylori & inflammation

In cases with mild density of bacteria, 70 cases (41.1%) showed moderate inflammation, 62 cases (36.5%) with mild inflammation and 38 cases (22.4%) with marked inflammation. Among biopsies with moderate density of bacteria, 13 cases (4.9%) showed mild inflammation, 116 cases (43.3%) showed moderate inflammation and 139 cases (51.9%) showed severe inflammation. In severe density of bacteria, ten cases (5.9%) showed mild inflammation, 34 cases (20.1%) showed moderate inflammation and 125 (74%) cases showed severe inflammation (Chart 4).

Chart 4: Distribution of H. pylori & inflammation in helicobacter associated gastritis.

Eosinophil count and lymphoid aggregate density in helicobacter gastritis

Eosinophil infiltrate were noted in 32 biopsies. Occasional eosinophils were found in 4 cases (0.7%), few eosinophils found in 18 cases (3%) and many eosinophils found in 10 cases (1.6%) whereas the majority was negative for eosinophils (575) cases (94.7%). The gastric biopsies of 260 cases revealed lymphoid aggregates (Figure 3). Occasional lymphoid aggregate was found in 30 cases (4.9%), few lymphoid aggregate in 82 cases (13.5%), many lymphoid aggregate was found in 148 cases (24.4%) and 347 cases (57.2%) were negative.

Among the cases with lymphoid aggregates, severe lymphoid aggregates appeared to show marked density of bacteria, moderate lymphoid aggregates in majority of cases appeared to show marked bacteria and about one third cases with mild to moderate bacteria. Even majority of cases with mild lymphoid aggregates, also revealed severe bacterial density (Chart 5).
Chart 5: The relation of the density of lymphoid aggregate with the density of H. pylori.

Figure 3: Photomicrograph reveals lymphoid aggregate (x5 power) (H & E stain).

The presence of intestinal metaplasia in helicobacter gastritis

56 cases (9.2%) showed intestinal metaplasia (Figure 4). Incomplete metaplasia was found in 53 cases (94.6%) whereas complete metaplasia was rare and detected in 3 cases only (5.4%) and 551 cases (90.8%) was negative. Two cases of metaplasia was in the body, 10 cases was in the antrum and 44 cases the site was not mentioned (Chart 6).
The presence of dysplasia in helicobacter gastritis

Twenty one (3.5%) of gastritis cases showed gastric dysplasia. Low grade dysplasia was found in 14 cases (66.7%) and high grade dysplasia was found in seven cases only (33.3%). Among these, eleven cases of low grade dysplasia and 3 cases of high grade showed associated incomplete metaplasia.
Discussion

Since the discovery of H. pylori in 1983 by Warren and Marshall, there were several reports stating the prevalence and various lesions associated with the bacteria. In current gastric biopsy based study, we demonstrated H. pylori in 48.3% of gastric biopsies. This incidence appears less than other North and West African countries. Jemilohun and his coworkers [9] showed H. pylori in 63.5% of 52 gastritis patients with dyspepsia and in Dooley study [10] H. pylori was detected in 85.7% of 42 (40-70 %), which increases with age to 85-90 % [13]. Prevalence of gastritis increased significantly in advancing age with maximum(47%) in 7th decade [10]. We basically studied H. pylori in symptomatic patients who underwent endoscopic biopsy. Adults who currently harbour the organism are more likely to have been infected in childhood.

Sultan and Li[14] noted the infection rate similar in male and female and the prevalence rate is less than 10% among children less than 12years, 20% in less than 30years and above 50% in aged more than 60. In current study, in male, the maximum cases were seen in 7th decade (18.2%) and in female the maximum cases were seen in 3rd decade (23.2%). We noted higher incidence among female(54.7%) than male; similar to Jemilohun study [9]. On the other hand Zhang and his co-workers [15] reported the prevalence of H. pylori infection among males higher than that among females. Ramshoo and his colleagues [16] found a high significant association between H. pylori infection with chronic gastritis both in peptic ulcer patients and healthy volunteers. He noticed 90% association of H. pylori infection and chronic gastritis in peptic ulcer patients. Colonization of the asymptomatic gastritis cases. The prevalence of H. pylori infection appears to vary in different countries, though in general higher in children than in adults, Mohammad and his colleagues [11] noted the prevalence of H. pylori of 72.3% among Egyptian school children, possibly due to lower standards of personal hygiene in younger populations [12]. The prevalence of H. pylori in Israel, Algeria, Saudi Arabia, Turkey with a high level of infection in childhood gastric mucosa with H. pyloriresulted in the development of chronic gastritis in all cases of Kandinskystudy [2]. Our study revealed histological frank chronic gastritis in all except two cases (with minimal inflammatory cells) among 607 patients and chronic ulcer was noted in 22.6% of these patients. The ulcer incidence appears to be high comparing to Nigerian study revealing 6.4% [17]. Most H. pylori infected individuals show antral predominant gastritis, which predisposes them to duodenal ulcers, but rarely causes gastric cancer. On the contrary, patients with corpus-predominant gastritis are likely to develop gastric ulcers, gastric atrophy, intestinal metaplasia and eventually gastric cancer [18]. In our study the ulcer was in gastric corpus in 5 cases (3.6%) and 31 cases (22.6%) in antrum and in 101 cases (73.7%), the information about site was not mentioned. Zhang and his coworkers [15] identified glandular atrophy and intestinal metaplasia in 40.3% and 39.9% respectively among antral biopsy, and 14.1% and 13.6% among corpus biopsy. We observed atrophic gastritis and intestinal metaplasia in 12.6% and 9.2% of Helicobacter positive cases respectively. The intestinal metaplasia is mainly seen in antral biopsies (93.3%). Incidence of intestinal metaplasia is similar to Naeem’s study [19]
(9.52%), which also noted severe inflammation in 47.6% cases, eosinophil infiltrate in 33.3% cases and lymphoid infiltrate in 23.8% cases. We also found severe chronic inflammation in 49.6% cases, but noted lymphoid aggregates in 42.8% cases and eosinophils only in 5.3% cases. Carilho and his colleagues [20] noted gastric atrophy in 8.3% and intestinal metaplasia in 8.3%. While eosinophils have been observed to comprise part of the inflammatory reaction in acute H. pylori gastritis, the role of the eosinophil in the pathogenesis of chronic gastritis is usually associated with severe inflammation (74%), or moderate inflammation (20.1%). Topal and coworkers investigated the relation of H. pylori with chronic atrophic gastritis, intestinal metaplasia, and bcl-2 in 52 cases. They found that more the H. pylori intensity, the greater the degree of chronic gastritis, activity and atrophy. The majority of our cases are of non-atrophic gastritis (87.4%) cases. Though several cases were with high density of bacteria, cases going for glandular atrophy were relatively less. Follow-up study among 3433 adults in China, a region with very high rates of gastric cancer demonstrate that H. pylori was associated with significant increased risk of progression to dysplasia or gastric cancer with odd ratio 1.8% [24]. Gastric cancer developed in 36 (2.9%) of 1526 Japanese H. pylori-infected patients, especially those with severe gastric atrophy and intestinal metaplasia [25]. Our study showed twenty one (3.5%) of gastritis cases with gastric dysplasia, which is very low in comparison to other study. Naeem [19] from Pakistan detected 19% and Yeh [7] from Malaysia noted 31.2%. Low grade dysplasia was found in 14 cases (66.7%) and high grade dysplasia was found in seven cases only (33.3%). Among these, eleven cases of low grade dysplasia and 3 cases of high grade showed associated incomplete metaplasia. In conclusion, our findings acknowledges the global fact that H. pylori is a known common associated agent for chronic gastritis and appear to follow Correa cascade [26] progression of atrophy, metaplasia and dysplasia. The high bacterial load possibly results in lymphoid proliferation. The prevalence of atrophy and intestinal metaplasia is in accordance with some other African countries and that of dysplasia is very low, indicating the further study of CagA genotyping of H. pylori in this region. But the dysplasia is strikingly associated with incomplete dysplasia, which warrants the need of endoscopic surveillance. The possible role of other dietary, environmental and genetic cofactors for gastric oncogenesis remainsto be elucidated.

References


Abdominal Tuberculosis presented with Ileocaecal Stenosis – A case report with review of literature

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Abstract

Extra pulmonary tuberculosis occurs in 5 to 15% of all cases of tuberculosis whereas tuberculosis affecting gastrointestinal tract occurs only in 1% of all cases of tuberculosis. The gastrointestinal tuberculosis most commonly presents as peritonitis.

In this article we are going to report a case of abdominal tuberculosis. A 29-year-old Libyan female presented with sub acute intestinal obstruction due to thickening of terminal ileum and ileocaecal stenosis. She underwent surgery and histopathological study revealed abdominal tuberculosis.

We reviewed the articles related to abdominal tuberculosis published in literature.

Key words
Abdominal tuberculosis, gastrointestinal tuberculosis, thickening of terminal ileum and ileocaecal stenosis

Introduction

Tuberculosis (TB) is a common infectious disease in the developing world and caused by Mycobacterium tuberculosis. An estimated two billion people are infected with Mycobacterium tuberculosis worldwide, out of which about 8.8 million people develop active tuberculosis each year. In addition to this, tuberculosis kills 1.6 million people each year.

It commonly affects the lungs causing pulmonary tuberculosis. But, it can also affect outside the lungs causing extra pulmonary tuberculosis. Extra pulmonary tuberculosis can affect any site in the body. The most common sites affected in order of their frequencies of occurrence are lymphatic system, genitourinary system, bones and joints, blood, meninges and abdomen. Abdominal tuberculosis occurs in about 2% of all cases of tuberculosis. These figures are different in cases of patients with immunodeficiency. Immunodeficient patients are more likely to have extra pulmonary tuberculosis than those with normal immune system. For example, about 50% of HIV patients with tuberculosis have extra pulmonary tuberculosis whereas about 11% of HIV patients with tuberculosis have abdominal tuberculosis.

Case presentation

A 29-year-old Libyan female presented to the department of surgery with pain abdomen for the last ten days. It was sudden in onset, severe, continuous and colicky in nature. It started at the right lower abdomen and then spread over the entire abdomen. She also complained of similar previous episodes. The pain was associated with vomiting of a large amount of green material and it was not related to meal.

She revealed a history of chronic dry cough, shortness of breath and wheezing. She was diagnosed as a case of pulmonary tuberculosis about 6 years back and received treatment for one year. Her brother is a known case of pulmonary tuberculosis. She had a past surgical history of appendectomy about 6 years back.

On examination, she was conscious and oriented with a pulse rate 96 bpm, blood pressure 120/85 mm Hg, respiratory rate 28 per minute and temperature 37.5°C. No abnormality was found on examination of heart and lungs. Abdomen was soft, lax with centrally placed umbilicus, not
distended, moving normally with respiration, but there was generalized tenderness on palpation with positive bowel sound. Her nervous system was normal.

On admission, her CBC revealed WBC 7.1 x 10³/µL, RBC 4.4 x 10⁶ /µL, HGB 11.8 g/dL, HCT 37%, MCV 84.1 fL, MCH 26.8 pg, MCHC 31.9 g/dL, PLT 489 x 10³ / µL. Her blood biochemistry reports included random blood sugar 89 mg/dL, urea 20 mg/dL, creatinine 0.5 mg / dL, total bilirubin 0.5 mg/dL, AST 24 U/L, ALT 18 U/L and ALK 70 U/L. Her blood group was ‘O’Rh (-). Serology tests for HIV, HBV and HCV were found negative.

Chest x-ray (fig 1A), x-ray abdomen in erect posture (fig 1B) and contrast-enhanced CT scan of abdomen and pelvis (fig 2) were done.

Figure 1. A. Normal chest x-ray (metal artefact visible) B. X-ray abdomen in erect posture showing no significant abnormality (metal artefact visible)
Fig 2. Contrast enhanced CT scan of abdomen and pelvis using intravenous, oral and rectal contrast showing focal thickening of the walls of terminal ileum. Three views are taken. A. axial B. coronal and C. sagittal

The patient underwent laparotomy. The thickened segment of terminal ileum was excised and sent for histopathological studies. Intraoperative and postoperative images of the respected segment of the terminal ileum were taken (fig 3).

Fig 3. A. Part of the terminal ileum showing homogenous grayish-yellow cut section with cassation-like material and ulcers with thickened edges and shallow floor B. Part of the ascending colon with the surrounding momentum showing multiple nodules of variable sizes (lymph nodes)

The histopathological report revealed tuberculosis of ileocaecal junction with free surgical margins, involvement of 26 lymph nodes and tuberculosis peritonitis. In addition, mental tissue showed tuberculosis deposits and eight mesenteric lymph nodes in the sample were all involved.

Discussion

In abdominal tuberculosis, *Mycobacterium tuberculosis* can infect the peritoneum, gastrointestinal tract, intraabdominal solid organs and mesenteric lymph nodes.\(^5,6\) This is more common in adults as well as in children under the age of ten years representing about 10% of the cases of abdominal tuberculosis.\(^7\) The most common age group affected by this condition is between 25 and 45 years.\(^8\) *Mycobacterium tuberculosis* infects the abdomen either through blood from the primary tuberculosis in the lung or through direct spread from an adjacent infected tissue.
Ingestion of infected sputum is also a possible mechanism of abdominal infection.\(^9\)

The symptoms associated with abdominal tuberculosis are usually non-specific and may lead to misdiagnosis or delay in diagnosis.\(^10\) The delay in diagnosis is estimated to be at least four months in 70% of peritoneal tuberculosis in adults.\(^11\) The most common symptoms of abdominal tuberculosis include fever, abdominal pain and weight loss.\(^12\) Additional symptoms in chronic cases are anorexia, constipation, diarrhoea, malaise and night sweat.\(^5,12\) About one third of the patients may present with acute problems such as intestinal obstruction, perforation and peritonitis.\(^5\)

On examination of abdomen, no abnormality is usually detected. Sometimes a firm mass may be palpated in the right lower quadrant of the abdomen which is usually caused by hypertrophy of the iliac region of the small bowel.\(^5\) Sometimes a mass may be palpated in the central abdomen and it results from enlargement of the mesenteric lymph nodes.\(^6\) A chronic abdominal problem of unknown origin, for example unexplained intestinal obstruction or unexplained ascites or mass, should raise a high index of suspicion for tuberculosis.\(^6\)

The most common site of intestinal obstruction in abdominal tuberculosis is the ileocaecal region and comprises of 52% to 85% of the cases.\(^13\) Other less common sites of intestinal obstruction in abdominal tuberculosis include jejunoleum, colon and anorectal area.\(^14\) The causes of ileocaecal region being the most common site of intestinal tuberculosis and tuberculous intestinal obstruction are thought to be the presence of large amount of lymphoid tissue in this region, physiological stasis in that area and high rate of absorption (especially of fluid and electrolytes) with minimal digestion taking place in that region.\(^4,5,14\)

Diagnosing abdominal tuberculosis is not easy and needs a high index of suspicion. Routine haematological tests usually have little value in diagnosing abdominal tuberculosis.\(^9,15\) They usually reveal mild normocytic, normochromic anaemia, thrombocytosis and lymphocytosis with elevated ESR.\(^15\) Imaging plays an important role in diagnosing abdominal tuberculosis. A chest x-ray showing pulmonary tuberculosis supports the diagnosis of abdominal tuberculosis.\(^2,6\) However, the chest x-ray in patients with abdominal tuberculosis mostly appear normal,\(^6\) except in one study where about 50% of the patients with abdominal tuberculosis had abnormal findings in their chest x-ray.\(^9\) A normal chest x-ray with no evidence of pulmonary tuberculosis does not rule out the diagnosis of abdominal tuberculosis.\(^2,6\) X-ray abdomen may show intestinal obstruction and bowel perforation.\(^6\)
Ultrasonography of abdomen may show dilated loops of small bowel, diffuse or focal thickening of bowel wall, thickened peritoneum with enlarged mesenteric lymph nodes in addition to the ability of ultrasound of detecting ascites if it is present. CT scan of abdomen can show the thickening of bowel wall, inflammation and stricture outside the bowel wall, matted bowel calcification, irregular ureteral thickening and hydronephrosis can be seen when tuberculosis affects the urinary tract. Tuberculosis rarely affects the liver, spleen, pancreas and adrenal glands, but when it does, hypodense masses (tuberculoma) can be seen in these organs and in cases of military tuberculosis numerous very small hypodense foci can be seen in liver and spleen.

Acid fast bacilli can be detected in ascitic fluid and with more difficulty in lymph nodes and gastrointestinal tissues. In case of tuberculous peritonitis, examination of ascetic fluid reveals lymphocytosis, high protein content (>30 g/L), high LDH level (>90 IU/L) and low serum-ascitic fluid albumin gradient (SAAG) (<11 g/L).

Colonoscopy is useful in visualizing ulcerative, stricturous and hypertrophic bowel lesions and for taking biopsy samples from them while loops and mesentery, omental mass, enlarged intra-abdominal lymph nodes and it can detect ascites also. Ascitic fluid is rich in protein and cells in case of tuberculous peritonitis and this is the reason why it usually has high attenuation (25-45 HU). Focal nephritis, nodular masses in the kidney, renal parenchymal scarring and laparoscopy or laparotomy can help in visualizing the intraabdominal lesions and sampling them. Histopathological examination of the biopsy samples can reveal granuloma with caseation.

A combination antituberculous drug therapy for 6-9 months with isoniazid, rifampin, pyrazinamide and ethambutol is the recommended first line of management unless there is a need for surgical intervention. Surgery is recommended when there is strong suspicion of abdominal tuberculosis, but no tissue could be obtained for examination or in cases of acute abdomen. If surgery is indicated, usually conservative procedures such as limited segmental ileocecal resection or strictureplasty are preferred and the acute complications of abdominal tuberculosis such as peritonitis, perforation, can be treated surgically as needed.

References

Effect of Cigarette Smoking on Lipid Profile
In Male in the City of Zawia – Libya

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Abstract
Objective
The present study aimed to compare the lipid profile between smoker and non smoker and to evaluate the durational significance on lipid profile in the smokers and to clarify the influence of daily cigarette smoking on the components of lipid profile in Libyan males.

Methods
The study was conducted on 50 healthy cigarettes smokers and compared with 50 healthy age and weight matched non-obese non-smokers who served as controls. Subjects in both groups were in the age range of 30-60 yrs., having no history of alcohol abuse, or diseases like diabetes mellitus, hypertension, hepatic impairment, renal disease, gout, hypouricemia, and obesity, and were neither on drugs like β-blockers, lipid lowering drugs, or thiazide diuretics. Clinical data were obtained from the history and record on questionnaire sheet. The clinical assessment was done by (physician) a medical doctor.

Results:
A significantly increased of serum total Cholesterol, Triglyceride and Low density lipoprotein cholesterol with significant decreased in serum High density lipoprotein cholesterol level in smokers as compared to non smokers and same results were found in smoker group with > 15 cigarettes smoked per day while with increase duration of smoking the TC & LDL-C were increased, TG showed no difference while the HDL-C was decreased showing greater risk of these persons to atherosclerosis and coronary heart disease.

Conclusions:
This study concluded that cigarette smoking causes alteration in lipid profile. Increased duration of smoking and number of smoked cigarettes / day causes more dyslipidaemia. This smoking might be related in the alteration in serum lipid profile levels, and may be the major causes of increases risk for coronary artery and hence cardiovascular disease among cigarettes smokers.

Key words:
Cigarette Smoking, Coronary Heart Disease, Dyslipidaemia, Lipid profile and Tobacco consumption.

INTRODUCTION:
Cigarette smoking is generally considered as associated with increased risk of a variety of medical disorders. Such as chronic bronchitis and emphysema, carcinogenesis and for cardiovascular disease¹ ². Several studies provide the evidence that cigarette smoking is strongly associated with altering...
the normal status of the lipid profile

Cigarette smoking increases risk for death from all causes in men and women. The risk of dying from cigarette smoking has increased over the last 50 years in men and lung cancer. Smoking is estimated to increase the risk for coronary heart disease by 2 to 4 times for stroke by 2 to 4 times, of men developing lung cancer by 25 times, of women developing lung cancer by 25.7 times. Smoking causes diminished overall health, increased absenteeism from work, and increased health care utilization and cost. There is increasing experimental evidence that oxidation of low density lipoprotein cholesterol (LDL-C) plays a major role in the pathogenesis of coronary artery disease (CAD) among smokers. Nicotine and other toxic substances from Tobacco smoke are absorbed through the lungs into the blood stream and are circulated throughout the body. These substances damage the blood vessel walls, which allow plaques to form at a faster rate than they would in a non smoker. Nicotine increases the amount of bad fats (total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG)) circulating in the blood vessels and decreases the amount of good fat (high-density lipoprotein cholesterol (HDL-C) availability. Nicotine induces oxidative stress, generates free radicals that attack on the membrane lipids resulting in the formation of malondialdehyde (MDA), which causes peroxidative, tissue damage.

MATERIALS & METHODS:

The study was conducted from January 2016 to May 2016 at. Az Zawiyah Research Center –Libya. A total of 50 healthily male were enrolled in this study in the age of 30 – 60 Years, 50 were smokers [44.2±20Years] and 50 were non smokers [42.10±13.39Years]. The local ethics committee approved the study. Before participation, volunteers were fully informed of the nature and purpose of the study and written consent was obtained from each. The smoker group was sub classified according to smoking number of cigarette /day into two group smoking less than 20 cigarettes/ day and smoking more than 20 cigarettes/ day, and according to the duration of smoking sub classified to smoking for less
than 20 years and smoking for more than 20 years. Blood samples were obtained following an overnight fasting. Samples were withdrawn from a cubital vein into blood tubes (plain containers). The serum was then separated from the cells by centrifugation at 3000 r/min for 10 min and immediately stored on ice at 4°C. Serum Cholesterol, Triglyceride, HDL-C and LDL-C was measured by using the enzymatic method using Cobas integra 400 plus - Roche, the reference value are [TC < 200mg/dl, TG < 200mg/dl, HDLC > 55mg/dl and LDL 49 -172mg/dl].

STATISTICAL ANALYSIS:

Data were expressed as mean ± standard deviation (SD). The means were compared using independent sample t.test. Analysis was two-tailed and a p -value ≤ 0.05 was considered as statistically significant.

RESULTS:

Baseline characteristics of the 100 participants (male), 50% of them were non smoker (n = 50) aged 42.10±13.39 years and 50% were cigarettes smoker (n = 50) and they were 44.20±20 years of age. The mean ± SD values for serum cholesterol, triglycerides, LDL-C and HDL-C are given in Table 1. All the components of lipid profile studied (Cholesterol, triglycerides and LDL-C) were found significantly increased for smokers compared to the healthy control non- smoking subjects, while the HDL-C were decreased in smoker group compared to the non smoker group. The values of significance for various comparisons are given in Table 1. Table 2 In this set of data, the subjects were categorized according to average number of cigarettes / day] table 2 showed that there was significant increased in the mean levels of cholesterol, Triglyceride while the number of cigarettes smoked per day has no effect on serum LDL-C. HDLC was significantly decreased among smoking group [>15cigarettes/day versus <15 cigarettes/day. Table 3 In this set of data, the subjects were categorized according to the duration of smoking [Less than 20 and above 20 Years] table 3 showed that the mean levels of total cholesterol and Triglyceride were increased with increase in the duration of smoking, HDL-C shows a decreased with the increase duration of smoking, while the duration of smoking has no effect on serum LDL – C.
Table 1: Comparison of lipid profile between smoker and non smoker

<table>
<thead>
<tr>
<th>Serum Level</th>
<th>Smokers [n =50]</th>
<th>Non smokers [n =50]</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol [mg/dl]</td>
<td>199.5</td>
<td>167</td>
<td>0.00</td>
</tr>
<tr>
<td>HDL –Cholesterol [mg/dl]</td>
<td>38.68±11.68</td>
<td>47.06±68</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL –Cholesterol [mg/dl]</td>
<td>158.56±39.69</td>
<td>99.64±34.78</td>
<td>0.00</td>
</tr>
<tr>
<td>Triglyceride [mg/dl]</td>
<td>184.26±50.13</td>
<td>144.74±25</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The table show the mean ± SD and probability (P). T- test was used for comparison. P value ≤ 0.05 was considered significant.

Table 2: Influence of daily number of smoking cigarettes on lipid profile

<table>
<thead>
<tr>
<th>Serum level</th>
<th>Smoking&gt;20 cig/ day [N=14] Mean±</th>
<th>Smoking &lt;20 cig./day [n=36] Mean±</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol [mg/dl]</td>
<td>218.04±24.68</td>
<td>198±26.39</td>
<td>0.00</td>
</tr>
<tr>
<td>LDL –cholesterol</td>
<td>139.02±19.42</td>
<td>149.53±21.51</td>
<td>0.11</td>
</tr>
<tr>
<td>HDL –cholesterol</td>
<td>37.50±5.27</td>
<td>40.32±5.277.70</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglyceride [mg/dl]</td>
<td>185.04±40.53</td>
<td>155.43±54.36</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The table show the mean ± SD and probability (P). T- test was used for comparison. P value ≤ 0.05 was considered significant.

Table 3: Influence of duration of smoking on lipid profile

<table>
<thead>
<tr>
<th>Serum level</th>
<th>smoking &gt; 20 years [N=19] Mean±</th>
<th>smoking&lt;20 years [n=31] mean±</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>202.50±15.46</td>
<td>180.34±8.55</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL –cholesterol</td>
<td>161.00±24.0</td>
<td>154.05±15.20</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL –cholesterol</td>
<td>41.32±4.82</td>
<td>47.50±1.40</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglyceride [mg/dl]</td>
<td>257.12±64.24</td>
<td>146.42±7.78</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The table show the mean ± SD and probability (P). T- test was used for comparison. P value ≤ 0.05 was considered significant.

Discussion:

Cigarette smoking harms nearly every organ of the body, causes many diseases, and reduces the health of smokers in general. 9,10 Cigarette smoking is the leading...
adult males. In our study Serum TC, TG and LDL were significantly higher in smokers as compared to non-smokers and the serum HDL level was significantly lower in smokers as compared to non-smokers. Our findings are in accordance with the findings of many research workers. The change in the serum cholesterol & lipoprotein levels became more marked with the number of cigarettes smoked per day and duration of smoking in years. This finding has been substantiated by Imamura et al.\textsuperscript{5}, N .S Neki\textsuperscript{6} Contrary to the above findings Diricana M et al\textsuperscript{7} did not find significant differences in serum TC, TG, LDL-C, and HDL-C levels between smokers and nonsmokers. Nesje LA. et al\textsuperscript{8} also found no significant difference between smokers and non-smokers concerning triglycerides and total cholesterol .These differences may due to ethnic’s variation in population in previous studies. Dyslipidemia is a well-established risk factor for the development of coronary artery disease. Our study demonstrated presence of Dyslipidemia in chronic Libyans smokers. The main limitation of this study is that important factors which may contribute to the cardiovascular risk factors among males Libyans such as dietary habits, physical activity and genetics were not included.

CONCLUSION:

increased duration / Years and number of cigarette/day smoked. Smoking plays the key role for atherosclerotic process and with coronary artery disease.

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Detection of bacterial contamination of red meat discs
Displayed shops selling meat Butchers

Abstract:

Red meat is considered worldwide as one of the essential foods for human diet, because it is the main source of vital proteins as well as lipids and salts. Beef meat burgers communally sold in fast food shops due to its pleasant taste despite the possibility of causing food poisoning and illness if it has been prepared from contaminated raw meat. Our study was planned to detect the microbial quality of beef burger sold in Libyan’s butchers. The investigation was based on 75 samples which were collected randomly from various sources; small and large butchers. Extracts from the samples were propagated on different agar media in order to determine the total number of aerobic bacteria, Salmonella, Staphylococcus aureus and Escherichia coli. The results of this study showed that all beef burgers were free of contamination with Salmonella. The results also showed the presence of varying numbers of bacteria, such as Gram positive and Gram negative bacteria. The total number of aerobic bacteria was found to be between $5 \times 10^3$ to $6 \times 10^3 \log (\text{cfu/ml})$, and the total number of Staphylococcus aureus was between $2 \times 10^2$ to $5 \times 10^4 \ \text{cfu/ml}$ and lowest number recorded was Escherichia coli ($4 \times 10^2$ to $7 \times 10^3 \ \text{cfu/ml}$). All of these recorded numbers of Bacteria are within the Libyan specification standard (2009).

Key words: Lean red meat, salmonella, poisoning and disease, the general census of bacteria. Libyan standards for meat in 2009.
الكشف على التلوث البكتيري لأقراص اللحوم الحمراء المعرضة بمحلات بيع اللحوم بصبراته

المقدمة:

تعد اللحوم الحمراء من الأغذية الضرورية للإنسان في جميع أنحاء العالم حيث تعتبر أحد مصادر البروتين الأساسية إلى الدهون والأملاح. وتستخدم أقراص اللحوم المتاحة في بعض المطاعم، والتي قد تكون موضوعة بشكل خاص للحصول على اللحوم الطازجة، والتي قد تعاني من التلوث البكتيري.

وتهدف هذه الدراسة إلى تحديد مدى التلوث البكتيري لأقراص اللحوم الحمراء المتاحة في محلات بيع اللحوم في بصبرة. يتم فحص عينات من أقراص اللحوم باستخدام تقنيات مختلفة لتحديد مدى التلوث البكتيري، بما في ذلك التحقق من تعداد العامل بكتيري، والكائنات المسببة للاستجابة الميكروبية للمادة العضوية، وعوامل أخرى.

وتظهر النتائج أن بعض العينات تحتوي على تعدادات عالية من البكتيريا، بما في ذلك Salmonella، Staphylococcus Aureus، و Escherichia Coli. وتشير النتائج إلى عدم وجود تطابق بين إعداد العاملات البيئية والكائنات المسببة في بعض العينات، مما يشير إلى ضعف في التحكم في عمليات حماية اللحوم.

وعلى هذا الأساس، يُعتبر من الضروري اتخاذ إجراءات تعليمية لضمان عدم وجود التلوث البكتيري في العينات المستخدمة في محلات بيع اللحوم في بصبرة. كما يجب على المصنعين و المتورطين بالعملية أن يكونوا على علم بالإجراءات اللازمة لضمان خالية اللحوم من التلوث البكتيري.

الخلاصة:

الكشف على التلوث البكتيري لأقراص اللحوم الحمراء المعرضة بمحلات بيع اللحوم بصبراته.

الكلمات المفتاحية: اللحوم الحمراء، Salmonella، Escherichia Coli، التسمم، التوزيع، والمصادر، وسلطات الأغذية والطبية في ليبيا.
تعتبر دول اللحوم من ملاءمات نبض الاقتصاد العالمي في إنفاذ ما يشمل ذلك الاحترافounters الباطنية عالية، والملوثات البكتيرياية والمركبات المعدنية، مثلاً الحديد والفسفر، كما أن العناصر المعنية بجودة اللحوم تحتوي على عناصر كيميائية، بما في ذلك البوتاسيوم، الفيتامينات B، عناصر مثل المغنيسيوم والكالسيوم، والمعادن الضرورية للصحة العامة.

وقد أصبحت الدراسات الميدانية التي تأتي في شكل أوراق اللحم المفروم المختلطة، وتدخل مكونات اللحم المفروم في تركيبها، وهو المكون الرئيسي لها، من الوجبات المرغوبة جداً والمشروعة على نطاق واسع في مجتمعنا، وخاصة بين شرائح الشباب، وتتمثل هذه الدراسة في التعرف على جودة أقراص اللحم المعروضة لمحال بيع اللحم، وذلك من الناحية الميكروبيولوجية، والكشف عن وجود البكتيريا المرضية باللحم في صناديق الأغذية ولأغراض البقاء والعمل، وكمالمات الأنواع والأشكال والمصادر، والقياسية السرية لأقراص اللحم المفروم (البرجري) لسنة 2009.

المواد والطرق:

للحيوانات المجهري المختلفة (1).

ومجمل المجموعة مجملات الأحياء المجهريات التي توجد فيها، وكذلك تصنيفها، مخطط شكل (1) يوضح الطرق العملية للكشف عن الأحياء المجهري عزلاً أو تعرفها.

الطريقة العملية

جمع العينات:

عينات أقراص لحم، من محلات 75 جمعت اللحوم والأسواق المركزية داخل مدينة صفاقس، وتقلت في حافظة مبردة مباشرة إلى مختبر الأحياء الدقيقة للأغذية بالكلية. وجهزت العينات لتجميل التعرف والكيميائي.
جامعة أقراس لحم 75

تجهيز العينات للتحليل الميكروبيولوجي

الكشف عن وجود جراثيم الهوائية

تقييم عدد الجراثيم الهوائية

S. aureus  Salmonella  E. coli  (API) 20 E  صبغة الجرام

شكل (1) يوضح الطرق العملية للكشف عن الأحياء المجهرية وعزلها وتعريفها.

- **تجهيز العينات للتحليل الميكروبيولوجي:**

  تم استخدام سلسلة من التخفيفات العشرة مع استعمال القفازات المعقدة خلال جميع مراحل عمليات الجمع والتجهيز. مخطط شكل (1) يوضح التقدير الكلي للأحياء وعزلها وتعريفها.

  24 إلى 106 وخصوصياً درجة 37° لمدة ساعتين بعد المواسمات

  **Total viable count**

  بعد كل النمط الميكروبات اللاهوية اختيارية.

- **تقييم عدد الجراثيم الهوائية الحية:**

  تم تقدير عدد الجراثيم الهوائية الحية باستخدام رج (Nutrient broth) مُستننَتا مرصا مُغَذ ّي

  (Selenite broth) وال تقديري جيداً ونقل بواقعة 1 مل إلى 9 مل لتضييق النتائج الأول ومنه حضرت بقية التخطيطات، وزرع هذه التخفيض على الأطباق

  **pour plate method 3**

- **الكشف عن بكتيريا Salmonella**

  تم الكشف على بكتيريا Salmonella بنقل 0.5 سم³ من محلول التخريض إلى 5 سم² على ملامح التخريض المقشر والمغشَّي و Selenite broth

  بوصفها الأبوسطة المتقدمة المستخدمة لعزل الالميلسية وخصب في درجة حرارة 37° لمدة 48 ساعة. ثم خططت على

  سطح بيئة Shigella Agar - Salmonella

  24 م³ لمدة 37 وخصوصياً الأطباق جميعها في درجة

  ساعة (7).
الكشف عن بكتيريا Escherichia coli

وتلك باستعمال SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS
وحجة تلوث عاش بالجراثيم في جميع العينات
وحجة أن عدد الجراثيم الهوانية الحية في
إلى أكثر 10١٣ الألقوان كانت ما بين
2.56 10٤ - 2.56 10٥/غرام.
أوضح تناضح دراسة مصرية أجريت لتقييم
الجودة الميكروبية للمنتجات اللحم المفروم
الطازجة وغير المطهية (16) ، بأن التعداد الكلي
10٥ و 10٦ للميكروبات.
متوتعة سجل 5.01X 10٣ غرام لكل كيلو. كما تشير بعض
الدراسات بأن وجود جراثيم الإشريكية
القولونية Escherichia coli O157: H7
المفروم قد تسبب في أحداث العديد من
الأمراض (17-18).

<table>
<thead>
<tr>
<th>التعداد الأومني (كتلغرام)</th>
<th>التعداد الألقي (كتلغرام)</th>
<th>نمط التعداد</th>
</tr>
</thead>
<tbody>
<tr>
<td>10٤×6</td>
<td>10٣×5</td>
<td>تعداد الأحياء المجهرية الهوائية (Aerobic plate count)</td>
</tr>
<tr>
<td>10٤×7</td>
<td>10٣×4</td>
<td>تعداد الإشريكية القولونية (count E. coli)</td>
</tr>
<tr>
<td>10٤×5</td>
<td>10٣×2</td>
<td>تعداد المكورات العنقودية الذهبية (S. aureus count)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>تعداد السالمونلا (Salmonella)</td>
</tr>
</tbody>
</table>

تعداد الأحياء المجهرية الهوائية في الطبقات، ونعدادات الإشريكية القولونية، والمعكورات (1): الجدول
العنبية للتغطية في الجوانب، ونعدادات الإشريكية القولونية، والمعكورات (1): الجدول
المتغطية الميكروبية للأعراض اللحم الحمراء.

 Escherichia coli
Serratia Spp.
Proteus Spp.
Citrobacter Spp.
Klebsiella Spp.

العدود والشكل (2) يوضح أعداد الأحياء المجهرية المزعولة وأنماطها. تأثير
العدود والشكل (2) يوضح أعداد الأحياء المجهرية المزعولة وأنماطها.
## أعداد الأحياء المجهريّة المعزولة وأنماطها نسبيتها

<table>
<thead>
<tr>
<th>صبغة الجرام</th>
<th>أنواع البكتيريا المعزولة</th>
<th>عدد العزلات</th>
<th>نسبة العزلات إلى مجموع العزلات</th>
</tr>
</thead>
<tbody>
<tr>
<td>موجبة لصبغة الجرام</td>
<td>S. aureus</td>
<td>25</td>
<td>25%</td>
</tr>
<tr>
<td>سلبة لصبغة الجرام</td>
<td>E. coli</td>
<td>20</td>
<td>20%</td>
</tr>
</tbody>
</table>

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>Salmonella</td>
<td></td>
</tr>
<tr>
<td>%21</td>
<td>21</td>
<td>Serratia Spp</td>
<td></td>
</tr>
<tr>
<td>%19</td>
<td>19</td>
<td>Proteus Spp</td>
<td></td>
</tr>
<tr>
<td>%10</td>
<td>10</td>
<td>Citrobacter Spp</td>
<td></td>
</tr>
<tr>
<td>%05</td>
<td>05</td>
<td>Klebsiella Spp</td>
<td></td>
</tr>
<tr>
<td>المجموع الكلي</td>
<td>100</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

الجدول (2): الأحياء المجهريّة المكتشفة في عينات أقراص اللحوم الحمراء.
الشكل (2): نسب الأحياء المجهرية المكتشفة في عينات أقراص اللحوم الحمراء

يرجى ملاحظة أن هناك اختلافات بين الأنواع البيولوجية في هذه الدراسة، إذ أن النتائج تتضمن

- ت互动 الشروط الصحية والمراقبة المستمرة على اللحوم والوقاية العاملة في المجازر وموصلات البيع.

الوصفات:
- متاحة الرقابة الصحية على اللحوم.
- توعية المحافظين بال📝قليلات الملاحظات أخرى.

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المراجع/ باللغة الإنجليزية


شكر وتقدر
إلى أصحاب التميز والأفكار المبتكرة

• البروفسور أسامة الشامخ – مدير مركز أليافا بدولة فنلندا

• البروفسور رجب عون – مدير مكتب البحوث بالمعهد القومي لعلاج الأورام بصراته
Salmonella Enteritidis’ Proteins produce in Vitro and in Vivo Protection against Colonization

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⁴ Department of Infection and Immunity, Vet school, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, UK.

Abstract:

Salmonella enterica can be considered as one of the most important causes of food-poisoning with poultry thought to be the main source. Although S. Typhimurium, S. Enteritidis and the vast majority of other Salmonella serovars generally produce little systemic disease in adult chickens, they are able to colonize the alimentary tract of poultry. The two caeca are the main sites of the colonization of Salmonellae in chickens, and the bacteria can be easily harvested from the caeca for analysis. Bacterial proteins analysed utilizing SDS-PAGE showed differences between in vitro and in vivo that out of about 40 protein bands of in vitro preparation only a few (3-5) bands can be visualized from in vivo preparations. We suggested that some avian proteases might be responsible. Accordingly, and to investigate the hypothesis that bacterial-precipitated protein harvested from chickens is thought to be more protective than bacteria grown in broth culture, the immunogenicity of protein-precipitated vaccines harvested from chicken intestine and those from broth culture (in vitro), were compared using bacterial proteins as an orally inoculated vaccine candidate in chicken. The results demonstrated that the in vitro sonicated proteins obtained from a nutrient broth culture had a much better protective vaccine effect than the in vivo sonicated proteins preparations harvested from bacteria grown in chickens.

Introduction:

Many bacterial pathogens such as Clostridium, Staphylococcus, Campylobacter and many other bacterial strains are capable of causing food-poisoning, and Salmonella enterica can be considered as one of the most important causes with poultry thought to be the main source. Although S. Typhimurium, S. Enteritidis and the vast majority of other Salmonella serovars generally produce little systemic disease in adult chickens, they are able to colonize the alimentary tract of poultry, resulting in contamination of poultry carcasses and entry into the human food chain. However, there is a great demand to control food-poisoning salmonellosis at both breeder and layer levels at the national and global level in
order to produce *Salmonella*-free poultry products, due to the current correlation between *S. Enteritidis* PT4 and poultry products. Salmonellosis costs the European Union a minimum of 500-900 million Euros annually. Salmonellosis in food animals is a major target for reduction of human infection by the European Union. Legislation has been introduced to monitor the most important *Salmonellae* serovars. The major *Salmonellae* serovars of public health consequence are *S. Typhimurium* and *S. Enteritidis* (causing 15% and 60% respectively of all cases in Europe in 2002). Our team studied newly-hatched chickens infected with *S. Enteritidis*. We analyzed proteins of *S. Enteritidis* in the caeca of 1-day old checks (*in vivo*) together with a comparison with nutrient broth medium (*in vitro*) in order to detect changes in the pattern of protein expression during infection. The preliminary exploratory study of individual bands identified major proteins (flagellin of *S. Enteritidis* and *Typhimurium* *fliC*) and mixtures of proteins including 60 kDa chaperonin *groEL* and glyceraldehyde-3-phosphate dehydrogenase *gapA*. Some proteins may be expressed equally both *in vivo* and *in vitro* (e.g. fimbrial, flagellar, outer membrane protein, metabolic, regulatory, and LPS-synthesis encoded genes). These proteins are predicted to play a major role in colonization. Chicken caecal colonization by paratyphoid *Salmonella* (e.g. Enteritidis, Typhimurium and others) has been linked to the physical attachment by fimbriae(1) motility (2), type three secretion system (T3SS) of *Salmonella* Pathogenicity Islands “SPI-1 and SPI-2” (3), bacterial cell wall component lipopolysaccharide “LPS” (4, 5) and outer membrane proteins “OMPs” (6). This comparison showed differences between the two profiles and indicated that it is difficult to make a reasonable comparison as out of about 40 protein bands of *in vitro* preparation only a few (3-5) bands can be visualized from *in vivo* preparations, the reason behind that thought to be the degradation of *in vivo* protein with some avian proteases. Then we hypothesized that vaccine prepared from bacteria grown *in vivo* in chickens will give better protection than a vaccine prepared from bacteria cultured in vitro because they will be expressing antigens normally expressed during infection/colonization. Accordingly, and to investigate the hypothesis, the immunogenicity of protein-precipitated vaccines harvested from chicken intestine and those from broth culture (*in vitro*), were compared using bacterial proteins as an orally inoculated vaccine candidate in chicken.

**Material and methods:**

**Preparation of protein-precipitated vaccines from bacterial**

**Cells cultured in vitro in nutrient broth:**
A single colony of S. Enteritidis PT4 (antibiotic sensitive parent strain) was inoculated into 10ml NB and incubated overnight at 37 °C and 1ml of this broth culture was transferred into 2 x 100ml NB in 250ml flasks and incubated for two hours at 37 °C in a shaking incubator at 200rpm. Each flask was then decanted to three 50ml Falcon tubes each containing 33.3ml, the tubes then centrifuged at 5000g for 30min at 20°C and the supernatants were discarded. Subsequently the pellet from each tube was re-suspended with 3.33ml NB, then the content of three Falcon tubes were mixed into one Falcon tube. The contents (10ml NB 10⁹ bacterium/ml) which is equivalent to 10⁸/0.1ml = 3 x 10⁸ bacterium/0.3ml = 5 x 10⁸/0.05ml which used for chicken injection (i.m) to both breast sides.

**Preparation of in vivo S. Enteritidis protein vaccine:**

A total of 60 newly hatched chickens were inoculated orally within 18 h of hatching. Chickens were infected orally with 0.1 ml of a culture of the antibiotic-sensitive parent S. Enteritidis PT4, grown for 16 h in nutrient broth at 37ºC and diluted in sterile nutrient broth to contain 10⁷ cfu/ml. After 16 – 18 hs post-infection chickens were killed one-by-one, and the caecal contents were harvested from both chicken caeca of each bird. The caecal contents of three randomly chosen chicks were transferred to three separate sterile universal tubes, placed on ice to test viable bacterial number on MacConkey agar and nutrient agar. The caecal contents of the remaining chickens were put in 50 ml Falcon tubes, stored at -80ºC until needed. Three chicks were left without inoculation, their caecal contents were used to streak on MacConkey agar and nutrient agar plates, incubated for overnight at 37ºC to ensure that there were no contaminants with other bacteria. For the vaccine preparations, the S. Enteritidis-infected caecal contents were diluted in nutrient broth and then centrifuged at 20,000 x g for 5 min at 4ºC (Avanti®J-E Beckman centrifuge coulter), then the supernatant was discarded and the pellets were resuspended in NB, followed by sonication (Sonics VCX500) for 5 min immediately after adding the protease inhibitors (Sigma P8465). This sonicates was then centrifuged at 15,000 g for 10 min at 4ºC. Subsequently, the supernatant was filtered using 0.45 μm filters and stored in 1 ml aliquots in Eppendorff tubes at -20ºC until required.

**Preparation of invitro S. Enteritidis protein vaccine:**

A single colony of the parental S. Enteritidis PT4 sensitive strain was picked and used to inoculate 10ml NB in a universal bottle which was then incubated overnight at 37ºC. On the following day 250ml flasks, each containing 100ml nutrient broth, were inoculated with 1ml of the overnight broth culture of S. Enteritidis PT4 and incubated overnight at 37ºC shaking incubator (150 rpm). The contents of these broth cultures were divided into four 50ml centrifuge tubes
and centrifuged at 20,000 x g for 5 min at 4ºC (Avanti®J-E Beckman centrifuge coulter). The pellet from each tube was resuspended in 5ml NB and sonicated for 5 min (Sonics VCX500) after adding the protease inhibitors (Sigma P8465), followed by centrifugation at 15,000 x g for 10 min at 4ºC and filtration as mentioned above. The proteins preparations were then stored at -20ºC until required.

**Vaccine quality control:**

Protein sonicates harvested from both *in vivo* and *in vitro* environment were streaked on MacConkey and nutrient agar plates which were incubated overnight at 37 ºC to check for any Salmonella growth.

**First vaccination experiment**

A lot of 60 1-day commercial layer chickens obtained from Millennium Hatchery Hy-Line UK Ltd (Studley Warwickshire), were utilized in this experiment. On the day of arrival birds were divided into three groups each of 20 birds, being placed in separate cleaned rooms (Trigene Disinfectant 20L Clear from Scientific Laboratory Supplies Ltd (CLE1320). Followed by chemical fogging with Virkon disinfectant from Sigma (Z692158). Chicks were distributed between the rooms as follows (*in vivo* sonicated proteins group – Room I; *in vitro* sonicated proteins group – Room II; unimmunised group – Room III). All birds in all groups were inoculated with 0.1 ml of neat Avigard gut microflora (Microbial Developments Limited, UK), then at the fifth day of age all chickens were inoculated intramuscularly (i.m), into the breast muscle, with 0.05 ml containing protein preparation. Chickens were also inoculated orally with 0.1 ml of the corresponding vaccine for each group as shown in Table 1. The unimmunized group (control) was inoculated with sterile NB. At three weeks of age the vaccination program was repeated with all birds inoculated with 0.3 ml orally and 0.1 ml i.m using the corresponding vaccine for each group. All birds were challenged with 0.5 ml of NB culture (3 x 10⁸ cells) of a nalidixic acid resistant (NalR) mutant of *S. Enteritidis* strain at week 5 of their age. Cloacal swabs were collected from all birds at 1⁰, 2⁰, 3⁰, 4⁰, 7⁰, 14⁰, 21⁰ and 28⁰ day post- challenge for a semi-quantitative estimation of bacterial shedding(7, 8) of the challenge *S. Enteritidis* NalR by plating on BG agar supplemented with nalidixic acid (20 µgm/ml⁻¹) and novobiocin (1 µgm/ml⁻¹). On day 28 post-infection after collection of cloacal swabs, all birds were slaughtered and their caecal contents were collected for a semi-quantitative *S. Enteritidis* NalR count estimation.
<table>
<thead>
<tr>
<th>Day/Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In vivo sonicated proteins</td>
<td>In vitro sonicated proteins</td>
<td>Unvaccinated Control group</td>
</tr>
<tr>
<td>1</td>
<td>0.1ml Avigard orally</td>
<td>0.1ml Avigard orally</td>
<td>0.1ml Avigard orally</td>
</tr>
<tr>
<td>5</td>
<td>0.05 ml in vivo proteins; i.m</td>
<td>0.05 ml in vitro proteins; i.m</td>
<td>0.05 ml sterile NB</td>
</tr>
<tr>
<td></td>
<td>0.05 ml in vivo proteins; orally</td>
<td>0.05 ml in vitro proteins; orally</td>
<td>0.05 ml sterile NB</td>
</tr>
<tr>
<td>21</td>
<td>0.1 ml in vivo proteins;</td>
<td></td>
<td>0.1 ml in vivo proteins;</td>
</tr>
<tr>
<td></td>
<td>0.3 ml in vivo proteins; orally</td>
<td>0.3 ml in vitro proteins;</td>
<td>0.3 ml in vivo proteins; orally</td>
</tr>
<tr>
<td>31</td>
<td>Challenged orally with 0.1ml (5 x 10^8) live SE NalR</td>
<td>Challenged orally with 0.1ml (5 x 10^8) live SE NalR</td>
<td>Challenged orally with 0.1ml (5 x 10^8) live SE NalR</td>
</tr>
<tr>
<td>Post challenge sample collection</td>
<td>Five randomly selected birds from each group were killed at day 1, 4, 6 and 8 post infections; tissue portion of their spleen, liver and caecal contents were collected for salmonella count</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Experiment-I of vaccination and challenge regime (orally challenged). NB = nutrient broth

**Second vaccination experiment**

Signs, then collected in pre-labelled, pre-weighed sterile universal bottles. The caecal contents for each bird were then collected separately in pre-weighed sterile universal bottles. The three bottles for each bird were kept on ice prior to reweighing and diluting in x 9 the weight of the sample in PBS. All tissue samples (liver and spleen) were kept on ice until they weighed and then proportional amounts (10 x weight expressed as volume) of PBS (pH 7.2) were added into each tube. Each tissue portions was This experiment was different from the first experiment only in the route of challenge and types of sample collected. The birds, groups, vaccination programs were identical to those in the first experiment. Subsequently, all birds were challenged intravenously via the wing vein with 0.1 ml (1 x 10^6 cells) of S. Enteritidis NalR at 5 weeks age. Five birds from each group were selected randomly and killed at 1, 4, 6 and 8 days post-challenge. Immediately after killing, spleen and liver samples were observed for any clinical
homogenised in a Griffiths tubes in PBS (pH 7.2) to obtain homogenous suspension (2) prior to dilution for counting. This together with a x 9 dilution of the caecal contents were used for bacterial count estimations.
<table>
<thead>
<tr>
<th>Day/Group</th>
<th>Group I \textit{in vivo} sonicated proteins</th>
<th>Group II \textit{in vitro} sonicated proteins</th>
<th>Group III Unvaccinated Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1ml Avigard orally</td>
<td>0.1ml Avigard orally</td>
<td>0.1ml Avigard orally</td>
</tr>
<tr>
<td>5</td>
<td>0.05 ml \textit{in vivo} proteins; i.m</td>
<td>0.05 ml \textit{in vitro} proteins; i.m</td>
<td>0.05 ml sterile NB i.m</td>
</tr>
<tr>
<td></td>
<td>0.1 ml \textit{in vivo} proteins; orally</td>
<td>0.1 ml \textit{in vitro} proteins; orally</td>
<td>0.1 ml sterile NB Orally</td>
</tr>
<tr>
<td>21</td>
<td>0.1 ml \textit{in vivo} proteins; i.m</td>
<td>0.1 ml \textit{in vitro} proteins; i.m</td>
<td>0.1 ml sterile NB i.m</td>
</tr>
<tr>
<td></td>
<td>0.3 ml \textit{in vivo} proteins; orally</td>
<td>0.3 ml \textit{in vitro} proteins; Orally</td>
<td>0.3 ml sterile NB Orally</td>
</tr>
<tr>
<td>35</td>
<td>Challenged intravenously with 0.1ml ((5 \times 10^8)) live SE NaI\textsuperscript{R}</td>
<td>Challenged intravenously with 0.1ml ((5 \times 10^8)) live SE NaI\textsuperscript{R}</td>
<td>Challenged intravenously with 0.1ml ((5 \times 10^8)) live SE NaI\textsuperscript{R}</td>
</tr>
<tr>
<td>Post challenge sample collection</td>
<td>Five randomly selected birds from each group were killed at day 1, 4, 6 and 8 post infections; tissue portion of their spleen, liver and caecal contents were collected for salmonella count</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Experiment-II of vaccination and challenge regime (intravenously challenged).

NB = nutrient broth
Enumeration of bacteria in chicken faeces (Experiment I)

After collection of all swabs 2 ml selenite broth (Oxoid, CM0395) were added to each tube, followed by brief vortexing. Each swab was plated in a standard manner on brilliant green agar plate (BGA) supplemented with nalidixic acid (20 µgm / ml⁻¹) and novobiocin (1 µgm / ml⁻¹) (9). The inoculated plates and the selenite broths were incubated overnight at 37°C. Then the swabs were left into selenite broth tubes for overnight incubation at 37°C prior to plating on BGA, to encourage the growth of Salmonellae and inhibit the growth of other flora. Then the overnight incubated swabs were plated again on the antibiotic-containing BGA media and incubated overnight at 37°C. Plates inoculated directly were read and observed for Salmonella growth using a semi-quantitative estimation of faecal shedding and caecal colonisation of Salmonella from infected chickens (1, 7, 10-12). Next day the enrichment plates were also checked for Salmonella growth. Xylose Lysine Deoxycholate (XLD) media (Oxoid, CM0469) was used as a confirmatory test for any Salmonella growth. Suspect colonies were sub-cultured on this media and incubated overnight at 37°C, and the plates were checked for black colonies indicating Salmonella as a result of H₂S production, in addition to slide agglutination tests.

Bacterial enumeration in tissues samples (Experiment II)

The bacterial count of S. Enteritidis NalR in spleen, liver and caecal contents for the 5 birds of each group (at day 1, 4, 6 and 8 post challenge), were estimated by serial dilution and plating aliquots of dilutions (13). Aliquots of each dilution were plated on BGA plates supplemented with nalidixic acid (20 µgm / ml⁻¹) and novobiocin (1 µgm / ml⁻¹) and incubated overnight at 37°C. Bacterial colonies were counted and the viable count converted into Log₁₀ numbers. The xylose lysine deoxycholate medium XLD (Oxoid, CM0469) and slide agglutination tests were also used as confirmatory test to confirm any Salmonella growth.

Data analysis

Analysis of data obtained from experiment I

Cloacal swabs were taken from each bird two days previous to challenge inoculation for culture to guarantee that the chicks are free from Salmonellae. Differences in percentage excretion rates between groups of birds were compared using χ², and this was considered as statistically significant if the P value was ( <0.05).

Analysis of data obtained from Experiment II

As in experiment I cloacal swabs were taken from each bird two days before being challenged for culture to guarantee that the chicks are free from Salmonellae. The bacterial counts of S. Enteritidis NalR (challenge) of the tissues (spleen and liver) and caeca in different groups on BGA plate, in different time points were
recorded and the P value of each group compared to the control group were calculated using Student’s unpaired t test (Microsoft Office 2010). A P value of (< 0.05) was considered as statistically significant.

RESULTS:

It was decided to carry out experimental in vivo infection using 1-day old chicks primarily to avoid the development of intestinal microflora, which would be likely to have a significant effect on interference in interpreting the patterns of protein expression in S. Enteritidis as well as to enable the bacterium of interest (S. Enteritidis) to multiply extremely well in the absence of competitive colonizers (Barrow et al., 1987; Barrow et al., 1988). Using birds aged from 2-6 weeks is the best model to study Salmonella colonisation of chicken, as their gut flora is mature (Barrow, personnel communication), but for studying Salmonella proteins this might give a false results due to cross contaminations of gut flora. A protein analysis of S. Enteritidis in the caeca of 1-day old checks (in vivo) together with a comparison with nutrient broth medium (in vitro) was used to detect changes in the pattern of protein expression during infection and in particular to identify proteins that enable this strain to colonise the caeca. We compare the immunogenicity of bacteria (S. Enteritidis) harvested from the intestine with those grown in vitro in nutrient broth cultures. The preparations would include (i) whole cellular proteins prepared from in vivo-cultured bacteria and (ii) whole cellular proteins prepared from in vitro-grown bacteria in NB, all of which would be tested for their ability to protect against Salmonella colonisation in chicken.

Results for experiment I (orally challenged chicks)

No Salmonella organisms were isolated from the chickens on receipt. The percentage excretion rates of the challenge Salmonella strain in the different groups are shown in Table 3. When Salmonella was cultured by direct plating if the colony numbers present per plate was 1 or more this was designated as ≥ 1, while when they were 50 colonies or more this was designated as ≥ 50 (14). The bacteria cultured by enrichment followed by plating were shown as the percentage of positive swabs, which had been confirmed by XLD agar and slide agglutination tests as shown in Table 3.
Percentage of chickens (20 birds per group) excreting *S. Enteritidis Nal^R* (challenge strain) from direct plates, and number of positive birds (Positivity %) from enriched plates at different time points post-infection

<table>
<thead>
<tr>
<th>Sample</th>
<th>In vivo proteins</th>
<th>In vitro proteins</th>
<th>Unvaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Enriched</td>
<td>Direct</td>
</tr>
<tr>
<td>Birds (No&amp; %)</td>
<td>≥50</td>
<td>≥1</td>
<td>≥50</td>
</tr>
<tr>
<td>Days PI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cloacal swabs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0%</td>
<td>10%</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>4</td>
<td>14%</td>
<td>38%</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>7</td>
<td>5%</td>
<td>5%</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>14</td>
<td>0%</td>
<td>0%</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>21</td>
<td>0%</td>
<td>0%</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>28</td>
<td>0%</td>
<td>0%</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Caecal content</td>
<td>28</td>
<td>0%</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 1: Effect of vaccinating with whole-cell sonicated protein preparation on faecal excretion of *S. Enteritidis Nal^R* (challenge strain), results obtained from direct plates; plus results of BGA enriched plates shown number of *S. Enteritidis Nal^R* positive birds (positivity %), from cloacal and caecal sample collected at different time points post infections, chicks were orally inoculated
Based on evaluation of the results of all samples collected (either caecal contents or cloacal swabs), the percentage of chickens positive for S. Enteritidis NalR challenge strain for the first day post-infection was 52% for the birds in group one which were treated with in vivo sonicated proteins preparation and 26% in group two which were treated with in vitro sonicated proteins preparation compared with 45% in untreated control (group three). Then there was a noticeable decrease in the percentages of faecal excretion in all groups two weeks post-infection as the percentages were 14%, 0% and 15% in groups from one to three respectively. Moreover, three weeks post-challenge the percentage of positive birds’ faecal proteins treated groups respectively, which were considered as statistically significant. The in vivo protein preparation unexpectedly had a lower immunogenic effect than did the in vitro proteins preparation. From the caecal samples collected at week four post-infection Salmonella was detected only in unimmunized control (group three), while no growth of any Salmonella were observed in the two treated groups (< 1 x10^2 cfu/ml) as shown in Table 3 and Figure 1.

In summary, both in vitro and in vivo protein preparations had a much greater immunization effect. The $P$ values were ($\chi^2=16.77, P<0.001$) and ($\chi^2=28.3, P<0.001$) for the in vivo and in vitro...
Figure 1: Faecal excretion of challenge S. Enteritidis strain following vaccination with Salmonella proteins produced from bacteria cultured either in chickens (in vivo) or in nutrient broth (in vitro) compared with unvaccinated control, this figure also shown that no growth of any Salmonella were detected in caecal contents of all treated groups 4 weeks post infection.

Results for experiment II (Intravenously challenged chicks)

were detected in the control group. Moreover, as shown in Figure 2 below there was no difference of the viable counts (Log₁₀) of Salmonellae in the spleen on the 1st day post-infection between all groups. Surprisingly, the count of Salmonellae in spleen tissues on the 4th day post-infection in immunized groups (Log5.2 and 5.1 cfu/ml) respectively, were all higher than unimmunized control group which was Log 4.7 cfu/ml

At 1 day post-infection the bacterial count in liver were Log 3.8 and 4.0 cfu/ml in

The results presented in Table 4 shows the averages of Log₁₀ Salmonellae counts in liver and spleen of five chickens taken at different time points post-infection from the two groups of immunised birds plus the control group with the P values. No Salmonellae were detected in caecal contents of any bird from the group either immunised with in vivo sonicated proteins, or group treated with in vitro sonicated proteins; although some other bacterial growth such as E. coli, Klebsiella were observed as illustrated in Table 5 and Figure 4. Salmonellae challenge organisms

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groups immunized with *in vivo* and *in vitro* proteins preparations respectively. This result was unexpected as the counts in the two vaccinated groups were again higher than that of unimmunized birds (Log 3.4 cfu/ml) as illustrated in Table 4 below. *Salmonella* counts in liver on 1<sup>st</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> day post infection steadily decreased in all vaccinated and unimmunized birds as shown in Table 4 and Figure 3 below. Consequently, however, on the last day of sample collection (the 8<sup>th</sup> day post infection), the mean \( \log_{10} \text{Salmonella} \) count in liver was 2.06 and 1.0 for *in vivo* and *in vitro* protein vaccines respectively and 2.3 for unimmunized birds as shown in Figure 3 below. In addition, bacterial counting was performed on caecal contents for all birds and with the exception of some lactose fermenter bacteria cultured from different group bird’s caeca, no *Salmonellae* were detected (< Log 2 cfu/ml) in caecal contents of any bird.

<table>
<thead>
<tr>
<th>Days PI</th>
<th>( \log_{10} )</th>
<th>SE</th>
<th>( \log_{10} )</th>
<th>SE</th>
<th>( \log_{10} )</th>
<th>SE</th>
<th>( \log_{10} )</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.8</td>
<td>0.1</td>
<td>0.0</td>
<td>7</td>
<td>5.0</td>
<td>0.2</td>
<td>4.0</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>0.1</td>
<td>0.0</td>
<td>8</td>
<td>5.2</td>
<td>0.2</td>
<td>4.0</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>2.6</td>
<td>0.2</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td>0.8</td>
<td>4.0</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>0.6</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td>0.6</td>
<td>2.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 4: The protective effect of protein preparation from S. Enteritidis harvested from chickens in vivo or nutrient broth in vitro measured by liver and spleen counts of chicks inoculated intravenously by the parent strain. \( \log_{10} \) mean viable counts of Salmonella per ml of homogenized liver tissue of 5 birds from each group/time point.
Figure 2: The number of *Salmonella* Log$_{10}$ cfu/ml in chicken’s spleen tissue in the groups of birds (each of 20 birds) treated with *S.* Enteritidis whole cellular *in vivo* and *in vitro* sonicated proteins preparations compared with unimmunised control post challenge with parent strain (*S.* Enteritidis Nal$^R$) inoculated intravenously.

Figure 3: The number of *Salmonella* Log$_{10}$ cfu/ml in liver tissue in the groups of birds treated with either *S.* Enteritidis whole cellular *in vivo* and *in vitro* sonicated proteins preparation compared with unimmunised control post challenge with parent strain (*S.* Enteritidis Nal$^R$) intravenously.
**Figure 4:** The number of lactose fermentor bacteria Log10 cfu/ml-1 in caecal contents in the two groups of birds treated with *in vivo* or *in vitro* protein preparations of *S.* Enteritidis compared with unimmunised group at 1st, 4th, 6th and 8th day post intra-venous infection with challenge strain *S.* Enteritidis NalR

**Table 2:** The effect of protein preparation of *S.* Enteritidis harvested from *in vivo* and *in vitro* conditions on colonisation of chicken caeca with lactose fermentor bacteria when challenged intravenously by the parent strain *S.* Enteritidis NalR. (Average viable counts log$_{10}$ per 1ml of caecal contents). SE=Standard Error, P=P value

<table>
<thead>
<tr>
<th>Days post Infection</th>
<th><em>In vivo</em> proteins</th>
<th><em>In vitro</em> proteins</th>
<th>Unvaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log$_{10}$</td>
<td>SE</td>
<td>p</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>2</td>
<td>0.07</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>0.3</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>2.6</td>
<td>0.2</td>
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<td>8</td>
<td>2.9</td>
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**Discussion:**

Control of *Salmonella* infections in salmonellosis. Legislation has been introduced by the European Union (Directive 2003/99/EC, Regulation
2160/2003) to monitor the most important *Salmonella* serovars with timetabled requirements for submission of action plans to control infections in major hosts, particularly poultry and pigs. As a part of this both live and inactivated vaccine are now used in many countries both in the EU and around the world. Nevertheless, live vaccines used in the EU are produced by chemical mutagenesis, and are antibiotic resistant. In the present study two types of vaccine were produced from *S. Enteritidis* PT4. The vaccines were a sonicated protein preparation from (i) bacteria harvested from *in vivo*- and (ii) from *in vitro*-cultured bacteria. The hypothesis was that the type of vaccine prepared from *Salmonellae* harvested directly from the chicken intestine would be more immune-protective than those cultured *in vitro* in contents and compared at the end of vaccination experiment I (orally challenged), there appeared little correlation with the semi-quantitative measures determined by cloacal swabbing. This phenomenon is well known, and is probably associated with intermittent caecal evacuation (18). In the present work when birds were challenged orally to assess *Salmonella* caecal colonisation by cloacal swabbing (Experiment I), the response was great enough to significantly prevent caecal colonisation completely in the groups of birds challenged orally with a virulent *S. Enteritidis* NalR strain when the vaccine was the protein preparation harvested from either the *in vivo* condition (in chickens) or *in vitro* (in nutrient broth culture). The results show that protection by both *in vitro* and *in vivo* proteins preparation were statistically significant ($P<0.001$) in their ability to protect against *Salmonella* colonization. However, the level of protective immunity induced by the *in vitro* protein preparation was higher than that induced by the *in vivo* preparation.

The good level of protection induced by the *in vitro* preparation is in agreement with the previous work conducted by (Khan et al (2003), who found that outer membrane proteins of *Salmonella* when inoculated with adjuvant are effective against *S. Enteritidis* in chickens. At 4th week post infection no *Salmonellae* were detected from caecal swabs from the vaccinated groups ($<1 \times 10^2$ cfu/ml) compared with unimmunized group that show the percentage of *Salmonellae*
positive to be 35%. However, these results showed that the better protection induced by the proteins from the \textit{in vitro} cultured bacteria in comparison with the proteins from the \textit{in vivo} harvest bacteria were unexpected, since it was anticipated that the \textit{in vivo} preparation would have been at least as immunogenic as the \textit{in vitro} preparation, as the protein concentrations of both \textit{in vivo} and \textit{in vitro} preparation were similar. The \textit{in vivo} preparation would have contained a number of antigens that are expressed in the very earliest stages of infection and these may and liver, no bacteria were observed in the caecal swabs collected from all birds. This observation is different from what has been reported previously where S. Enteritidis was shed in faeces after intravenous challenge (17, 22 and 23). The clearance of the challenge strain from internal organs in both vaccinated and unvaccinated birds were similar. During systemic infection following intravenous challenge the macrophage interaction with Salmonellae is the key in the progress of the systemic infection (24). Salmonella clearance into gastrointestinal tract from the tissues is through gall bladder (17). It has been previously reported that in chickens biliary antibodies are involved in S. Typhimurium clearance from the gut (25). This observation correlated with the results of Woodward et al. (2002) who reported that the Salmonella count in gall bladder is higher in unimmunized group compared with vaccinated birds (26). However, other authors used a similar route of challenge and reported that bacterial shedding in the faces reached have been important. Moreover, certain genes that encode some important antigens such as LPS and flagella were down-regulated in the intestine of chickens (16, 19).

The result of this study is in agreement with previous work conducted by Toyota-Hanatani et al. (2009), suggesting that a part polypeptide in S. Enteritidis Fli-C (SEP 9) inhibits S. Enteritidis colonization in the intestine of chickens two weeks after challenge, similarly to commercial inactivated S. Enteritidis vaccine (20). It is thus likely that the antigenic profile of Salmonella during the infection of antigen-presenting cells is very different from that of Salmonellae during intestinal colonisation, or that the proteins may have some immune-suppressive effects (21). The immunogenicity of bacteria harvested from macrophage infections has not been assessed but given that the biology of Salmonella organisms is very different in the gut and in macrophages.

As we ensured that the protein concentrations in the vaccine preparations prepared from the \textit{in vivo} and \textit{in vitro} cultures were similar and obtained from a similar number of bacteria and we can state that the \textit{in vitro} bacteria did not produce larger amounts of protein compared to the \textit{in vivo} bacteria. So, the difference may lie in the levels of specific proteins expressed under the different conditions of culture.

In this study (experiment II), when birds were challenged intravenously (systemic infection), to assess Salmonella systemic invasion of internal organs such as spleen...
(27), which indirectly should reduce the number of human food-borne salmonellosis cases (28). In poultry vaccines against Salmonella infection are thus incompletely effective, and must be seen as a single component in Salmonella control regimens involving a combination of vaccination programs together with hygienic measures. The highest number 1 – 2 weeks post infection (23), which might explain the absence of Salmonella from the caecal sample at day 8. Poultry immunization against Salmonellae is considered as an important contributory measure to infection control. In chickens vaccination may reduce the severity and period of infection and help avoid re-infection.
References


Research Support, U.S. Gov't, P.H.S
Peutz-Jeghers Syndrome
Case Report
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Abstract:
Hamartomatous polyps in GIT are found in all patients of PJ, most commonly the jejunum. One third of patients have symptoms before the age of 10 years and one half before the age of 20 years. Patients most commonly present with obstruction or abdominal pain related intussusception induced by polyps. Intestinal bleeding from the polyps is the second most common GI symptom. Treatment for serious bleedings and intussusceptions in the small bowel related to PJS is resection. [1, 2, 5]

Introduction:
A combination of hamartomatous polyps of smooth muscles and mucocutaneous pigmentsations was described by Hutchinson in 1896. The familial hamartomatous polyps was described originally by Peutz and later followed by Jeghers and colleagues. [4] It is an autosomal dominant inherited disorder most commonly resulting from truncating mutations in a serine threonine kinase gene on chromosome 19q (STRK11-LKB1). [10] According to John Hopkins registry, the diagnosis requires two of the following:
• Small bowel polyposis.
• Mucocutaneous pigmentation.
• A family history suggesting autosomal dominant inheritance. [1, 2, 3, 8]

Report of case
An 18 years old Libyan female, presented to the surgical ER with colicky abdomen pain associated with vomiting, the pain started 2 weeks prior to presentation. Episodes of recurrent abdominal pain over the course of 4 years with multiple hospitalizations were reported in her past medical history. Initial examination revealed a dark-brown pigmented maculae on perioral peri-nasal and peri-ocular skin Figure A. A mildly distended abdomen, Soft lax upon palpation, diffuse tenderness and guarding upon central palpation (paraumbilical area). Bowel sounds present. Digital rectal examination reported an empty rectum. Work up for the patient included blood works and radiology. Her CBC elicited marked anemia with a HGB level of 7
mg/dl. Her biochemistry reports sodium and potassium levels of 134, 3.9 Mmol/L respectively. Radiology: Erect abdomen x-ray reveals centrally located dilated bowel loop, Figure B. Further assess with abdomen CT scan with Contrast which illustrated segment of small intestine, mesenteric fat and blood vessels invaginate into adjoining intestinal lumen causing bowel obstruction and giving target sign of intussusception just anterior to left kidney Figure C. Urgent laparotomy was of merit to relieve the obstruction, and upon exploration, the bowel health at the intussuscepted segment mandated removal of the segment. So, Resection and Primary Reanastomosis was done. Figure D. Patient recuperated quickly and discharged on the 6th postoperative day.
Discussion

To stand on solid ground a criteria made by John Hopkins registry was followed to confirm the provisional diagnosis, which requires the presence of the following:

1. Histologically verified hamartomatous polyps.
2. With 2 of the following:
   • Polyp at small bowel.
   • Melanotic pigmentation.
   • A FH of (PJS), \[2, 7, 8\]

The criteria were met when the histopathology reported the intussuscepted segment of the small intestine has two infarcted polypoid lesions consistent with Peutz Jeghers polyps, the polypoid lesions composed mainly of extensive infarcted intestinal tissue with few preserved area of epithelium and lamina propria intervened by arborizing fascicles of smooth muscles \textbf{FigureD}, the histopathology reports absence of any malignant changes.

Conclusion:

To minimize relaparotomies and avoid complications of repeated resections and also halt any malignant growth related to the aforementioned, affected people are advised to undergo regular upper endoscopy, colonoscopy, and small bowel and pancreatic imaging. Polyps > 1 cm in size should be removed as a precautionary measure to prevent complications such as anemia related to chronic GIT bleeding and obstructions due to polyps and intussusceptions that might eventually result in complications such as short bowel syndrome or malnutrition due to repeated resections of recurrent intussusceptions. \[6\]

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

References: