Bacteriological and Enzymatical Study on Rheumatoid Arthritis Patients

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Abstract

The current study included the collection of 175 samples (blood-urea) of patients suffering from rheumatism, collected from Baghdad Teaching Hospital (Educational Laboratory), Al- Kindy Teaching Hospital, Al-Imamian Al-Kadhimya in Medical City in Baghdad at different duration between 2016/10/1 -2017/2/1. The bacterial growth results showed that 80% of urea samples positive for bacterial culture, while the rate of samples did not show any bacterial grow this 20%. The isolation subjugates to morphological, microscopically and biochemical tests, as also diagnosis by Api system. The most frequent bacterial pathogenic is *E. coli* which appeared highly rate(41.97)% followed by *E. cloacae* (21.25)%, *P. aeruginosa* (12.5)%, *Salmonella* (10)% and the proportion of *K. pneumonia* (7.5)%, while *S. marcescens* showed (6.25)%. When the measurement of the concentration of liver enzymes Glutamic Oxalate Transaminase (GOT), Glutamic Pyruvate Transaminase (GPT), Alkaline phosphates’ (ALP), the results showed a significant decreas P≤0.05 in the level of enzyme GPT in patients serum which reached 16.94±0.84 mg/ml, while its level in the healthy serum was 6.78 ± 6.78 mg / mL ALP enzyme results showed non-significant high at P≤0.05 in the level of patients serum with rheumatoid arthritis, as it reached the level in the serum 2.46±134.42 mg/ml, while the level in the healthy serum was 0.50±4.11 mg/ml. The enzyme GOT showed on-significant high at P≤0.05 in the level of patients serum as it reached the level in the serum 0.88±21.51 mg/ml, while the level in the healthy serum was 0.50±4.11 mg/ml.

Keywords: Liver enzymes, Rheumatoid arthritis, Bacteria.

Introduction

Rheumatoid arthritis is known as chronic autoimmune inflammatory and systematic disease infects different joints in the body, it is infects about 1% of the adult population could lead to an increase in the mortality rate at double, a global prevalence disease affects females more than male1.

The serious situation of the disease infected synovium region for large joints which contains a lot of synovial fluid resulting from the infection inflammation in synovial fluid container on the large number of macrophage and fibroblast2. Many of the causes interaction that lead to the occurrence of the disease, the effect of genetic factors, these factors constitute a proportion of 60-40% of the level of preparedness of the individual to the disease3, or as a result of an imbalance in the immune system or physical effort or the effect of environmental factors such as bacterial infection in addition to smoking, the hormones and lack of vitamins as assist factor in the development of rheumatoid arthritis4.

Rheumatoid arthritis, lead to many effects it cause to infection of other organs in the body such as the lung, eye, nervous system, heart and blood vessels5,6. Because of rheumatoid arthritis disease as systematic-inflammatory, there for the target organ at basics liver primarily, the liver is the largest organ in the body and has many of the basic functions, the metabolic processes that occur on the carbohydrate, proteins and fats as well as enter in synthesis of plasma proteins and the removal of the toxicity of drugs and toxic

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materials within the body\(^7\). The liver tissues in patients have hypertrophy in Kupffer cells, an increase inflation in the lymphocytes and monocyte and hepatomegaly\(^8\).

The effectiveness of liver enzymes change as a result of hepatocellular damages, resulting in increased concentration level of enzymes according to the severity of liver disease and potential toxicity with the increased incidence of various diseases of the liver disorders\(^9\), \(^10\). The reason for the rise of the enzymes at rheumatoid arthritis, to the expansion of the bile ducts of the liver under the influence of the effectiveness of the disease\(^11\).

Experimental

Study Samples

A total of 175 blood and urea samples were collected at age 20-60 years old from the clinic consultation at the following hospitals: Baghdad Teaching Hospital (Educational Laboratory), Al-Kindy Teaching Hospital, Al-Imamian Al-Kadhimyain Medical City in Baghdad, at the period 1/10/2016 to 1/2/2017. The current study included 75 blood samples and 100 urea samples.

Collecting Blood Samples

Withdrawn 5 ml of venous blood from patients with rheumatoid arthritis, and blood put on the test tube empty of any substance, for the purpose of the separation of the blood and get the serum. To prepare the serum the tube after the clotting put on centrifuge for 3 minutes at 3000 RPM, then the withdrawal of the serum and neglected the deposit and kept at a temperature 20\(^\circ\)C for the enzymatic tests.

Collecting Urea Samples

Samples were collected from 100 patients covered by the current study from both sexes, and at different ages. The samples had been cultured directly by a streak method on the Mac Conkey agar medium and blood agar medium. The plates were reversed and incubated at 37\(^\circ\)C for 24 hours.

Isolation and Diagnosis Bacteria

Diagnosed bacterial isolates depending on cultural, microscopically and biochemical tests adopted by\(^12\), as also the diagnosis by API20E system and according to the manufacturer instructions.

Antibiotic Susceptibility Test

Antibiotic sensitivity test was conducted to disc method on the agar-Müller-Hinton agar by using following antibiotics: Amikacin, Impenem, Cefotaximb, to bramycin, Cefixime, Tetracycline, Gentamycin, Amoxicillin, Ceftazidine, and Trimethoprim. The measurement of inhibition diameter zone by (mm) around antibiotics discs was depended and compared with global measurements according to CLSL (2013).

Results and Discussion

Isolation and Identification

80 a sample is positive for bacterial culture of a total of 100 samples main that (80\%) of urea of patients with rheumatoid arthritis, as in table 1.Interaction of genetic factors, age, sex, hormones and environmental factors such as infectious injuries to start rheumatoid arthritis\(^13\). The results of the current study, as indicated in the table (1) that Escherichia coli bacteria is the most frequent species of bacteria, where it was isolated from 34 patient at percentage (41.97)\%. These results of the present study agreed with local study by\(^14\). Followed by Enterobacter cloacae bacteria came in second by (17) sample at percentage (21.25)\%, as the results of the study differed the findings of\(^15\), while the percentage of the isolation of Pseudomonas aeruginosa(12.34)\%, which represents (10) cases, in the third site came our consistent with\(^16\), \(^17\). At fourth site the infection with Salmonella spp. by (10)\%, which represents (8) cases of infected and this result a violation with\(^18\). Serratiamarcescens bacteria appeared (5) isolates by (6.25) % as a result of the current study disagree with\(^19\), either Klebsiella pneumonia bacteria recorded (7.5)\% by (6) isolates which differed with the results of\(^20\).

Antibiotic Susceptibility Test

All isolated of E.coli bacteria showed that effectiveness against antibiotics Tobramycin, Amoxicillin and Gentamycin as shown in table (2). It was found that all isolates sensitive by (52.94) %, while the percentage of resistance Cefixime is (52.94) % as these results of the current study differed to\(^20\). While a decline in resistance of bacteria to the antibiotic Ceftazidime at (38.2) % and converged on the results of the current study with the findings\(^21\) as the percentage of resistance reached to (31.6) %.
As the antibiotic Cefotaxime the percentage of resistance (44.11) %, these results of the current study differed with\textsuperscript{15} that recorded a percentage of resistance reached (28) %. The carbapenems antibiotic group which includes antibiotic Impenem, the percentage of resistance (52.94) %, these results of the current study differed with\textsuperscript{17}, as well as resisting of E. coli bacteria to antibiotic Trimethoprim which record (50) %.

The results of the current study violated\textsuperscript{22}, who record the percentage of resistance (70.4)%. While resisting E. Cloaceae of Tobramycin, Gentamycin and Trimethoprim were (68.75, 62.5, 62.5) % respectively, the percentage of antimicrobial-resistant of Ceftaxime, Ceftazidime and Cefotaxime were(62.5, 62.5, 43.75) % respectively. The resistance of bacteria to Impenem was (68.75)% while the resistance of E. cloacae to Amoxicillin and Tetracycline were(68.75, 56.25) %. The results of the current study differed with\textsuperscript{23}. P. aeruginosa bacteria showed full resistance for Ceftazidime (100) %.

The percentage of their resistance to amino glycosides antibiotics Amikacin, Tobramycin and Gentamycin in (70, 70, 60) % respectively, while the resistance trend of Ceftaxime and Cefixime(70, 50)%and found that the percentage of resistance to the antibiotic Impenem (80) % and Tetracycline (60) %, the percentage of resistant bacteria to Amoxicillin and Trimethoprim (70) %. These results of the present study agreed with\textsuperscript{19} for the antibiotic Amikacin and our results for the antibiotic Tetracycline and Gentamycin consistent with\textsuperscript{24}, as it was found that the percentage of resistance was (60.71) %. Our results for Cefotaxime agreed with\textsuperscript{25}, as the percentage of resistance (70.4) %, while the results of the current study differed for Cefixime with\textsuperscript{26}as the percentage of resistance reached (80) %.

Our results for Trimethoprim, Impenem and Amoxicillin disagree with\textsuperscript{14, 27}, while our results agreed with\textsuperscript{18, 28}and for the antibiotic Ceftazidime, Tobramycin. While the resistance of the Salmonella bacteria Cefixime and Amoxicillin (100) %, the percentage of resistance to Impenem, Cefotaxime, Trimethoprim and Gentamycin (75) % and the percentage of resistance to the antibiotic Tobramycin and Ceftazidime(50) %, found that the percentage of resistance to the antibiotic Amikacin (25) % and the antibiotic Tetracycline (50) %, the results of the current study are consistent with the findings of\textsuperscript{29} for the antibiotic Gentamycin, which found that the resistance of the Salmonella bacteria isolated from diarrhea (75)%and disagree for Cefotaxime and Tetracycline the percentage resistance (75.6, 69)%. Our results differed for Impenem, Amikacin, Ceftazidime, Cefixime and Trimethoprim with\textsuperscript{30}, they found that the resistance of bacteria to antibiotics (0)%,\textsuperscript{31}found that the percentage of resistant bacteria to Amoxicillin (100)%.

The results of the current study disagree with\textsuperscript{32} for Cefixime which recorded low percentage of resistance (16.92)%, while the bacteria S. marcescens showed a full resistance to all antibiotics under study as the percentage of resistance (100)% as the results of the current study agreed to\textsuperscript{33} for Amikacin and disagree with them for the following antibiotics Tetracycline, Ceftazidime, Gentamycin ,Impenem, Trimethoprim and Cefotaxime and also differed for the antibiotics Amoxicillin and Cefotaxime with\textsuperscript{34}.

As well as bacteria K. pneumoniae showed resistance for all antibiotics under study, as the results of the study agreed with\textsuperscript{15} for the antibiotic Amoxicillin and Tetracycline which record (100)%,the results of this researcher disagree with the following antibiotics: Amikacin, Cefotaxime, Trimethoprim, Tobramycin and Gentamycin which sensitivity of bacteria to ward these antibiotics (0)%, and our findings contrary to\textsuperscript{15}who found that the sensitivity of the bacteria to ward Impenem, Ceftazidime and Cefotaxime (29.41, 64.70%,100).

Measurement of the Concentration of Liver Enzymes in the Serum of Patients with Rheumatoid Arthritis

Used several kite from Randox Company (USA) to measure the concentration of enzymes GPT, GOT and used the kite by a company Bio Merieux to measure the concentration of the ALP.

The Study of the Concentration of Liver Enzymes by Age Categories and Sex

The results of the current study revealed that the level of the ALP record high an increase in the first age category 20-30 years and the second age category 31-40 years followed by
the forth age category 51-60 years. As a research study results showed an increase in the level of the enzyme at the age above of 50 years as the enzyme ALP rise above the normal level in the growth stage, adolescence and adults over the age of 50 years. As for GPT and GOT enzymes found an increase in the level in the second age category 31-40 years and 41-50 category may the reason for the rise in the increase of enzymes GPT, GOT to the high body mass.

Measure the Concentration of Liver Enzymes Got, Gpt, Alp in the Serum of Patients

The results of the current study has shown that the rise in the average concentration of ALP significantly in patients. These results are consistent with who pointed to the reason for the high level of ALP returns to infect the disease small joints in the hand as well as the infected of all joints in the body. The immune system for patients with rheumatoid arthritis, attacking the bone through chemical inflammatory signals these signals rebuild the bone and raise the level of the enzyme in the patients.

Also founded significant increase P≤0.05 in average concentration of GOT in the serum of patients as it reached in the serum (21.51) mg/ml, while in the serum of healthy people (0.88) mg/ml. These findings agreed with the results of .The reason for the rise of GOT enzyme in the patients to damage in liver cells and necrosis which leads to a rise in the serum. The results of the current study differed which demonstrated a significant decrease in the average concentration of GPT as in patients (16.94) mg/ml compared with its level at control group (0.84) mg/ml.

These results agree with, who logged a significant increase in the average concentration of the enzyme and found that GPT enzyme highest percentage in the liver and less percentage in the serum and it differs from an object to another and from tissue to another.

Figure 1: Show the average concentration of liver enzymes CPT, GOT, ALP for patients with rheumatoid arthritis and control by mg/ml

Table 1: Numbers and percentages of bacterial species, isolated from the urea of rheumatoid arthritis patients

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. isolation</th>
<th>Percentage (%)</th>
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</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>34</td>
<td>41.97</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>17</td>
<td>21.25</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>Salmonella SPP</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>6</td>
<td>7.5</td>
</tr>
<tr>
<td>Serratiamarcescens</td>
<td>5</td>
<td>6.25</td>
</tr>
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</table>
Table 2: Resistance of isolated bacteria from urea of rheumatoid arthritis patients to antibiotics

<table>
<thead>
<tr>
<th>Antibiotic Bacteria</th>
<th>No.</th>
<th>AK 30ug</th>
<th>AX 25ug</th>
<th>CN 10ug</th>
<th>TOB 10ug</th>
<th>TMP 10ug</th>
<th>TE 10ug</th>
<th>IPM 10ug</th>
<th>CFM 10ug</th>
<th>CTX 10ug</th>
<th>CAZ 10ug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>34</td>
<td>17 (50)</td>
<td>16 (47.05)</td>
<td>16 (47.05)</td>
<td>16 (47.05)</td>
<td>17 (50)</td>
<td>17 (50)</td>
<td>18 (52.94)</td>
<td>18 (52.94)</td>
<td>15 (44.11)</td>
<td>13 (38.2)</td>
</tr>
<tr>
<td>Enterobacter Cloacae</td>
<td>18</td>
<td>12 (75)</td>
<td>11 (68.75)</td>
<td>10 (62.5)</td>
<td>11 (68.75)</td>
<td>10 (62.5)</td>
<td>9 (56.25)</td>
<td>11 (68.75)</td>
<td>10 (62.5)</td>
<td>10 (62.5)</td>
<td>7 (43.75)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10</td>
<td>7 (100)</td>
<td>7 (100)</td>
<td>6 (100)</td>
<td>7 (100)</td>
<td>7 (100)</td>
<td>6 (100)</td>
<td>8 (100)</td>
<td>5 (100)</td>
<td>7 (100)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Salmonella</td>
<td>4</td>
<td>3 (75)</td>
<td>4 (100)</td>
<td>3 (75)</td>
<td>2 (50)</td>
<td>3 (75)</td>
<td>3 (75)</td>
<td>4 (100)</td>
<td>3 (75)</td>
<td>2 (50)</td>
<td></td>
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<tr>
<td>S.marcescens</td>
<td>5</td>
<td>6 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>3 (100)</td>
<td>2 (100)</td>
<td>10 (100)</td>
<td></td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td>6</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The effect of age and sex in the level of concentration of liver enzymes at the patients suffering from rheumatoid arthritis

<table>
<thead>
<tr>
<th>Liver enzyme</th>
<th>Sex and age</th>
<th>GPT SE+M</th>
<th>GOT SE+M</th>
<th>ALP SE+M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>1.33±16.84</td>
<td>1.27±20.98</td>
<td>3.67±133.31</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.36±17.73</td>
<td>1.23±21.58</td>
<td>3.58±133.77</td>
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<tr>
<td></td>
<td>30-20</td>
<td>2.53±10.00</td>
<td>4.10±22.95</td>
<td>4.50±147.85</td>
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<tr>
<td></td>
<td>40-31</td>
<td>1.93±19.24</td>
<td>2.07±24.91</td>
<td>3.50±142.81</td>
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<tr>
<td></td>
<td>50-41</td>
<td>1.30±18.66</td>
<td>1.76±23.10</td>
<td>4.84±132.21</td>
</tr>
<tr>
<td></td>
<td>60-51</td>
<td>1.52±14.25</td>
<td>2.18±22.05</td>
<td>3.65±135.34</td>
</tr>
</tbody>
</table>

References

27. Idrees EKM (2012) Comparison between different phenotypic and genotypic methods for detection of metallo β-lactamases in Pseudomonas aeruginosa that has multidrug resistance the antibiotics. College of Science. Abdel Aziz University.