Ultrasonographic and biochemical Diagnosis of Experimental nephrotoxicity in Dogs

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Abstract:

This study was carried out on ten puppies which subjected to experimental nephrotoxicity by gentamycine. The clinical examination of puppies was dullness, restlessness, polyuria, polydipsia, vomiting, severe salivation and dehydration. Sonographic examination of the affected kidneys in puppies was changed to show greater echogenicity of the renal cortex. These changes were more distinct after 10th of injection and characterized by appearance of intensive hyperechogenicity and complete loss of corticomedullary junction. In relation to hematological parameters which revealed homoconcentration, leukocytosis and neutrophilia at 5th day and reach its maximum value at 10th day. At this time, serum analysis indicated increase serum urea, creatinin and uric acid at 5 and 10 days respectively. Serum total proteins and albumin decreased at 5th day of injection at 10 day respectively. Microscopically, examination of the affected kidneys revealed diffuse coagulative necrosis of most of the renal epithelial cells.

Key words: Dogs, Ultrasonography, Nephrotoxicity

Introduction:

Renal disorders were common diseases of the dog that associated with other organs, not all diseases or lesions of the kidney resulting in clinical signs (Chandler et al., 1995). Clinical signs and accumulated laboratory data may not always point towards existing renal diseases. Abdominal radiography can also uncover suspected or unsuspected renal abnormalities, which can be characterized further with ultrasonography (Konde et al., 1986). Ultrasound had great value in the planning of further diagnostic and therapeutic procedures. It could be repeated without risk to patient or operator (Mwanza et al, 1996). Ultrasonography has been considered to be one of the most valuable imaging techniques for evaluation of the abdominal cavity and its organs including liver, biliary system, kidney, urinary bladder, pancreas, stomach, small intestine, uterus and prostate (Cruz and Wriggly, 2003). Therefore, the aim of this work is to evaluate different methods used for diagnosis of nephrotoxicity through the following:

1- Induction of some renal affection as nephrotixicity by gentamicine.

2- Recording the clinical picture, hematological and biochemical alterations changes.
3- Recording of an ultrasound image on the affected animal.

**Materials And Methods**

Ten puppies with ages ranged between (three to nine months old), weighing 4-10 kg were individually kept in steel cages and fed on a diet composed of meat, bones and bread. They were subjected to complete de-worming. After a period of adaptation (6weeks), the puppies were subjected to **experimentally-induced** nephrotoxicity.

**A-Clinical examination:**

Firstly, deworming occurred by injection of ivermectin 1 % and was repeated after 3 weeks with continues dipping in a disinfectant. During this period, periodic clinical and laboratory examinations were applied. Determination of the body temperature, pulse and respiratory rates as well as examination of mucous membranes, lymph nodes and abdomen was conducted according to Kelly, (1984).

**B- Experimental work:**

Ten puppies were injected I/M with gentamycin 10% as toxic dose (30mg/kg) B.wt. daily for 10 days according to Helal (2005). All puppies were evaluated by clinical examination, ultrasonographic and laboratory studies before and at 5 and 10 days of injection.

**C- Ultrasonographic examination:**-According to (Barr, 1992).

**D- Haematological examination:**

Total erythrocytic count, total leukocytic count, hemoglobin, PCV differential leucocytic count according to (Coles, 1986).

**E- Biochemical analysis of serum:**

E.1. Serum urea level (mg/dl) was determined acc. to (Patton and Crouch., 1977).

E.2. Serum uric acid level (mg/dl) was determined according to (Wilding and Heath., 1975).

E.3. Serum creatininc level (mg/dl) was determined according to (Young, 1990).

E.4. Serum albumin level (g/dl) was determined according to (Drupt, 1974).

E.5. Serum total proteins level (g/dl) was determined according (Henry, 1964).

E.6. Serum alanine amino-transferase (ALT) and Serum aspirate amino-transferase (AST) levels (U/L) was determined according to (Reitman and Frankel, 1957).
E.7. Serum alkaline phosphatase (ALP) level (U/L) was determined according to (Rec. GSCC.DGKC, 1972).

E.8. Serum gamma-glutamle transferase level (U/L) was determined according to (Persijin And Van Der Silk, 1974).

E.9. Serum total cholesterol level (mg/dl) was determined according to (Ratliff and Hall, 1972).

E.10. Serum bilirubin level (mg/dl) was determined according to (jendrassik et al., 1938).

F-Macroscopic examination of kidney of the necropsied puppies:

Kidney of each necropsied puppy was carefully examined by naked eye for detection of any gross lesions.

G-Pathologic studies:-

Following complete necropsy sacrificed animals, fresh renal specimens were collected and examined histopathologically according to (Culling 1983).

H-Statistical analysis:-

Data obtained were statically analyzed to illustrate the results of his study using the methods of Norman and Baily (1997).

Results

1. Clinical features:

Puppies of this group showed inappetance, dullness, restlessness, polyuria, polydipsia, vomiting, severe salivation and dehydration. Clinical signs of anemia were detected after 5 days in puppies of this group.

2. Hematological examination:

2. 1 Whole blood picture.

The mean value of PCV % and TWBCs count were significantly (P<0.0.5) increased at the 5th day post injection in puppies injected with toxic dose of gentamicin but were highly significant at 10th day post injection (p<0.01). The mean value of Hb content and TRBCs count were significantly (P<0.05) decreased at the 5th day and highly significant at 10th day of injection (p<0.01). (Table 1).

2. 2. Leukocytic differential count.
The mean value of neutrophils % was significantly (P<0.05) increased at the 5th day and highly significant at 10th day of injection (p<0.01). The mean values of lymphocytes % and monocytes % were significantly (P<0.05) decreased at the 5th day and highly significant at 10th day of injection (p<0.01). (Table 2).

3. Serum analysis.


The mean values of serum urea, serum creatinine and serum uric acid were significantly (P<0.05) increased at 5th day and highly significantly (P<0.01) increased at 10th day of injection, (Table 3).

3.2. Serum Protein Profile.

The mean values of serum total protein, albumin and A/G ratio, in puppies injected with toxic dose gentamicin were significantly (P<0.05) decreased at 5th day post injection and they were highly significantly (P<0.01) decreased at 10th day post injection, (Table 4).

3. 3. Liver function tests.

The mean values of serum activities of ALP, GGT, ALT, AST, total cholesterol and total bilirubin were not significantly (P<0.05) changed during the experiment, (Table 5).

4. Sonographic examination:

Ultrasonographic appearance of the kidney before the Injection showed that the renal cortex has a homogenous echogencity and affine echo texture. The medulla was uniform in echogencity and hypoechoic relative to the cortex. The demarcation between the cortex and the medulla was crisp. (Fig.1) Ultrasonographic appearance of both kidneys of puppies before injection of toxic dose of gentamicin revealed that the renal cortical echogenicity of the left kidney was less echoic than that of the adjacent spleen and liver. Renal cortex of the right kidney is less echoic than the adjacent liver, (Fig 2, 3). At fifth day post injection ultrasonographic appearance of left kidney showed the same echogenicity of the renal cortex when compared with spleen, (Fig 4). The renal cortex of right kidney, appear more echoic when compared with liver, (Fig 5). At 10th of injection, ultrasonographic appearance of left kidney showed higher echogenicity of the renal cortex as compared with spleen, (Fig 6). And the renal cortex of right kidney appears more greater echoic when compared with liver, (Fig 7).

5. Macroscopic examination of kidneys of necropsied puppies:
The kidneys of puppies injected with toxic dose of gentamicin showed diffuse grayish white areas. On cut section there was pale renal cortex and medulla and some show a large area of congestion at corticomedullary junction. (Fig 8, 9).

1.2.1.6. Histopathological examination:

Histopathological examination of the kidneys of dogs injected with toxic dose of gentamicin after 10 days of injection showed diffuse coagulative necrosis of most of the renal epithelial cells. Proximal convoluted tubules with mild lymphocytic infiltration in the interstitial tissue and the glomeruli appear hyper cellular with obliteration of the Bowman space. (Fig 10, 11).

Table (1). Hematological parameters of puppies injected with toxic dose of gentamicin 10 % (Mean±S.E).

<table>
<thead>
<tr>
<th></th>
<th>Before the injection</th>
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<tr>
<td></td>
<td></td>
<td>5th days</td>
</tr>
<tr>
<td>PCV %</td>
<td>39.0±1.0c</td>
<td>42.33±0.33b</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>13.07±0.17a</td>
<td>6.73±0.008b</td>
</tr>
<tr>
<td>RBCs million/mm³</td>
<td>7.07±0.42a</td>
<td>6.27±0.003b</td>
</tr>
<tr>
<td>WBCs Thousand/mm³</td>
<td>9.45±0.17c</td>
<td>12.63±0.15b</td>
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Means within the same row having the same letters aren’t significantly different at (P<0.05).

Table (2). Differential leukocytic count of puppies injected with toxic dose of gentamicin 10 % (Mean±S.E).

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<tr>
<td></td>
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<td>5th days</td>
</tr>
<tr>
<td>N %</td>
<td>65.33±0.33c</td>
<td>72.33±0.33b</td>
</tr>
<tr>
<td>L %</td>
<td>24.0±0.58a</td>
<td>18.33±0.33b</td>
</tr>
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Means within the same raw having the same letters aren’t significantly different at (P<0.05).

Table (3). Kidney function tests of puppies injected with toxic dose of gentamicin 10 % (Mean ±S.E).

<table>
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<th>Before the injection</th>
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<tr>
<td></td>
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<td>5th days</td>
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<tr>
<td>Urea mg/dl</td>
<td>26.74±1.36c</td>
<td>43.19±3.98b</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.87±0.003c</td>
<td>2.52±0.11b</td>
</tr>
<tr>
<td>Uric acid mg/dl</td>
<td>2.35±0.009c</td>
<td>4.99±0.006b</td>
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</table>

Means within the same raw having the same letters aren't significantly different at (P<0.05).

Table (4). Serum proteins profile of puppies injected with toxic dose of gentamicin 10 % (Mean±S.E).

<table>
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<th>Before the injection</th>
<th>After the injection</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>5days after</td>
</tr>
<tr>
<td>Total proteins g/dl</td>
<td>6.83±0.30a</td>
<td>5.58±0.003b</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>3.81±0.21a</td>
<td>2.62±0.21b</td>
</tr>
<tr>
<td>Globulins g/dl</td>
<td>3.02±0.17a</td>
<td>2.93±0.38a</td>
</tr>
<tr>
<td>A/G ratio %</td>
<td>1.27±0.009a</td>
<td>0.94±0.17ab</td>
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Means within the same raw having the same letters aren’t significantly different at (P<0.05).
Table (5). Liver function tests of puppies injected with toxic dose of gentamicin 10 % (Mean±S.E).

<table>
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<th></th>
<th>Before the injection</th>
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<tr>
<td></td>
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<td>5th days</td>
</tr>
<tr>
<td>Alkaline phosphatase U/L</td>
<td>15.87±0.24a</td>
<td>15.94±0.004a</td>
</tr>
<tr>
<td>Gamma glutamyl transferase U/L</td>
<td>1.44±0.008a</td>
<td>1.37±0.003a</td>
</tr>
<tr>
<td>Alanin transferase U/L</td>
<td>26.8±0.21a</td>
<td>27.5±0.38a</td>
</tr>
<tr>
<td>Asparate transferase U/L</td>
<td>24.27±0.24a</td>
<td>24.37±0.27a</td>
</tr>
<tr>
<td>Total cholesterol mg/dl</td>
<td>148.14±2.37a</td>
<td>151.89±1.72a</td>
</tr>
<tr>
<td>Total biliruben mg/dl</td>
<td>0.279±0.004a</td>
<td>0.281±0.007a</td>
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Means within the same raw having the same letters aren't significantly different at (P<0.05).

Fig (1) Ultrasonogram and schematic representation of normal left kidney. C= renal capsule, A W=abdominal wall, P=parenchyma and M=medullary pyramid.
Fig (2). Ultrasonogram and schematic representation of left kidney before the injection of the toxic dose of gentamicin. Less echoic renal cortical echogenicity of the left kidney than that of the adjacent spleen. In addition, less echoic when compared with the liver  W=abdominal wall , L K =left kidney , S=spleen, L= liver and D =diaphragm.

Fig (3). Ultrasonogram and schematic representation of right kidney before the injection of the toxic dose of gentamicin. Less echoic renal cortical echogenicity of the right kidney than that of the adjacent liver.  W=abdominal wall, R K =right kidney, L= liver and D =diaphragm.
Fig (4). Ultrasonogram and schematic representation of left kidney after 5 days from the injection of the toxic dose of gentamicin. Isoechoic renal cortical echogenicity of the left kidney than that of the adjacent spleen. W=abdominal wall, L K =left kidney and S=spleen.

Fig (5). Ultrasonogram and schematic representation of right kidney after 5 days from the injection of the toxic dose of gentamicin. More echoic renal cortical echogenicity of the right kidney than that of the adjacent liver. W=abdominal wall, R K =right kidney, L= liver and D =diaphragm.
Fig (6). Ultrasonogram and schematic representation of left kidney after 10 days from the injection of the toxic dose of gentamicin. More echoic renal cortical echogenicity of the left kidney than that of the adjacent spleen. W=abdominal wall, LK =left kidney, E=enhancement and S=spleen.

Fig (7). Ultrasonogram and schematic representation of right kidney after 10 days from the injection of the toxic dose of gentamicin. Sever echoic renal cortical echogenicity of the right kidney than that of the adjacent liver. W=abdominal wall, RK =right kidney, L= liver and E=enhancement
Fig (8,9). Kidneys of puppies injected with toxic dose of gentamicin after 10th of injection showing diffuse grayish white areas and cut section of puppy kidneys injected with toxic dose of gentamicin after 10th of injection revealed (P) pale renal cortex and medulla with presence of hemorrhage at cortico-medullary junction (H).

Fig (10,11). Diffuse coagulative necrosis of most of the renal epithelial cells, proximal convoluted tubules with mild lymphocytic infiltration in the interstitial tissue and Glomeruli appear hypercellular with obliteration of the Bowman space.

Discussion

With respect to gentamicin intoxicated puppies group the clinical features were inappetance, dullness, restlessness, polyuria, polydipsia, vomiting, sever salivation and dehydration. Anemia was found after 5 days in puppies through paleness of visible mucous membrane of this group. These findings were prominent from the fifth day of the injection. Similar finding were previously recorded by Nelson and Couto (1992), Aguiar-Hennemann et al.(1997) ,El-Shawarby and Abd-el-Raof (1999). Moreover, Helal (2005), attributed these findings to toxic effects of gentamicin on renal tissues. The mean value of PCV % and WBCs were significantly increased at
the 5th day post injection. But highly significant at 10th day of injection. The increase in PCV% might be attributed to dehydration Schalm et al., (1975) and Helal, (2005) and the increase of WBCs may attributed to stress which occurred in dogs given toxic dose of gentamicin as a result of endogenous corticosteroids release secondary to nephrotoxicity. Those results were coincided with those of Brown et al. (1985), Rivers et al. (1996a), Shaw and Ihle (1997) and Helal (2005). The mean value of Hb content and RBCs count were significantly decreased at the 5th day of injection in puppies injected with toxic dose of gentamicin. Highly significant decrease was observed at 10th day of injection. Those results were coincided with those of Moorehead (1975), Wills (1977), Fried (1981) and Helal (2005), who stated that the rapid onset of acute renal failure precludes most of the hormonal changes seen in chronic renal failure, thus hyperthyroidism was not a feature, nor is defective erythropoietin production although, other factors (e.g. hemorrhage and RBC fragility) contribute to anemia. The mean value of neutrophils % was significantly increased in the 5th day and highly significant in 10th day of injection. The mean values of lymphocytes % and monocytes % were significantly decreased at the 5th day post injection in puppies injected with toxic dose of gentamicin, and highly significant at 10th day of injection. These results could be attributed to stress which occurred in puppies given toxic dose of gentamicin because of endogenous corticosteroids release secondary to nephrotoxicity. This was agreed by Brown et al. (1985). Rivers et al. (1996a), Shaw and Ihle (1997) and Helal (2005). The mean values of serum urea, creatinine and uric acid were significantly increased at 5th day post injection and highly significantly increased at 10th day. These results were in agreement with those of Brown et al. (1985).Rivers et al. (1996a), El-Shawarby and Abd-El-Raof (1999), Kim et al. (2000) and Helal (2005). The authors attributed this to fact that any damage of renal tissue was followed by increase urea, creatinine and uric acid. The mean values of total proteins and albumin in puppies injected with toxic dose gentamicin were significantly decreased at 5th day of injection and they were highly significantly decreased at 10th day of injection. This result was agreeable with Brown et al. (1985) and Helal (2005), who recorded that in amino-glycosides induced nephrotoxicosis there was hypo-protiemia and hypo-albuminemia. Globulin was not significantly changed during 10 days post injection. And A/G ratio was significant decrease at 10 after the injection. The mean values of serum ALP, GGT, ALT, AST, cholesterol and total biliruben were not significantly changed during 10 days. Ultrasonographic appearance of both kidneys of puppies before injection of toxic dose of gentamicin revealed that the renal cortical echogenicity of the left kidney was less echoic than that of the adjacent spleen. In addition to be less echoic when compared with the liver. The renal cortex of the right kidney was less echoic than the adjacent liver. At 5th day of injection ultrasonographic appearance showed greater echogenicity of the renal cortex. These changes were more distinct after 10th of injection and characterized by appearance of intensive hyperechogenicity and complete loss of
corticomедullary junction. These results coincided with Rivers et al. (1996a), El-Shawarby and Abd-El-Raof (1999), Macanovic et al. (1999), Jin et al.(2001) and Helal (2005) who stated that after treatment with toxic dose of gentamicin, the renal cortex was hyperechoic relative to the surrounding tissue. Increased renal cortex echogenicity was associated with significant nephrotoxicosis. The kidneys of puppies injected with toxic dose of gentamicin showing diffuse grayish white areas. On cut section there were pale renal cortex and medulla and some cases showed a large area of congestion at cortico-medullary junction. This appearance was coincided with the ultrasonographic images and was similar to that observed by Rivers et al. (1996a) and Helal (2005). Histopathological examination of the kidneys of dogs injected with toxic dose of gentamicin after 10 days of injection revealed diffuse coagulative necrosis of most of the renal epithelial cells. Proximal convoluted tubules were with mild lymphocytic infiltration in the interstitial tissue and the glomeruli appear hypercellular with obliteration of the Bowman space. This agreed with Rivers et al. (1996a) and El-Shawarby and Abd-El-Raof (1999).

References


المتخصص العربي
أجريت هذه الدراسة على عدد عشرة كلاب صغيرة واثبت الفحص الاكثنيك حدوث الم شديد وفقدان الشهية وبالفحص بالموجات فوق الصوتية أظهر زيادة في ومض الكلية البصري مقارنة بالطحال والكلية البصري مقارنة بالكلية وفحص صورة الدم اظهر زيادة في تركيز خلايا الدم زيادة في العدد الكلى لكرات الدم البيضاء وخاصة الخلايا المتعادة ونقص في الخلايا الليمفاوية و الهموجلوبين والعدد الكلى لكرات الدم الحمراء وفحص مصل الدم اظهر زيادة معتدلة في مستوى بوليما الدم والكرياتينين وحمض البوريك ونقص بروتينات الدم ووزان الداء و نسبة الزائدة.