Evaluation Of Ultrasonography As A Bioindicator of Renalotoxicity

El-Shawarby, R. M. and Abd-El-Roaf, Y. M.*
Dept. of Forensic Med. and Toxicology - * Dept. of Animal Medicine
Fact. of Vet. Med. - Zagazig Univ., Benha branch

ABSTRACT

Nephrotoxicity in dogs was experimentally induced by the oral administration of ethylene glycol 95% (10 ml/kg b.w. one dose) and IM injection of gentamicin (20 ml/kg b.w. daily for 10 days). It was diagnosed by the Ultrasonography in assistance of the clinical signs, some biochemical parameters of serum and the histopathological findings. Ultrasonography showed an increase in echogenicity of renal cortex and medulla in case of dog intoxicated by ethylene glycol opposite to that observed in case of dogs intoxicated by gentamicin. Levels of urea, uric acid, creatinine and sodium appeared significantly higher while potassium and chloride were significantly lower in serum of dogs intoxicated either by ethylene glycol and gentamicin when compared to that of control. Confirmatory results were obtained from the histopathological findings of kidneys in treated and control dogs.

INTRODUCTION

Ultrasonography produces image of tissue structures and from these images the size and nature of structures can be determined (1). The idea behind sonography was based on the pulse-echo-principles, as short electric pulses produced by a generator are converted by transducer into bursts of acoustic energy and the sound beam emitted proceeded into a typical divergent path produced different echoes. The ultrasound scanner convert this information into dots of light on the scanner (2).

Ultrasonography is one of the most recent techniques adapted for diagnosis of the renal affection of dogs (3). It is an easy, non invasive and does not associated with adverse effects (4).

The renal ultrasonographic changes of dogs intoxication by ethylene glycol were characterized by increase in echogenicity of both renal cortex and medulla. The changes were dose related (5-10). On other hand injection of gentamicin, one of the aminoglycoside group, is a nephrotoxic drug as indicated by the gray scale sonography (11), clinical signs (12), histopathological findings (13) and the biochemical analysis of serum (14-16). Therefore the present study was designed to justify implementation of sonography in diagnosis of nephrotoxicity, in addition to bioindicators and histopathological parameters. Gentamicin and ethylene glycol were chosen to represent nephrotoxic drugs.

MATERIALS AND METHODS

Chemicals

Ethylene glycol 95%, is a liquid commercial antifreeze obtained from Prestone antifreeze, Union Carbide Co., New York.

Gentamicin 10%, is one of the aminoglycoside antibiotic obtained from Memphis Co. for Pharm. & Chem. Ind., Cairo, Egypt.

Apparatus

Ultrasonography real time scanner with video printer, sector transducer, the prob with frequency 7.5 MHz (Linus) Japan.

Experimental design

The study was carried out on 15 healthy dogs aged 1.5-2 years and weighted 5-10 kg.

Animals were divided into three comparable groups each of 5 dogs, kept under observation for 2 weeks before experimental work.

The first group of dogs was fasted 24 hours. To avoid vomiting animals injected with chlorpromazine (1mg/Kg b.w.) before ethylene glycol administrated intoxication (9). Dogs in this group were administered orally with a single dose of 95% ethylene glycol as 10 ml/kg b.w.t. (9). The 2nd group of dogs was injected I.M. with gentamicin as toxic dose of 20 mg/kg b.w.t. daily for 10 days (17). The 3rd group of dogs was kept as control. All dogs were put under observation for recording the clinical signs.

At the end of the experiment for each group, blood samples from the cephalic vien (18) were collected without anti-coagulant to separate serum. Estimation of serum urea (19), uric acid (20), creatinine (21), sodium and potassium (22), as well as chloride (23) were carried out.

All dogs were examined by using 7.5 MHz focused transducer ultrasonography (2). Then after, all dogs were sacrificed and specimens from kidneys were taken. These specimens were fixed in 10% buffered formaline, dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin. Sections 5-
7 microns in thickness then stained by hematoxylin and eosin stain (24) for histopathological examination.

Data obtained were tabulated and statistically analyzed according to student's (t) test (25).

RESULTS

A) Ultrasonographic findings

The ultrasonographic examination of the kidneys of non treated dogs revealed hypoechoic renal cortex and anechoic renal medulla (Fig. 1). While the kidneys of dogs intoxicated with ethylene glycol showed an increase of echo-intensity of renal cortex than normal. Also the renal medullary echogenicity was increased, whereas the cortico medullary junction and central medullary region remained relatively sonoluent (Fig. 2). However after intoxication with gentamicin, the sonography showed a decrease of the echogenicity, specially at the interface between the pelvis and parenchyma (Fig 3).

B) The clinical signs

After intoxication of dogs by ethylene glycol, there were anorexia, ataxia, depression, vomiting and polydipsia. Another signs appeared after three hours in the form of difficult walking increased polydipsia and the dogs stood at their water bowls with their muzzles immersed and drank continuously. Rectal temperature was slightly decreased. Hyperpnoea and tachycardia were the early findings in all dogs while the bradycardia associated with the terminal stages.

The clinical signs concerning with intoxication of dogs treated by gentamicin appeared in form of inappetence, polyuria, polydipsia, vomiting, diarrhea and dehydration. Dyspnoea was observed in some cases. Anemia was obvious on dogs of this group. Dullness and ascites were clearly observed on some other cases. These findings were more prominent after 5 days of treatments.

C) The biochemical analysis

The serum analysis (table 1) of some kidney function tests re-caled a significant increase in the blood urea, creatinine values, uric acid and hyperkalemia. They were 42.67± 2.1, 1.21± 0.12, 3.44± 0.25 and 5.67± 0.34 mg/dl respectively when compared to 30.90± 1.40, 0.95± 0.08, 1.90± 0.09 and 3.89± 0.11 mg/dl in the control. However, significant decrease in sodium and chloride 99.30 ± 3.80 and 99.00 ± 2.08 mg/dl respectively was noticed after intoxication when compared to control (134.0 ± 3.20 and 112.30 ± 1.45 mg/分别).

Also a significant increase of blood urea, creatinine, uric acid and potassium (51.70± 6.50, 2.33± 0.33, 2.23± 0.18 and 5.05± 0.18 mg/dl respectively) were recorded post intoxication by gentamicin when compared to control (30.9± 1.40, 0.95± 0.08, 1.90± 0.09 and 3.89± 0.11 mg/dl respectively). Intoxication also showed a significant decrease in sodium and chloride (92.70± 9.10 and 103.1± 1.45 mg/dl respectively) when compared to the control (134.0± 3.20 and 112.30± 1.45 mg/dl respectively).

D) Histopathological findings

The microscopical examination of kidneys of control dogs showed pathological changes. (Fig. 4). While, kidneys in dogs intoxicated by ethylene glycol revealed tubular nephrosis by multiple areas cloudy swelling of convoluted tubules. Congestion of renal blood vessels with proliferation of glomerular tufts and disappearance of Bowman’s spaces were seen (Fig 5).

The microscopical examination of kidneys in dogs intoxicated with gentamicin showed focal multiple areas of tubular necrosis mainly in medulla represented by his eosinophilic cytoplasm and pyknosis of nuclei (Fig. 6). Congestion of some renal blood vessels and inter tubular blood capillary was also noticed.

Table 1: Biochemical parameters of kidney function serum of intoxicated dogs by ethylene glycol and gentamicin

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</th>
<th>Control</th>
<th>Dogs administrated Eth. gly. orally</th>
<th>Dogs treated with gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea nitrogen</td>
<td>30.90± 1.40</td>
<td>42.67± 2.10**</td>
<td>51.70± 6.50</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1.90± 0.09</td>
<td>3.44± 0.25**</td>
<td>2.7± 0.5</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.95± 0.08</td>
<td>2.18± 0.12**</td>
<td>2.3± 0.5</td>
</tr>
<tr>
<td>Sodium</td>
<td>134.0± 3.20</td>
<td>99.30± 3.80**</td>
<td>92.7± 9.10</td>
</tr>
<tr>
<td>Potassium</td>
<td>3.89± 0.11</td>
<td>5.67± 0.34**</td>
<td>5± 0.5</td>
</tr>
<tr>
<td>Chloride</td>
<td>112.30± 1.45</td>
<td>99.00± 2.08**</td>
<td>10± 1</td>
</tr>
</tbody>
</table>

± S.E. = Standard error
* Significant at P<0.01
** Significant at P<0.001
Fig. 1 (A & B) Kidney scan of apparently normal dog showing that renal medulla is the least echogenic followed by renal cortex. The renal cortex is hypoechogenic and finally granular in texture. The renal medulla virtually anechoic.
Fig. 2 (A & B) Kidney scan of a dog intoxicated with ethylene glycol showing an increase of echogenicity of renal cortex and medulla than the normal.
(A) Right kidney

(B) Left kidney

Fig. 3 (A & B) Kidney scan of dog intoxicated by gentamicin showing a decrease of echogenicity of renal cortex and medulla than the normal.
Fig. (4) Kidney of non treated dog showing normal histological renal tissue. H & E stain X 200.

Fig. (5) Kidney of dog intoxicated by ethylene glycol showing tubular necrosis represented by multiple area of cloudy swelling. H & E stain X 400.
DISCUSSION

The efficiency of using Ultrasonography and other tools in diagnosis of nephrotoxicity in dogs should be assessed. The present study is a trial to use the ultrasonography for the early diagnosis of acute nephrotoxicity by ethylene glycol and gentamicin in dogs. The ultrasonographic appearance of kidneys in case of ethylene glycol intoxication in dogs characterized by an increase echogenicity of the renal cortex than normal. Also, the renal medullary echogenicity was increased, whereas the corticomedullary junction and central medullary region remained relatively hyperechoic. These results partially agreed with previously cited reports (9,10) who attributed the increased echogenicity to cellular infiltration, mineral deposition and fat or fibrous replacement in kidneys. Also the increased echogenicity was confirmed in the present study by the histopathological findings which included tubular nephrosis. Concerning the ultrasonographic appearance of kidneys in gentamicin intoxicated dogs, there was a decrease of echo-intensity specially at the interface between pelvis and parenchyma. Such decrease in the echogenicity seemed to be the result of the tubular necrosis which displayed the present histopathological findings.

Regarding to the clinical observation in dogs intoxicated with ethylene glycol there were hypothermia, C.N.S. disorders, vomiting and oliguria. Hypothermia might be attributed to depression, coma and the sedative effect of ethylene glycol (6). Acute neurologic disorders probably attributed to aldehyde metabolites, metabolic acidosis and increased osmolality (26). The convulsion observed in the late stages of ethylene glycol intoxication might be the result of uremia (27). The vomiting and salivation signs were due to the irritant effect of ethylene glycol (28). In case of dogs intoxicated by gentamicin, the clinical signs were polydipsia, vomiting, diarrhea, dehydration, anemia, ascites and dullness. Similar findings were previously recorded (29) as toxic effects on renal tissues.

The significant increase of creatinine, urea, uric acid and potassium in serum of dogs intoxication with ethylene glycol indicates. Similar findings has been recorded in previous works (6,30). These can be attributed these changes to the direct effect of ethylene glycol on kidneys as showed from the present ultrasonographic examination and the histopathological findings. In case of gentamicine intoxication, significant increase of urea, uric acid, creatinine and potassium while serum sodium and chloride levels were significantly decreased. These results were in consistent with that previously reported (17,
30). These changes might be due to the nephrotoxic effect of gentamicin which causes destruction of glomeruli and proximal convoluted tubules. Confirmatory results were obtained by the sonographic examination and the histopathological findings in the present study.

From the present study, it can be concluded that the ultrasonographic examination can be used recommended in diagnosis nephrotoxicity of Dogs.

REFERENCES


المختص العربي

تقييم استخدام الموجات فوق الصوتية في تشخيص التسمم الكلوي في الكلاب

وجب الشواربي، ياسين عبدالرؤوف*

قسم الطب الشرعي والسموم، قسم طب الحيوان
كلية الطب البيطري - جامعة الزقازيق، فرع بنها

أجريت هذه الدراسة في محاولة لاستخدام الموجات فوق الصوتية في تشخيص التسمم الكلوي التجريبي الناتج من الأكينين جليكول والجنتاميسين على عدد 15 كلب تتراوح أعمارهم بين 1.5 - 2 سنة وأوزانهم بين 5 - 10 كجم حيث قسمت إلى ثلث مجموعات متماثلة.

المجموعة الأولى : جرعت بالفم الأكينين جليكول 10 سم / كجم مرة واحدة
المجموعة الثانية : حقن عضلياً بالجنتاميسين 2 مجم / كجم يومياً لمدة 10 أيام.
المجموعة الثالثة : فقد حُظيت كضابطة

وقد تم وضع هذه المجموعات الثلاثة تحت المشاهدة وسجلت الأعراض الظاهرة على الكلاب خلال فترة التجربة.

وبعد أنتهاء التجربة تم إجراء فحص كل الكلاب بالموجات فوق الصوتية وسجلت زيادة في经过在试验的实验室，使用超声波检测到临床症状，记录了各种药物的反应。此外，观察了药物混合物的毒性，并记录了皮肤上的反应。在实验的后期，使用超声波对所有药物进行了检查，记录了所有药物的反应。