Proceeding S.Z.P.G.M.I vol: 16(1) 2002, pp. 5-10.

Morphological and Histological Evaluation of Developing Kidney in Rats Exposed to Chloroquine

Aliya Zahid, Taquayya S. Abidi
Department of Anatomy, Allama Iqbal Medical College, Lahore

SUMMARY

A study was carried out to see the effects of chloroquine on kidneys of developing albino rats. In this study, 24 pregnant female albino rats were divided in 4 groups. They were kept in Animal House of Post Graduate Medical Institute, Lahore. Total gestational period in rats ranges from 20-22 days, which in this study was divided into three trimesters, each of seven days. Using oral dose of chloroquine 700mg/kg body weight in first, second and third trimesters of pregnancy, it was found that chloroquine induced nephrotoxicity in newborn rats which were exposed to drug during their intrauterine life. There was statistically significant decrease in kidney weight of offsprings in treated groups. Histological sections of kidney showed that there were degenerative changes in proximal and distal convoluted tubular cells. Some areas in cortex showed glomerular congestion. Demarcation of cortex and medulla was lost at some places. Subcapsular and parenchymal haemorrhages were also seen in few regions. All these changes were more marked in those animals which were exposed to drug during last trimester of gestation. It was concluded from the present study that chloroquine should be avoided during pregnancy, especially during last trimester.

Key words : Chloroquine, Developing kidney, Rats

INTRODUCTION

Chloroquine is the drug of choice for prophylaxis and treatment of malaria during pregnancy but it is surprising that few data have been published on its safety in pregnancy[1].

Chloroquine is well absorbed from gastro-intestinal tract. There is extensive sequestration of drug in tissues particularly liver and kidneys[2]. Chloroquine and its metabolites are eliminated predominantly by kidney[3,4]. It can cross the placenta to the foetus with foetal concentration same as in mother. Its nonpolar metabolites can be found in cord blood, neonatal blood and urine[5,6].

Musabayane et al.[7] studied that administration of chloroquine to rats induced extensive damage to proximal convoluted tubule and collecting duct cells, resulting in diminished renal function. In another study on rats, it was proved that chloroquine administration impaired kidney function resulting in Na^+ and Cl^- retention, increased aldosterone secretion and lowering of glomerular filteration rate[8,9].

Mulchinski et al.[10] studied that chloroquine produced adverse renal effects in vitro and caused inhibition of renal cortical slice oxygen consumption. In another study, it was proved that chloroquine can cause nephrotoxicity and impairment of renal function which is reflected in elevation of serum enzymes level and their corresponding decrease in kidney tissues[11]. Chloroquine caused decrease in urea and creatinine excretion in urine and impairment of renal electrolyte handling[12]. In another study on rats, chloroquine caused deposition of granular immune complexes in glomeruli and kidney showed features of focal glomerulonephritis[13].

MATERIALS AND METHODS

In this study, twenty four adult female rats and eight adult male rats of Albino Wistar strain were used. Weight of female rats used was between 250-

300 gms and male rats was between 300-350 gms. Animals were kept in the Animal House of Post Graduate Medical Institute, Lahore. For acclimatization, they were kept without treatment for 15 days. They were provided with normal feed and tap water ad libitum. Male and female rats were kept in separate cages. Care was taken regarding optimal light and temperature. For conception three female rats and one male rat were kept together in a cage for a week and then male rat was removed from the cage. Female rats were observed daily for signs of pregnancy which was confirmed by presence of vaginal plug. Presence of vaginal plug was taken as day one of pregnancy. After conception male rats were separated and 24 female rats were divided into four groups A, B, C and D, containing six animals each. Total gestational period in rats ranges from 20-22 days, which in this study was divided into three trimesters, each of seven days. The rats were weighed and marked. They were placed in their respective cages which were labelled by tags. Chloroquine phosphate in powdered form was used in this study.

Group A

This was a control group containing 6 animals each. They were fed on normal diet throughout pregnancy. They were allowed to complete their gestational periods without drug intake.

Group B, C and D

Each containing 6 animals, was given oral dose of chloroquine 700mg/kg body weight during first, second and third trimester of pregnancy, respectively.

After the control and experimental groups had delivered, their offsprings were selected at random (about 5/adult rat). Newborn rats on day one were anaesthetized by cotton pledget soaked with chloroform. After 3-5 minutes while the rats were still breathing, a ventral midline abdominal incision was made to expose abdomen. Both kidneys were then dissected out and placed on blotting paper to make them free of surrounding fluid. Each kidney was weighed on an electrical balance and weight was recorded in proforma. They were fixed in 10 % formaldehyde solution for at least 12 hrs. The kidneys were processed in an autoprocessor. Blocks were made, cut, stained and mounted. Sections were stained with haemotoxylin and eosin stains.

Gross Parameters

1. **Gross Appearance of offspring:**
   Gross appearance of offspring was noted to see any gross malformation .

2. **Gross appearance of kidneys:**
   The shape and colour of kidneys were observed. Detailed examination of external surface was performed.

3. **Weight of kidney**
   Weight of each kidney was recorded soon after dissection .

4. **Relative Tissue Weight Index (RTWI)**
   Relative Tissue Weight Index was obtained by this formula :

$$RTWI = \frac{\text{Mean weight of kidney}}{\text{Mean body weight}} \times 100$$

Histological Parameters

Sections were stained with haemotoxylin and eosin stains and following parameters were studied:

1. General architecture of Kidney(cortex and medulla)
2. Morphology of glomerulus
3. Morphology of Epithelial cells
4. Morphology of renal vessels
5. Presence of necrosis, haemorrhage etc.

RESULTS

Newborn rats were observed on the first postnatal day. Offsprings of control and experimental groups appeared normal. There was no gross malformation in any group.

Gross Appearance of Kidneys and Mean Kidney Weight:

In control group A, the external surface of kidney was smooth and shiny. The colour of kidney was whitish yellow. Mean kidney weight in control group was 0.071 gms ($\pm$0.014 SD).

In group B, the external surface of kidney was smooth and glistening but size was decreased as compared to control group. Mean kidney weight was found to be 0.05 gms ($\pm$0.05 SD) which was statistically significant ($P < 0.001$).

In group C and D, kidneys were small and external surface was dull. Mean kidney weights in group C and D were 0.045 gms and 0.032 gms respectively which were found to be statistically significant ($P < 0.001$) (Table 1 and Fig. 1).

Table 1: Effect of chloroquine on kidney weight of newborn rats (n=30)

Parameter	*Groups*			
	A Control)	*B*	*C*	*D*
Kidney weight (gms)	.071 ±.014	.050 ±.011	.045 ±.012	.032 ±.010
P Value	-	<.001	<.001	<.001

All values are expressed as: Mean+SD

P<0.001 (difference very significant) based on one way ANOWA 0.08

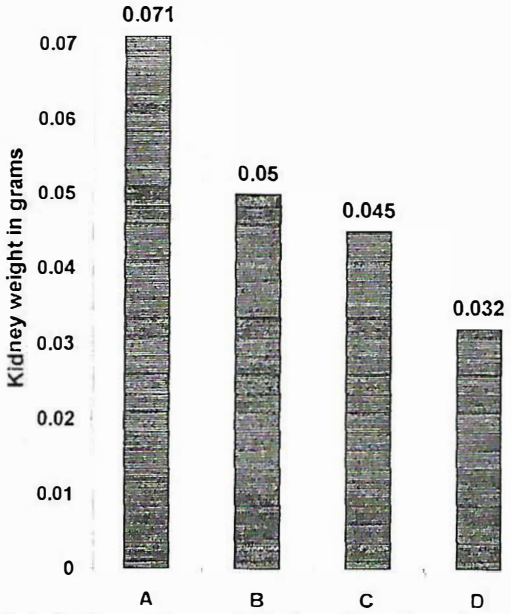


Fig. 1: Effect of chloroquine on kidney weight of newborn rats.

Relative Tissue Weight Index

Relative tissue weight index for kidney was calculated for each group which showed decrease as compared to control group. Relative tissue weight index of all groups is shown in (Fig. 2 and Table 2).

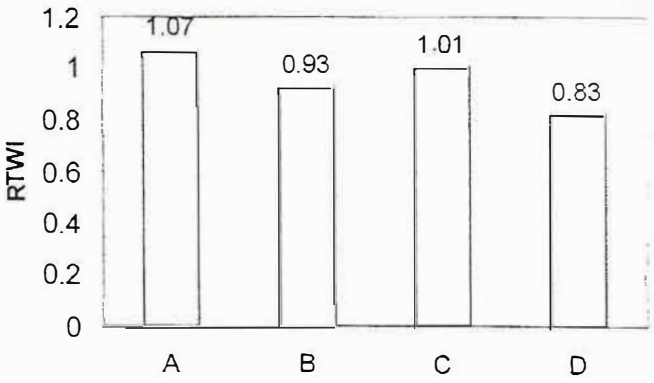


Fig. 2: Relative tissue weight index for kidney in different groups.

Table 2: Relative tissue weight index for kidney in control and experimental groups.

Groups	*RTWI*
A	1.07
B	0.93
C	1.01
D	0.83

Histological Observations:

1) Control Group (A):

The newborns recovered from control groups showed typical histological structure of the kidney. The parenchyma was divided into cortex and medulla. The renal corpuscles were numerous and completely formed in region of cortex. The lining epithelium of Bowman's capsule was simple squamous. The proximal and distal convoluted tubules were lined by continuous epithelium varying from low columnar to cuboidal in nature. Renal vessels were well formed.

2) Treated Groups (B, C & D):

Group B: This group was exposed to drug

during 1st week of their intrauterine life. They showed mild distortion of kidney architecture. The demarcation of renal parenchyma in cortex and medulla was prominent. Most of renal corpuscles were well formed with normal urinary space. At some places, congestion in glomeruli was seen. Proximal and distal convoluted tubule cells showed mild degenerative changes. Mild congestion of renal vessels was seen (Figs. 3 and 4).

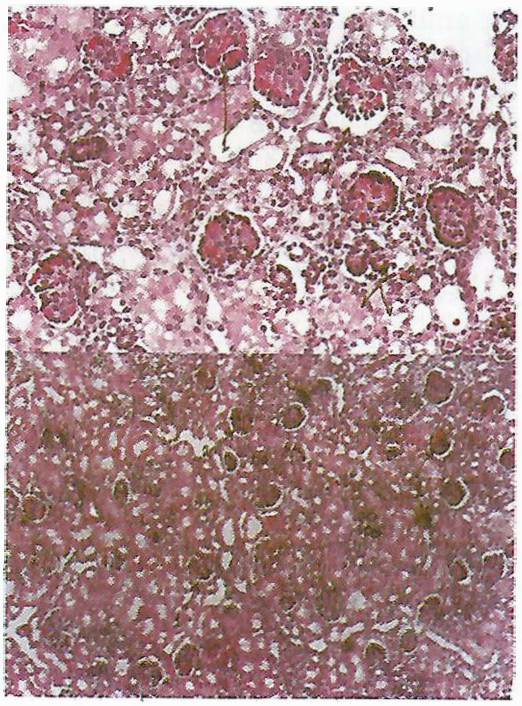
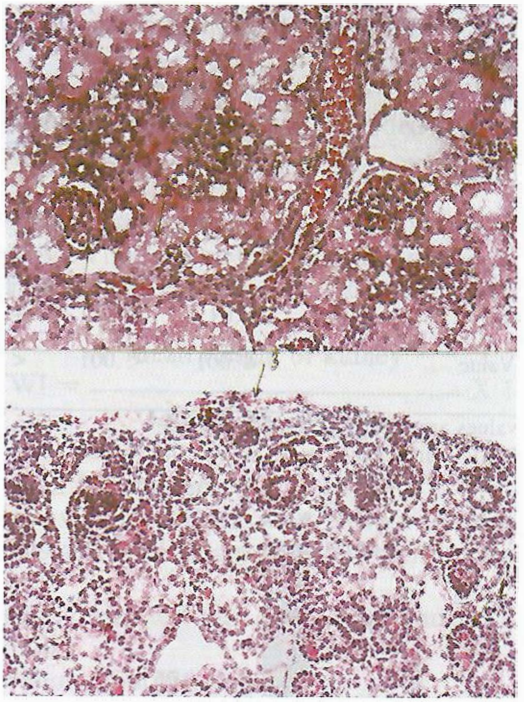


Fig.3&4: Histological section of kidney of newborn rat from Group B. 1, Glomeruli showing congestion; 2, urinary space; 3, Mild degenerative changes in tubule.

Group C: This group received the drug during 2nd week of their intrauterine life. The changes seen were same as in group B but intensity was mild to moderate (Figs. 5 and 6).

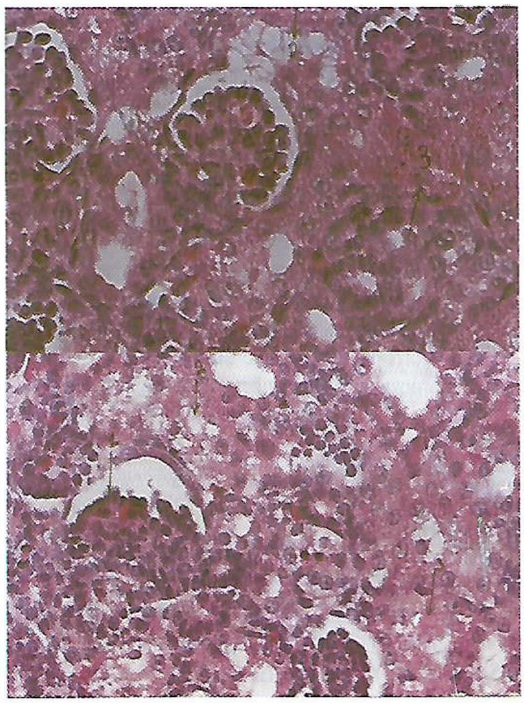
Group D: This group was exposed to drug during 3rd week of their intrauterine life. Demarcation of renal parenchyma in cortex and medulla was not prominent. Only few well formed glomeruli were found, mostly the cells were present in the form of clusters without any well defined urinary space. There was complete loss of renal tubular architecture at some places. Many necrotic foci were present. The proximal and distal tubules showed vacuolar degeneration at many places. Haemorrhage and congestion of renal architecture was more marked in the vicinity of glomeruli. Subcapsular haemorrhage was also seen at few places (Fig. 7 and 8).



Figs. 5 & 6: Histological sections of kidney of newborn rat from Group C. 1, Glomeruli showing congestion; 2, Vacoular degeneration in tubule cells; 3, Subcapsular haemorrhage; 4, Congested vessel.

DISCUSSION

There are very few published studies assessing the safety of medications during human pregnancies so data from animal teratogenecity studies are extremely valuable[14]. The results of present research revealed that chloroquine induces kidney damage in rats exposed to chloroquine during their intrauterine life. Kidneys from experimental animals showed significant decrease in weight as compared to their control. Decrease in weight was more marked in groups which were exposed to chloroquine during second and third week of gestation. The decrease in weight can be explained on embryological basis. In rats, nephrogenesis



Figs. 7 & 8: Histological sections of kidney of newborn rat from Group D. 1, Deformed Glomerulus; 2, Vacoular degenerative changes in tubule cells; 3, Necrotic foci in renal tubule.

begins on 14th day of gestation[15,16]. Chloroquine administration during this period interfered with the development and growth of kidney and caused destruction of already formed parenchymal tissue.

Chloroquine is a lysosomotropic agent that is selectively taken up into lysosomes causing inhibition of several lysosomal enzymes; secondary increase in the activity of some lysosomal enzymes; increased autophagy and fusion disturbances[17]. Autophagy by lysosomes may be responsible for reduction in kidney weight and degenerative changes in kidneys in present study. Mgbodile[18] also found that chloroquine administration during intrauterine and postnatal life resulted in marked decrease in neonatal and postweaning body and organ weights.

Chloroquine stimulates the synthesis of nitric oxide in endothelial cells that is a known mediator of tissue injury. Nitric oxide has cytotoxic properties and contributes to the process of tissue injury by directly damaging the tissue or by initiating additional immunologic reactions that result in tissue damage[19]. It is a known agent to inhibit endothelial cell proliferation. Proliferation of endothelial cells is a vital component of angiogenesis, that is inhibited by nitric oxide[20,21], thereby affecting the development of endothelial lining of vascular channels in rat kidney that may cause haemorrhages in parenchyma and glomeruli.

According to Jarzyna et al[22], nephrotoxicity of chloroquine is due to inhibition of glutamate metabolism in kidney. Chloroquine administration causes the poor development of the yolk sac vasculature resulting in low oxygen levels delivered to the embryo. Low oxygen tension in the tissues has been suggested to cause cell degeneration and necrosis[23].

CONCLUSION

The conclusion drawn from present research work is that chloroquine causes fetal kidney damage, particularly when given during later half of pregnancy. So its use during pregnancy should be discouraged.

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The Authors:

Aliya Zahid,
Assistant Professor
Department of Anatomy,
Allama Iqbal Medical College,
Lahore

Taquayya S. Abidi
Ex-Professor of Anatomy
Department of Anatomy,
Allama Iqbal Medical College,
Lahore

Address for Correspondence:

Aliya Zahid,
Assistant Professor
Department of Anatomy,
Allama Iqbal Medical College,
Lahore