

# Effect of Prenatal Doxycycline administration on Skeletal Differentiation in long Bones of Albino Rat

M. A. Siddiqui ( Department of Anatomy, Hamdard College of Medicine and Dentistry, Karachi. )  
M. Z. Janjua ( Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. )

## Abstract

**Objective:**To study the teratogenic risk of Doxycycline administration during gestation on skeletal differentiation. Design: An animal study carried out on experimental Albino rat.

**Setting:**The study was carried out in the Department of Anatomy Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre (JPMC), Karachi from June 1999 to May 2000.

**Methods:**Doxycycline was given to pregnant female Albino rats in a dose of 8mg/kg intraperitoneally from day 8th to 19th of gestation. The intact rat fetuses were isolated on days 15th to 19th of gestation and pups on 1st postnatal day. These animals were then killed by deep ether anesthesia and fixed in 80% alcohol. They were then bulk stained with alizarin red S and alcian blue. Finally they were cleared in 4% KOH and stored in glycerin. The fore and hind limbs were disarticulated from the axial skeleton and observed under stereomicroscope for evidence of skeletal differentiation in the form of presence of primary ossification centers in long bones. The time of appearance of these centers were noted and compared statistically with those in the control animals.

**Results:**The study revealed that the skeletal differentiation in long bones was delayed in experimental animals as compared with controls.

**Conclusion:**Doxycycline administration during pregnancy presents a risk to skeletal differentiation and therefore to its growth (JPMA 52:211,2002).

## Introduction

Doxycycline and Minocycline are the broad-spectrum antibiotics commonly used nowadays for different kind of infections. They belong to the group of Tetracyclines, which are contraindicated in pregnancy<sup>1</sup>, because they cross the placental barrier and get deposited in calcified tissues<sup>2,3</sup>. Their use in pregnancy has been associated with discoloration of teeth<sup>4</sup> and retardation in bone growth<sup>5</sup>. The evaluation of intended or unintended use of Doxycycline during pregnancy is a challenge for a doctor but unfortunately unqualified persons (quacks) are also using these drugs as commonly. Studies on the effects of tetracyclines on bone growth have been carried out but any effect on skeletal differentiation has not been reported so far. This study was done to evaluate the effect of Doxycycline administration during gestation on the differentiation of long bones in the extremities of prenatal laboratory albino rat.

## Material and Methods

One hundred and twenty rat fetuses and pups obtained from 32 pregnant female albino rats were used in this study. These 32 female albino rats were 10-12 weeks of age and

spontaneously ovulating when taken from the animal house of B.M.S.I., JPMC, Karachi for study. They were mated with fertile males of same strain, allowing one male rat with two female rats in one cage<sup>6</sup>. On next morning the female rats were examined for signs of mating in the form of blood stained vagina or a vaginal plug (a mucoid greenish white material). Presence of any one of these signs was considered as day I of pregnancy<sup>7</sup>. Pregnancy lasts 21 days in rat<sup>8</sup>.

Half of the pregnant female rats were given Doxycycline in a dose of 8mg /kg intraperitoneally from day 8th to 19th of gestation. The other half received normal saline. Intact rat fetuses were obtained on days 15, 16, 17, 18 and 19th of gestation by killing their mothers (the pregnant female rat in each group) and intact pups on 1st day of their birth from both experimental and control groups. Ten specimens were then randomly selected for study from each group for each of the days mentioned (total 60 from the group of female pregnant rats which received doxycycline and total 60 from the group which received normal saline). The sex of these offsprings was omitted.

All pre and postnatal animals were then killed by deep ether anesthesia and fixed in 80% alcohol after removing their skin and viscera. They were then bulk stained in alizarin red and alcian blue, cleared in 4% KOH (to reveal ossification centers) and stored in glycerin<sup>9</sup>. Left fore and hind limbs were separated from axial skeleton at their joints and viewed under a stereomicroscope. The presence or otherwise of primary ossification centers in humerus, ulna, radius, femur, tibia and fibula was observed and recorded. The said bones were observed throughout the experimental period (15th to 19th days of gestation and 1st postnatal day) even after the detection of primary ossification centers to see appearance of any additional ossification center in the shaft or ends of these long bones. The mean values of the time (day of gestation) of first appearance of these centers in experimental and control bones were subjected to student's T test.

## **Results**

The mean time of appearance of primary ossification centers in major skeletal components of both extremities in experimental and control animals are given in Table.

**Table. Time (Day) of first appearance of primary ossification centres in fore and hind limb long bones of Albino Rats.**

Bone	Control	Experimental	Delay
Humerus	15.00±0.00	16.40±0.16	1.4±0.16
Ulna	15.00±0.00	16.20±0.20	1.2±0.20
Radius	15.00±0.00	16.00±0.16	1.0±0.10
Femur	15.40±0.16	16.90±0.23	1.5±0.16
Tibia	16.00±0.00	16.70±0.21	0.7±0.21
Fibula	16.00±0.00	17.50±0.21	1.5±0.16

**Statistical Comparison**

Bone	Groups	P value	S / N.S.
Humerus	C vs E	< 0.001	H.S.
Ulna	C vs E	< 0.001	H.S.
Radius	C vs E	< 0.001	H.S.
Femur	C vs E	< 0.02	M.S.
Tibia	C vs E	< 0.01	M.S.
Fibula	C vs E	< 0.01	M.S.

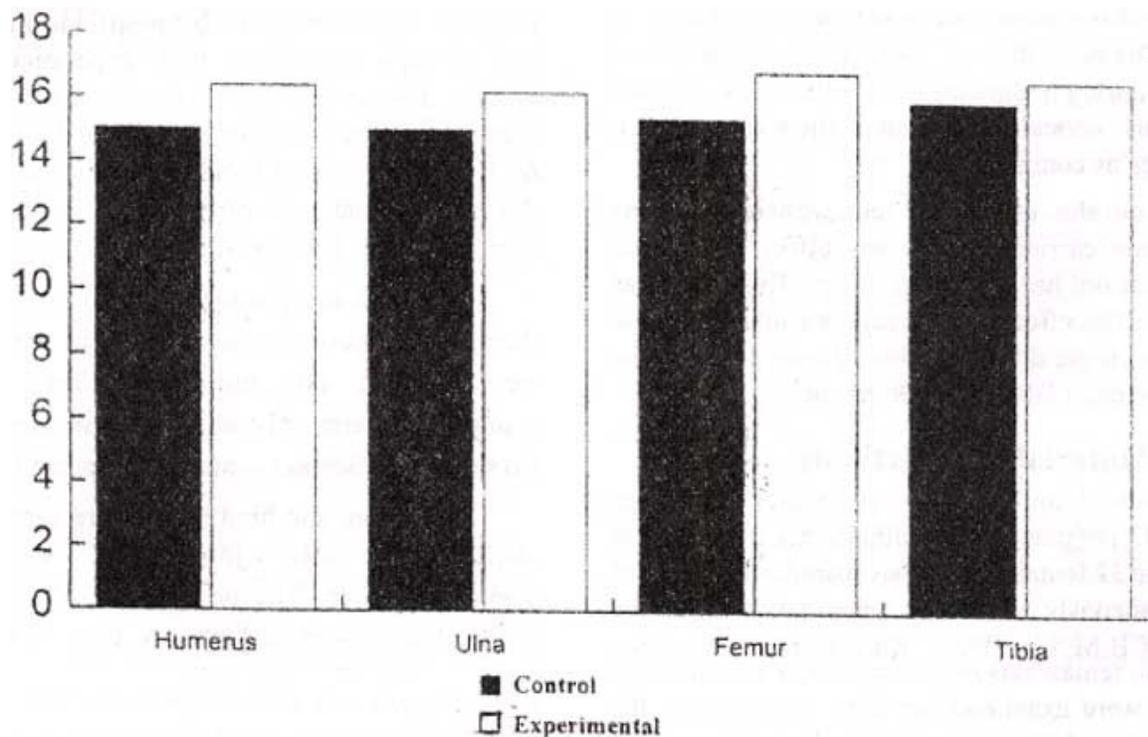


Figure. Bar chart showing time of first appearance of primary ossification centres in the control and experimental groups in the fore and hind limb long bones of albino rats.

### Fore limb

In the control animals, primary centers of ossification for humerus, ulna and radius were present on 15th day of gestation in all specimens observed (10 specimens of each bone). No additional ossification center was observed in any of the bones mentioned during the rest of the study period.

In experimental animals the primary ossification center in humerus was seen to be present on 16th day of gestation in 6 specimens and on 17th day in 4 specimens (average 16.4 day with a delay of 1.4 day). In ulna the center was seen on 15th day of gestation in 1 specimen, on 16th day in 6 specimens and on 17th day of gestation in 3 specimens (average 16.2 day with a delay of 1.2 day). In radius the same center was observed on 15th day of gestation in 2 specimens, on 16th day in 6 specimens and on 17th day in 2 specimens (average 16 days with a delay of 1 day). Any additional ossification center was not seen during the rest of the study period.

### Hind limb

In control animals the primary ossification center for femur was present on 15th day of gestation in 6 specimens and on 16th day in 4 specimens (mean =15.4 day).

In tibia the said center was present on 16th day of gestation in all 10 specimens while in fibula it was seen on 16th day of gestation in all specimens. Any additional prenatal ossification center was not observed in any of these bones during rest of the study. A secondary ossification center was however observed at the lower end of femur on 1st postnatal day in three specimens.

In experimental animals the primary ossification center for femur was seen on 16th day of gestation in 3 specimens, on 17th day in 5 specimens and on 18th day of gestation in 2 specimens (mean 16.9 day with a delay of 1.5 day). In tibia the said center was seen on 16th day of gestation in 4 specimens, on 17th day in 5 specimens and on 18th day in 1

specimen (mean 16.7 with a delay of 0.7 day). In fibula the primary ossification center was observed on 17th day of gestation in 6 specimens and on 18th day in 4 specimens (mean 17.5 days with a delay of 1.5 day). No other pre or postnatal ossification center was seen in any of these bones during the rest of study period.

## Discussion

Appearance of primary ossification center is the first indication of skeletal differentiation. The effect of Doxycycline treatment on skeletal differentiation was primordia of fore and hind limb long bones in experimental therefore studied by determining the time of first appearance of representative ossification centers in the and control animals.

Doxycycline delayed the appearance of primary ossification center in humerus by  $1.4 \pm 0.16$  days, in ulna by  $1.2 \pm 0.20$  days and in radius by  $1 \pm 0.00$  day as compared to those in control animals. This delay was highly significant ( $P < 0.001$ ). Similarly Doxycycline delayed the appearance of primary ossification centers in femur by  $1.5 \pm 0.16$  days, in tibia by  $0.7 \pm 0.21$  days and in fibula by  $1.5 \pm 0.2$  days when compared with those in control long bones. The delay averaged 1.3 days in fore limb and 1.1 days in hind limb bones. This indicates that the forelimb bones were subjected more to the adverse effects of Doxycycline. This may be attributed to the fact that the fore limb bones differentiate earlier than the hind limb bones during gestation<sup>10</sup>.

It has long been known that tetracyclines get fixed in calcified tissues and that they may have an inhibitory action on calcification process<sup>11</sup>. The mechanism by which tetracyclines bind to the calcified structure is stated by some workers to be simple chelation between them and calcium<sup>12</sup>. Others believe that fixation of tetracyclines is mediated by some more complex mechanisms<sup>13</sup>. Presently it is known that tetracyclines chelate calcium and form tetracycline-calcium-orthophosphate complex, which accumulates in bone by adsorption onto bone crystal surface and eventually incorporates into its crystal lattice<sup>2</sup>.

In this study the delay in appearance of ossification centers by Doxycycline may be attributed to its deposition in bones along with calcium resulting into their delayed demonstration by alizarin red. The alizarin red staining is the most valid technique of detecting early ossification (second only to histological technique).<sup>14</sup>

Our observation is in accordance with the findings of Balsamo et al<sup>15</sup>, who reported faulty and delayed calcification process of otoliths in developing chick embryo after tetracycline administration.

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