ORIGINAL ARTICLE

Histomorphometric Study on the Effects of Methotrexate on Gonads: An Experimental Study in Albino Rat.

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ABSTRACT

Background: Prolonged use of methotrexate in juvenile arthritis and multisystem involvement due to its toxicity prompted us to find out detailed histo-morphometric study after exposure to drug. The concern became alarming because of reported gonadal damage due its direct effect on fertility. **Methods:** Methotrexate was injected in both male and female rats (1mg/kg, intraperitoneally), weekly for 6 weeks. Thin sections of testis and ovary of both control and experimental groups were stained with H/E stains for light microscopic examination. **Results:** Histo-morphometric observations on seminiferous tubules included, external diameter, thickness of the wall and the diameter of the lumen. Such observations for ovarian tissue included diameters of ovarian follicles and their ova. Highly significant changes in both testicular and ovarian microscopic parameters confirmed the damage of tissue in experimental groups. **Conclusion:** The study suggests that utmost precaution should be taken while using methotrexate in younger patients.

Keywords: *Histomorphometry, Methotrexate, ovary, rat, testis.*

INTRODUCTION

Methotrexate has been recently used as a drug of choice for the majority of the patients with rheumatoid arthritis including cases of juvenile rheumatoid arthritis.[1] Unfortunately the drug is used for very long periods to get satisfactory therapeutic results posing the damages of severe toxicity.[1]

Multiorgan involvement due to methotrexate toxicity is well documented e.g., gut[2], central nervous system[3] and gonads.[4]

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Since the latter directly affects the fertility, the gonadal toxicity has become the topic of research of many scientists. Several cases of sterility in men using methotrexate are well documented due to oligospermia.[5-9] Reports available on the effects of methotrexate on spermatogenesis are scanty.[10,11]Battan et al[12] for the first time reported a detailed account of histopathological changes in gonads of both male and female rats after exposing them to methotrexate for six weeks.

Quantitative parameters have always an upper hand over qualitative observations as the former approach provides readings, which can be analyzed statistically to confirm the reliability in changes due to toxicity. The aim of present study is to measure sizes of different structures in testis as well as in ovary in both control and experimental rats and analyse them statistically to detect the changes if any, in the latter

MATERIALS ANDMETHODS

Twelve male and twelve female albino rats

[RattusNorwegicus] weighing 190-200 gms, were procured from animal house, J.N. Medical College, AMU, Aligarh. Ethical committee of the institution granted permission for the experimental work on rats. The rats of both sexes, were divided into equal number of control and experimental groups. Animals received standard pellet laboratory diet (Lipton India Limited) and water ad-libitum. Experimental rats received methotrexate (Biotrexate, Biochem PI) 1mg/kg, intraperitoneal injection, weekly for 6 weeks. Normal saline injection was given to control rats in a similar manner. At the end of 6^{th} week, all the animals were anaesthetized by giving injection ofNembutol, 30 mg per kg, intraperitoneally. Karnovsky's fixative was infused in each rat till the body showed signs of fixation. Testes and ovaries were dissected out by sagittal incisions bilaterally in the anterior walls of scrotum and abdominal wall respectively. Tissues of 2-3 mm size from both the gonads were processed for wax embedding technique to obtain sections of 10 µm thick. All the sections were stained with hematoxylin and eosin. By using stage and ocular micrometers manually, three measurements of seminiferous tubules (1, external diameter; 2, thickness of wall; 3, diameter of lumen) and seven measurements of ovarian tissue (diameters of ,1.primordial follicle; 2, primary follicle; 3, secondary follicle; 4, Graafian follicle; 5, ovum in primordial follicle; 6,ovum in primary follicle; 7, ovum in secondary follicle). Readings were tabulated and analysed by using Student's 't' test.

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RESULTS

All the parameters of seminiferous tubules, i.e., external diameter, thickness of the wall, diameter of lumen were significantly increased statistically [Table1]. There was no change in the size of the Graafian follicle in experimental group when compared with control one. Interestingly, there was a reduction in size of primary and secondary follicles of experimental rats and these differences were highly significant statistically [Table 2]. There was no statistically significant change in the size of the ovum of primordial follicle [Table 3]. But the sizes of ova in primary and secondary follicles showed statistically significant increase in experimental rats [Table 3].

Table 1: Measurements of seminiferous tubules (µm).

S.No.	Parameter	Group	N	Mean	SD(±)	SE	t	df	Sig. (2-tailed)
1.	External diameter	Control	40	209.65	20.173	3.190	-14.068	78	0.000
		Experimental	40	308.00	39.343	6.221			
2.	Thickness of wall	Control	40	38.85	11.203	1.771	-3.977	78	0.000
		Experimental	40	53.55	20.519	3.244			
3.	Diameter of lumen	Control	40	135.45	28.602	4.522	-9.906	78	0.000
		Experimental	40	211.05	38.879	6.147			

Table 2: Measurements of ovarian follicles (µm).

S.No.	Follicular type	Group	N	Mean	SD(±)	SE	t	df	Sig.(2-tailed)
1.	Primordial follicle	Control	20	28.00	000	000	Not applicable		
		Experimental	20	28.00	000	000			
2.	Primary follicle	Control	20	196.00	11.126	2.488	30.512	38	0.000
		Experimental	20	107.80	6.582	1.471			
3.	Secondary follicle	Control	20	312.90	51.843	11.593	3.137	38	0.003
		Experimental	20	273.00	23.382	5.228			
4.	Graafian follicle	Control	20	855.40	154.026	34.441	1.136	38	0.263
		Experimental	20	810.60	85.930	19.215			

Table 3: Diameter of ovum in different follicles (µm).

S.No.	Follicular type	Group	N	Mean	SD(±)	SE	t	df	Sig.(2-tailed)
1.	Primordial follicle	Control	20	28.00	0.000	0.000	-1.453	38	0.154
		Experimental	20	29.40	4.309	0.964			
2.	Primary follicle	Control	20	40.60	2.873	0.642	-12.089	38	0.000
		Experimental	20	54.60	4.309	0.964			
3.	Secondary follicle	Control	20	56.35	5.314	1.188	-12.525	38	0.000
		Experimental	20	74.20	3.518	0.787			

DISCUSSION

There was an overall increase in the parameters of seminiferous tubules. Increment in diameter and thickness of wall is explained by excessive edema as reported by Battan et al.[12] Increase in luminal diameter might be due to excessively reduced or nil sperm heads in its wall. A study by Bacci et al[13] investigated the long-term effects of combination chemotherapy for osteosarcoma. All the chemotherapy regimen contained methotrexate and other chemotherapeutic agents. Azoospermia was confirmed in 10 of the 12 men who underwent spermatography. Our report contradicts the previous concept of reduction in diameter of seminiferous tubules in rats after intravenous methotrexate intoxication.[10]

Our findings in ovary in experimental rats are very interesting. There was no effect of drug on the sizes of primordial follicle and its ovum as well as Graafian follicle. On the other hand, there was reduction in sizes of primary and secondary follicles. According to one report oocytes were relatively resistant to methotrexate prior to LH surge, but sensitive to the compound after this event.[14] This explains our observations on primordial follicles. But the effect was quite visible in primary and secondary follicles including their ova. Increase in size of the ova is explained by an early degenerative effect leading to swelling.[15] Reduction in size of follicles might be due to the slowed proliferation of granulosa cells due to toxicity. The authors are unable to explain the normal size of Graafian follicle. Excessive proliferation of granulosa cells in late developing follicles might be predicted, but needed further experiments at a higher level of organization.

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CONCLUSION

Methotrexate does effect histophysiology of gonads therefore, its use should be judicious and in younger patients the risk of infertility should be kept in mind.

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