Original Article

'Vitamin A' a Potential Teratogen

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ABSTRACT

Objective: To determine the teratogenicity of Vitamin A excess on intrauterine development of thymus in albino rats.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Anatomy/ Histopathology, Shaikh Zayed Postgraduate Medical Institute, Lahore from September 2008 to September 2009.

Materials and Methods: Study was conducted with 18 pregnant female albino rats, of Sprague-dawley variety. These female rats were randomly separated into equal groups, A, B and C (n=6). Vitamin A was used in the form of Isotretinoin (13-cis retinoic acid). The dose of isotretinoin used in this study was constant i.e, 2.5mg/kg body weight of rats for every experimental group. Taking the trimester of pregnancy as variable of the study dose was given on 9 (mid trimester) and 17 (late trimester) days of pregnancy to the mother rats. The sample size was obtained by collecting 18 foetuses from each group. Thymuses were collected from the rat foetuses of each group after dissection.

Results: The foetuses of experimental group whose mother rats received the dose of Vitamin A in mid trimester showed thymic ectopia in a significant number of foetuses ($P<0.01$), while dose of Vitamin A given in late trimester caused thymic hypoplasia ($P<0.01$) in fetuses of albino rats. **Conclusion**: It is obvious with these findings that single dose of Vitamin A can cause deleterious effects on developing thymus in albino rats.

Recommendations: Caution must be taken while administration of Vitamin A to a pregnant woman.

Key Words: Vitamin A, Albino rat, thymus, development, teratogenesis.

INTRODUCTION

Vitamin A is an essential human nutrient. It is essential for the maintenance of visual and reproductive function and for proliferation and differentiation of epithelial tissues.[1]

Vitamin A is a generic term used for a large number of related compounds. Retinol (an alcohol) and retinal (an aldehyde) are often referred to as preformed Vitamin A, found in animal food sources, such as liver, kidney and milk.[2]Vitamin A is also found in different vegetables (like carrots, spinach, peas) in the form of carotenoids (especially B-carotene). [3] These carotenoids can be converted by the body into retinal.[8] Retinal can be converted by the body to Retinoic acid, the form of the Vitamin A known to affect gene transcription. Retinol, retinal, retinoic acid and related compounds are collectively known as retenoids.

The effects of retinoids, biologically active derivatives of Vitamin A, are transduced by nuclear receptors, the Retinoic Acid Receptors - RARs (α-β-γ) and Retinoic X Receptors - RXRs (α-β-γ).[5,6]

The recommended daily amount of Vitamin A for male is 3000 IU/day and for female is 2300 IU/day.[7] However Teratology Society (1987) recommended daily intake of 8000 IU/day for pregnant women as maximum intake during pregnancy.[8]Excessive intake of Vitamin A produces a toxic syndrome called hypervitaminosis A.

Vitamin A is widely used in clinical practice to treat Xerophthalmia, dermatological problems, such as acne and psoriasis and chronic infections.[9]

The excess or deficiency of Vitamin A causes abnormal morphological development (teratogenesis).[10] Too much or too little retinoids at the wrong stage or at the wrong time can adversely affects the developing embryo.[11]In the case of excess the teratogenic effects of Vitamin A appears to occur at an undetermined level above 8000 IU/day. Vitamin A is especially harmful during early pregnancy when organogenesis occurs. Embryonic exposure to RA causes a wide spectrum of severe malformation in the offspring of human and rodents.

Due to the known teratogenic effect of retenoids, therapeutic doses are contra indicated during pregnancy.[12] Much of the knowledge about the toxicity of Vitamin-A in pregnancy arose from research with the drug isotretinoin.[8] A constellation of birth defects termed retinoic acid Embryopathy (RAE) resulted from oral administration of 13-Cis-retinoic-acid (isotretinoin).[13]

Several mouse studies have demonstrated potential adverse effects of RA when administered during mid and late pregnancy in mice.[11]

MATERIALS AND METHODS

This experimental study was conducted in Department of Anatomy/ Histopathology, Shaikh Zayed Postgraduate Medical Institute, Lahore.

In this experimental study 18 female rats, weighing about 200-250 g and 6 adult male rats, weighing 250-300 g of Sprague-dawley variety of albino rats were used. They were obtained from National Institute of Health, Islamabad. All animals were kept separately in animal house of the Punjab Postgraduate Medical Institute, Lahore. The food and water was provided ad libitum.

Study Design: After conception 18 female rats were randomly separated in to equal groups, A, B and C (n=6). Total gestational period in rats is of 21 days and each trimester was of 7 days. Vitamin A was used in the form of Isotretinoin (13-cis retinoic acid), given in oral form to the rats by nasogastric tube (N/G Tube). The dose of isotretinoin used in this study was constant i.e, 2.5mg/kg body weight of rats for every experimental group. Taking the trimester of pregnancy as variable of the study dose was given on 9 (mid trimester) and 17 (late trimester) days of pregnancy to the mother rats, while Group A was the control group in this group pregnant rats were given 1ml of olive oil as vehicle.

Sample collection: Cesarean sections of rats were carried out on gestational day 21. Their foetuses were removed, weighed and, killed by euthanasia. Three foetuses from each animal were then selected randomly and labeled as sub groups A1,B1 and C1 respectively. These selected foetuses were then immersed in 10% formalin for ten days and examined under a binocular dissecting microscope for external congenital anomalies. Afterward, their thymuses were dissected under binocular dissecting microscope and weighed then external morphological study of thymus was carried out. These thymuses were then fixed in zenker's solution. After fixation thymus was embedded in paraffin. Serial 5µm sections were cut and stained with hematoxylin /eosin and reticulin stains for detailed histological study of thymus.

Statistical Analysis: Qualitative data was analyzed statistically by Chi-square (X2) method, while quantitative data was analyzed by analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) Version 16.

All the quantitative variables were described by Mean, ± SD and all qualitative variables were described by frequency and percentages. P-value of < 0.05 was considered significant.

RESULTS

The gross congenital anomalies of thymus (small and ectopic thymus) were noticed. In group B1 (mother rats received dose in 2nd trimester), 4 out of 18 thymuses were ectopic (22.2%). Further analysis showed that mean weight of thymus and RTWI was not affected in this group.

Table No.1: Effects of Vitamin A on Gross Appearance of rat thymuses of control and experimental groups

Group	Normal	Abnormal	Total	Gross mal-formations observed	
				Ectopia	Thin thymus
A1	18	0	18	0	0
B1	14	4	18	4	0
C1	10	8	18	0	8
Total	42	12	54	4	8

p < 0.001**

A1: Control Group B1: Mid trimester Group
C1: Late trimester Group
** Highly significant difference(P<0.01)

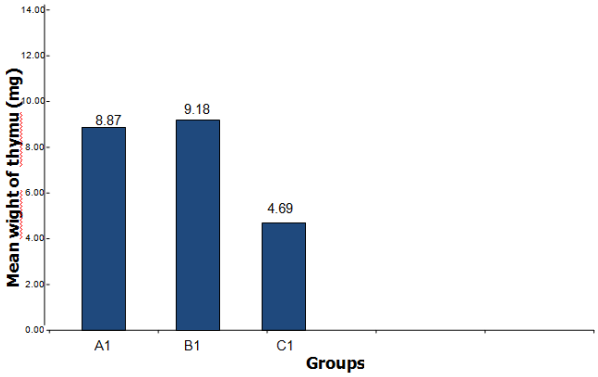

Figure No.1: Mean weight of thymuses of control and experimental groups exposed to Vitamin A during gestation

A reduction in mean thymocyte population was not observed in this experimental group.In group C1 (mother rats received dose in 3rd trimester), 8 out of 18 thymuses were found small and thin (44.4%). The mean weight of foetal thymus in experimental group C1 was significantly reduced when compared with control group A1 (P<0.01).

Table No.2: Weight of thymus (mg) of foetuses of control and experimental groups exposed to Vitamin A during gestation

Groups	Mean Weight	S.D	Minimum Weight	Maximum Weight
A1	8.8667	±1.67650	5.45	12.60
B1	9.1778	±2.89821	4.80	15.20
C1	4.6911	±1.61691	3.18	8.50

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A1: Control Group B1: Mid trimester Group
C1: Late trimester Group
SD: Standard Deviation

Table No.3: Comparison of effects of Vitamin A on Weight of thymus (mg) of control and experimental groups Contrast Tests

Contrast	Value of Contrast	Std. Error	t	df	P – Value
A1 Vs B1	-.3111	0.78917	-.394	27.231	0.696++
A1 Vs C1	4.1756	0.54899	7.606	33.956	0.000**
B1 Vs C1	4.4867	0.78223	5.736	26.648	0.000**

A1: Control Group B1: Mid trimester Group
C1: Late trimester Group
** Highly significant difference(P<0.01)
* significant difference (P<0.05)
++ Non significant difference(P<0.05)
Based on one way ANOVA

Table No.4: Relative tissue weight index of rat foetuses of control and experimental groups treated with Vitamin A during gestation

	Mean RTWI	S.D	Minimum RTWI	Maximum RTWI
A1	0.1670	±0.34880	0.119	0.268
B1	0.1775	±0.57267	0.107	0.320
C1	0.1103	±0.50801	0.074	0.236

A1: Control Group B1: Mid trimester Group
C1: Late trimester Group
SD: Standard Deviation
RTWI: Relative Tissue Weight Index

Table No.5: Comparison of effects of Vitamin A on Relative Tissue Weight Index of control and experimental groups Contrast Tests

Contrast	Value of Contrast	Stdandard Error	t	Df	P – value
A1 Vs B1	-0.0738	0.15805	-0.467	28.088	0.644++
A1 Vs C1	0.5983	0.14525	4.119	30.114	0.000**
B1 Vs C1	0.6722	0.18044	3.725	33.523	0.001**

A1: Control Group B1: Mid trimester Group
C1: Late trimester Group
** Highly significant difference(P<0.01)
* significant difference (P<0.05)
++ Non significant difference(P<0.05)
Based on one way ANOVA

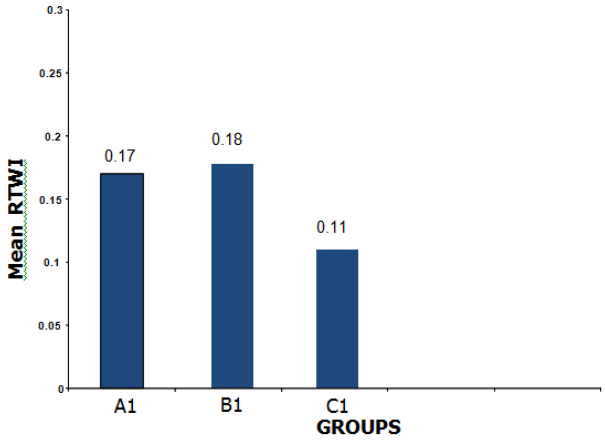

Figure No.2: Relative tissue weight index for thymuses of rat foetuses of control and experimental groups exposed to Vitamin A during gestation

Table No.6: Thymocyte Population in foetal thymuses of control and experimental groups treated with Vitamin A in different trimesters of pregnancy

Groups	Mean/ mm^2	S.D	Minimum/ mm^2	Maximum/ mm^2
A1	32.2778	±6.71088	23.00	44.00
B1	29.0000	±8.11679	20.00	45.00
C1	29.1111	±4.28251	24.00	37.00

A1: Control Group B1: Mid trimester Group
C1: Late trimester Group
SD: Standard Deviation

Table No.7: Comparison of effects of Vitamin A on Thymocyte Population of control and experimental groups Contrast Tests

Cont-rast	Value of Contrast	Standard Error	t	df	P -Value
A1 Vs B1	3.2778	2.48236	1.320	32.840	0.196++
A1 Vs C1	3.1667	1.87640	1.688	28.876	0.102++
B1 Vs C1	-.1111	2.16310	-.051	25.784	0.959++

A1: Control Group B1: Mid trimester Group
C1: Late trimester Group
** Highly significant difference(P<0.01)
* significant difference (P<0.05)
++ Non significant difference(P<0.05)
Based on one way ANOVA

Relative tissue weight index is an important parameter which should also be taken under consideration whenever the weight of tissue is discussed. It was found out that decrease weight of thymus in group C1 was also confirmed by decreased relative tissue weight index of

experimental group C1 as compared to control group A1. A reduction in mean thymocyte population was observed in experimental group C1, but this reduction had no statistical significance.

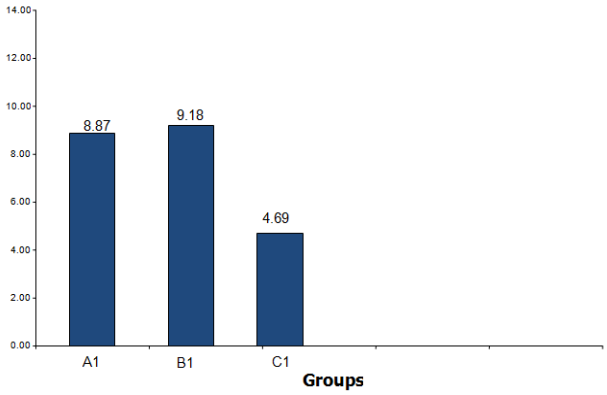

Figure No.3: Mean thymocyte population of experimental and control groups exposed to Vitamin A during gestation

DISCUSSION

Although RA is required for normal embryonic growth and development, it is also a powerful teratogen in excessive dose. Infants born to mothers exposed to retinoids during pregnancy have a 25-fold increased risk for malformations, nearly exclusively of cranial neural crest-derived tissues.[14,15] The neural crest, a transient, multipotential population of cells, originates from the dorsal neural folds, and cells migrate to a variety of sites within the developing embryo. Crest cells move in coherent streams and follow highly defined migratory patterns, a hallmark behaviour of these cells.

The present study was intended to assess the effects of Vitamin A on prenatal development of thymus in albino rats exposed to the drug during various trimesters of pregnancy.

In the current study Vitamin A was used in the form of isotretinoin. It was eminent that even in therapeutic dose, 2.5 mg /kg body weight, given in different trimesters of pregnancy Vitamin A exhibited teratogenic potentials.[16,17]

In this study exposure to rat foetal thymuses to Vitamin A on gestational day 9 (2nd trimester) resulted in ectopic thymus. Retinoids decrease neural crest cell adhesion to the substrate and their ability to migrate; this inhibition is dose dependent. Following in vivo exposure, RA also disrupts migratory pathways such that crest cells end up at the wrong target.[15]

In group C1, dose was given in third trimester on gd:17. In rats during development by the gd:15 thymus migrates into thorax and it increases in weight afterwards. So interference at this stage by Vitamin A on thymic development may lead to hypoplastic thymus, which was evident in group C1. These hypoplastic thymuses had shown significant reduction in weight, insignificant decrease in size of thymic lobules and insignificant decreased thymocyte population. These results coincide with the study of Makori *et al*. who found same hypoplastic thymuses in monkeys exposed to 2.5 mg/kg of 13-cis-RA (isotretinoin). Histological analysis of hypoplastic thymus tissues from exposed fetuses of these monkeys indicated a slight decrease in size of thymic lobes, but no identifiable changes in cellularity.[17]

Researchers have proved that in mice RA causes anatomical and functional thymic anomalies (thymocyte dysmaturation) that are probably related to abnormal expression of HOXA3 and Pax-1 genes.[18]

This study suggests that the administration of RA to pregnant rats results in the rapid transfer across the placenta to the developing embryo and teratogenic effects depends on the dosage and time of gestation.

CONCLUSION

The result of this research work clearly indicates that Vitamin A is capable of having direct influence on developing thymus (thymic ectopia and hypoplasia) even in single dose administration in prenatal period in mid and late trimesters of pregnancy. So caution must be taken while administrating Vitamin A to a pregnant woman.

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