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MINOCYCLINE INDUCED MORPHOMETRIC AND HISTOLOGICAL CHANGES IN THYROID GLAND OF GUINEA PIG

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ABSTRACT:

Background: This study has been undertaken to see the Minocycline induced histological and morphometric changes in thyroid gland of Guinea pigs.
Purpose of study: The study is design to observe the effects of Minocycline on thyroid gland of guinea pig.
Study Design: An experimental study.
Place: Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi.
Duration: October to November, 2010 for a period of 8 weeks.
Material and Methods: 20 adult male Guinea pigs weredivided into two groups A and B on the basis of treatment given. The animals of group A served as the control group and group B was administered 0.02mg/g/day minocyclinedaily with the help of nasogastric tube. The Guinea pigs were sacrificed at the end of the experimental period, their thyroid glands dissected, fixed in alcoholic formalin and processed further for histological examination.
Results: The microscopic examination of thyroid follicles revealed marked changes in area of follicles, epithelial cells, and in nuclei in Minocycline treated group B when compared with the control group A.
Conclusion: In present study long term use of Minocycline harmful to certain organs of body and should be avoided.

Key words: Minocycline, thyroid gland, follicles, Guinea pigs.

INTRODUCTION:

The thyroid gland is a bioactive endocrine gland. It regulates the metabolism and homeostasis (Tortora GJ and Derickson B, 2009)[1]. In this environment the gland is mostly affected by many drugs which are used to treat different diseases.Minocycline belongs to long acting tetracycline its serum half-life is 16 -18 hours (Katzung, 2009)[2].Minocycline inhibit bacterial protein synthesis by binding to the 30 S bacterial ribosome and preventing access of aminoacyl t RNA to the acceptor site on the m RNA ribosome complex. The percentage of an oral dose that is absorbed with an empty stomach is high for Minocycline i.e.100% (Brunten et al., 2009)[3]. Minocycline with proven safety, which has been commonly used to treat pneumonia and acne as well as infections of the skin genital and urinary systems, Central nervous system, gonorrhea, meningitis, shigellosis, conjunctivitis, psittacosis, Q fever, relapsing fever, and syphilis (Elewa et al., 2006)[4]. It is highly lipophilic compound and penetrates the blood brain barrier easily (Yang et al., 2010)[5]. The most relevant side effect of Minocycline is macroscopic black discoloration of the thyroid gland designated 'black thyroid syndrome'. This black discoloration is almost pathognomic for use of Minocycline (Bruins et al., 2007)[6].In the thyroid gland minocyline accumulates in the follicular epithelium and

colloid, in benign and even hyperplastic tissue as a result of the oxidative interaction between the drug and the enzyme thyroid peroxidase.(Pantanowitz and Tahan 2003)[7]ultrastructurally, pigment is confined to Lysosomes. This granular pigment stains like melanin on Fontana-Masson staining, it can also be bleached. In aspiration cytology specimens, the degenerative changes in follicular epithelial cells found in black thyroid gland can cause nuclear hyperchromatism and chromatin clumping. It was also reported that chronic administration of large doses of minocycline to rats, dogs, and monkeys induced a black discolouration of the thyroid gland (Taurog et al., 1996)[8].

MATERIAL AND METHODS:

This study was conducted in the Department of Anatomy, Basic Medical Sciences Institute, JPMC, Karachi and microscopy was done in Department of Anatomy, Karachi Medical and Dental College. In the present study, 20 adult male guinea pigsweighing between 450-650grms were taken from the Animal house of BMSI and were kept under observation for 1 week before commencement of study.The animals were maintained on standard laboratory diet and water ad libitum and were divided into two groups. Group A was served as control and group B received 0.02mg/g/ day minocycline (Steifel Laboratories Pakistan (Pvt) Ltd, Steifel Laboratories Inc.Coral Gables, FL33134, USA orally.The Minocycline was given daily to group B with the help of nasogastric tube. Amount calculated for each animal is 0.02mg/g/day for 8 weeks based on the human dose of 200mg/day (Silveira et al., 2009)[9].

Throughout the study period the animals were observed for their general health, activity and behaviour. Weight of the animals was done at the commencement of study, and at the end of experimental procedure. After completion of study period (at the end of 8 weeks) the animals were sacrificed in a glass container under deep ether anaesthesia. The incision was made in neck region from chin to xiphisternum, infrahyoid muscles were retracted to expose the thyroid, and thoracic viscera will be exposed by careful removal of skin, fascia and muscles. Thyroid gland was removed from the site and washed with normal saline. Its gross appearance was examined with the help of magnifying glass for colour, consistency, shape, contour and any dilated vessels.Thyroid glands were fixed in 10% buffered formalin for 24 hours. After that tissues will be processed (Bancroft et al., 1994)[10]by keeping them in 70% alcohol overnight,then tissue was dehydrated by passing through ascending grades of alcohol, starting from 80%, 90% and two changes of absolute alcohol (100%) for one hour each and cleared in two changes of xylene for one hour each. Infiltration was done with paraffin at 58 degree centigrade in laboratory oven and paraffin blocks was made with paraffin embedding system 4 micron thick sections was cut on rotatory microtome. After that tissue sections was placed in water bath at 42 degree centigrade and then placed on albumenized glass slide. Tissue was fixed on hot plate at 30-32 degree centigrade. Tissue was mounted and stained with Hematoxylin and Eosin, Masson Fontana for a detailed morphological examination of thyroid follicles under light microscope.

The lumen of thyroid follicles was examined for pigmentation under different magnifications. Various quantitative changes between the experimental group and control group were evaluated by applying student t- test. The difference was regarded statistically significant if the p-value was less than or equal to 0.05. All calculations were done by utilizing computer software SPSS version 16.

RESULTS

Group A

On gross examination, the thyroid gland of control group A appeared reddish brown in colour and soft in consistency with a smooth shiny surface. The microscopic examination of Hematoxylin and Masson Fontana stained sections of thyroid gland of control group revealed a normal pattern of thyroid follicles. The follicles were showing normal architecture, the larger ones measuring from 80 to 100 µm in diameter.The lining epithelial cells of the follicles were mostly low cuboidal to squamous due to the accumulation of colloid in the lumen of follicles. The nuclei of the follicular cells were flattened in shape located centrally.The mean values of area of follicles, height of follicular cells and height of nuclei were 23999.2 ± 140.43, 1.06± 0.00 and 1.18 ± 0.01 µm.

Group B

On gross examination, the thyroid gland of Minocycline treated group B appeared brownish in colour and soft in consistency. The microscopic examination of Hematoxylin and Eosin and Masson Fontana stained sections of thyroid gland of this group showed that most of the thyroid follicles were empty from colloid and the follicles which contained colloid were shrinked and deposition of the pigment. This pigment was mostly deposited in the apical region of follicular epithelial cells and also inside the colloid of follicles in scattered form. The height of follicular

MEAN VALUES OF AREA OF FOLLICLES, HEIGHT OF CELLS AND HEIGHT OF NUCLEI IN ALL GROUPS

Groups	Treatment received	Area of follicle (µm)	Height of cell (µm)	Height of nucleus (µm)
Group A	Normal Diet (ND)	23999.2* ± 140.43	1.06* ± 0.00	1.18* ± 0.01
Group B	Minocycline	14137.5** ± 392.18	6.95** ± 0.04	3.23** ± 0.11

Each value is Mean ± S.D for 10 Guinea pigs in each group.

Key:

*P< 0.05 significant

**P < 0.001 highly significant as compared to control.

FIGURE: 1
Photomicrograph of 4 microns thick Hematoxylin and Eosin stained section of thyroid gland in group B (Minocycline treated) Guinea pig showing darkly stained and shrinked colloid material (Arrow 1) and nucleus of follicular cell (Arrow 2). X 400.

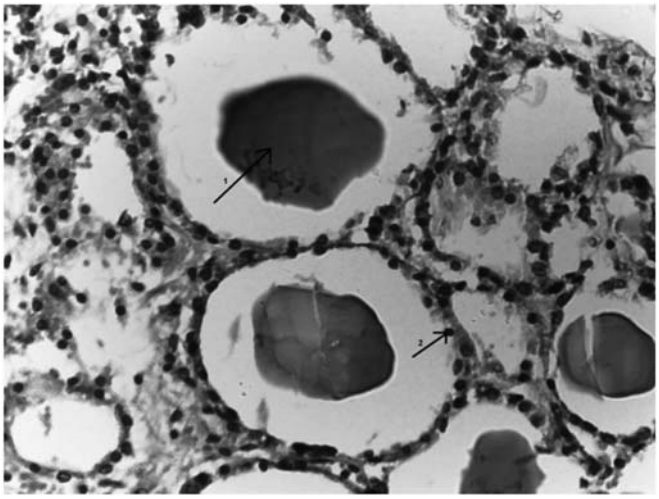


FIGURE: 2
Photomicrograph of 4 microns thick masson fontana stained section of thyroid gland in group B (Minocycline treated) guinea pig showing pigmentation inside the colloid material (P). X 400.

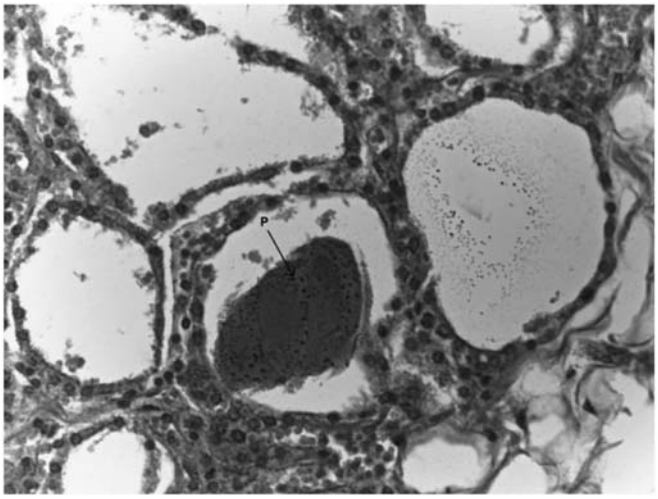


FIGURE: 3
Photomicrograph of 4 microns thick mason Fontana stained section of thyroid gland in group A (Control) guinea pig showing normal architecture of thyroid follicles filled with colloid material (CM). X 400.

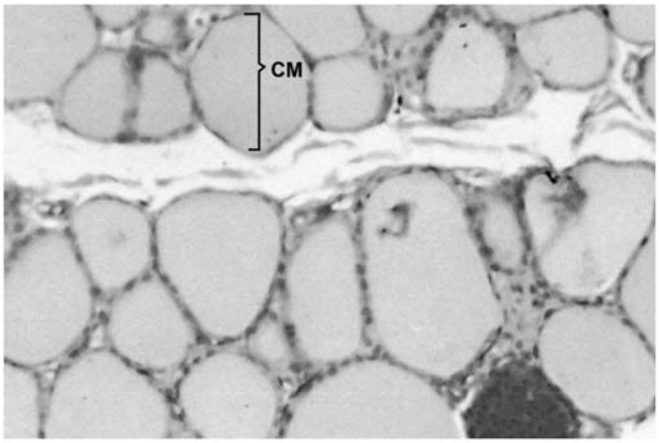
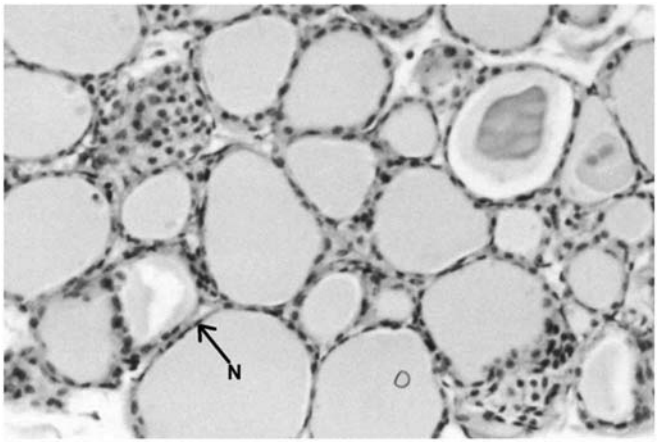


FIGURE: 4
Photomicrograph of 4 microns thick Hematoxylin and Eosin stained section of thyroid gland in group A (Control) guinea pig showing normal architecture and flattened nucleus (N). X 400.



epithelial cells was high cuboidal to low columnar and nuclei of these follicular epithelial cells were rounded in shape and increase in height. The mean values of area of thyroid follicles were 14137.5 ± 392.18 µm, which shows highly significant decrease ($P<0.001$) when compared with control group. The mean values of follicular cells and their nuclei were 6.95 ± 0.04 µm and 3.23 ± 0.11µm, which shows highly significant increase ($P<0.001$) in height of follicular cells and their nuclei (Table-1).

DISCUSSION:

Disorders of the thyroid gland are common, they consists of two general presentations i.e. changes in the size, shape and structure of the gland and changes in secretion of hormones from the gland. Overt hyperthyroidism and hypothyroidism often exhibit dramatic clinical manifestations of this gland (Farwell AP and Braverman LE, 2009)[11]. The lipophilic properties of minocycline, with complete oral absorption and good sebum penetration, make it the most widely prescribed systemic antibiotic used to treat acne vulgaris, often given for months or years (Ramakrishna et al., 2009)[12].

This study was carried out by observing and recording the histological and morphometric changes in thyroid gland. The

TABLE: 1

Mean of weight (G) of male guinea pigs in Groups A and B
(Data expressed as MEAN and SEM)

Animal No.	Group A (Control)			Group B (Minocycline)		
	Ini (g)	Fin (g)	Dif (g)	Ini (g)	Fin (g)	Dif (g)
1	650	660	10	550	450	-100
2	630	615	-15	600	510	-90
3	550	560	10	500	430	-70
4	600	590	-10	450	360	-90
5	580	585	5	480	375	-105
6	632	640	8	620	490	-130
7	590	610	20	618	510	-108
8	653	660	7	595	535	-60
9	610	615	5	580	498	-82
10	618	625	7	610	520	-90
Mean	611.3	616	4.7	560.3	467.8	-92.5
SEM	10.21	10.19	2.49	19.69	19.49	6.28

TABLE: 2

Mean of the area of follicles (µm) in different groups of guinea pigs
(Data expressed as Mean ± SEM)

Animal No.	Group A (Control)	Group B (Minocycline)
1	23945.25	12595.91
2	24733.78	15218.14
3	24181.14	13657.82
4	23009.92	13143.06
5	23950.35	15980.72
6	23890.35	14957.78
7	24280.16	13480.69
8	24280.25	15760.73
9	23930.19	12786.83
10	23790.3	13793.72
Mean	23999.169	14137.54
SEM	140.43	392.18

follicles of the thyroid gland were seen under light microscope to observe the area of follicles, height of epithelial cells, height of nuclei and changes in the colloid material in different groups. Microscopic examination of minocycline treated animals of group B in this study reveals that most of the thyroid follicles had no colloid and the follicles which contained the colloid were shrinked and darkly stained and epithelial cells were high cuboidal or low columnar. This is in agreement with the study of Inuwa.I and Williams M A (1996)[13] who explained that in hypothyroidism normal follicular structure of the thyroid gland is disrupted, the cuboidal follicular cells become columnar, obliterating the Lumina of the follicles, and colloid almost totally disappears in rats.This increase was clearly due to both hypertrophy and hyperplasia of follicular cells as evidenced by an increase in their height as well as the presence of numerous mitotic figures. Pantanowitz L and Tahan SR (2003) provided evidence that minocycline

TABLE: 3

Mean of height of follicular cells (µm) in different groups of guinea pigs (Data expressed as Mean ± SEM)

Animal No.	Group A (Control)	Group B (Minocycline)
1	1.06	7.08
2	1.06	7.03
3	1.06	6.86
4	1.05	7.1
5	1.04	6.89
6	1.05	6.93
7	1.07	6.79
8	1.08	7.05
9	1.07	6.9
10	1.09	7.04
Mean	1.063	6.967
SEM	0	0.04

TABLE: 4

Mean height of nucleus (µm) in different groups of guinea pigs (Data expressed as Mean ± SEM)

Animal No.	Group A (Control)	Group B (Minocycline)
1	1.15	3.11
2	1.18	3
3	1.17	3.01
4	1.2	3
5	1.18	2.94
6	1.16	3.9
7	1.2	3.7
8	1.14	2.9
9	1.19	3.5
10	1.18	3.19
Mean	1.175	3.225
SEM	0.01	0.11

accumulates in the follicular epithelium and colloid in benign and even hyperplastic tissue as a result of the oxidative interaction between the drug and the enzyme thyroid peroxidase.

The mean values of area of follicles were decreased highly significantly in group B animal when compared with the group A. This might be due to the long term use of minocycline which possesses potent antithyroid effects probably by inhibiting the iodination of thyroglobulin and also by inhibiting the coupling of diiodotyrosine residues to form triiodothyronine and thyroxine (Bowles et al., 1998)[14].

There is highly significant increase in the mean values of height of follicular cells and their nuclei in group B animals when compared to control group. This is in agreement with Inuwa and Williams (1996) who explained that normal follicular structure of the minocycline treated thyroid gland is disrupted, the cuboidal follicular cells become columnar and obliterating the Lumina of the follicles in rats.

Masson Fontana stained sections of group B animals showed deposition of pigment in the apical region of the follicular cells and also inside the colloid of the follicles in scattered form. This

FIGURE: 1

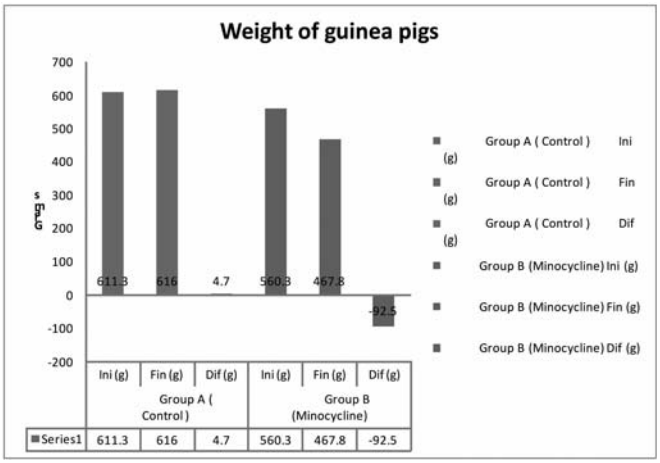


FIGURE: 2

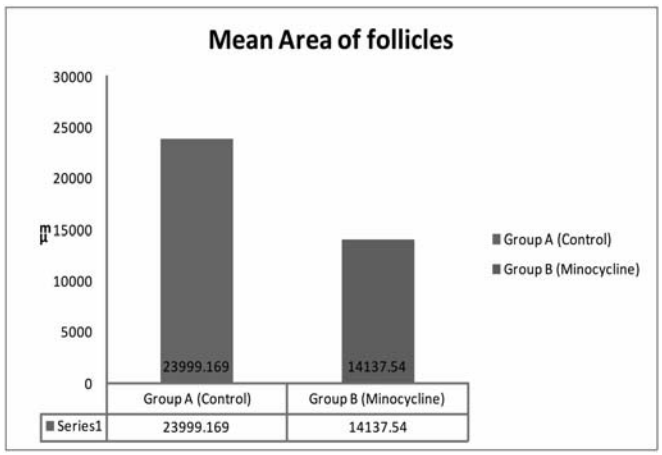


FIGURE: 3

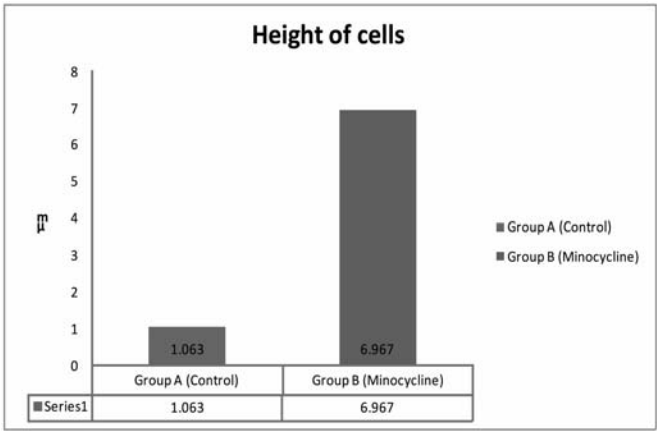
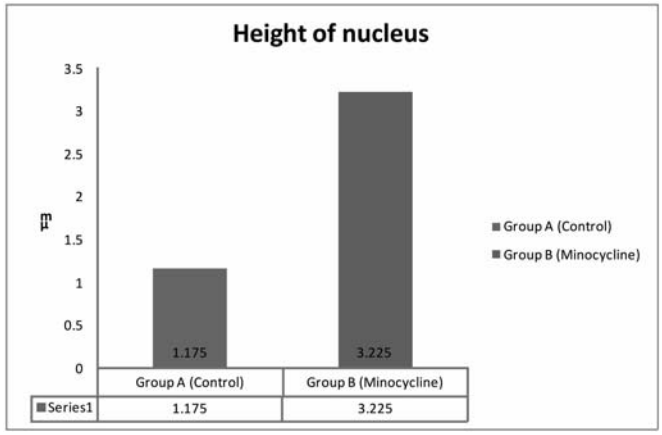


FIGURE: 4



is in agreement with Hecht DA (1999)[15] who discovered that the pigment was seen within follicle Lumina as large black deposits admixed with colloid. Onyia JN et al (1996)[16] reported that these granular pigments both in the follicular cells and in the colloid are strongly positive by Fontana – Masson stain, nonbirefrigent, and negative by iron stain.

The apical region of cells showed deposition of the pigment. This is in agreement with Hecht (1999)[15] who described that in black thyroid the pigment appeared within the cytoplasm of follicular epithelium as granular and black pigment. Capen (1997)[17] also stated that the pigment granules stain similarly to melanin and were best visualized on thyroid sections stained with the Fontana- Masson procedure Our result also match with Taurog et al (1996)[8] who reported that Black pigmentation of thyroid gland has also been observed in human on long term minocycline therapy.

The pigment was in scattered form.This is in agreement with Hecht (1999)[15] who discussed that in black thyroid the pigment appeared within follicle Lumina as large black deposits admixed with colloid. Pigment deposition, demonstrated by Fontana- Masson stained sections, and was pronounced in this group it might be due to oxidation degradation of the drug itself, drug interaction and alteration of tyrosine metabolism and lysosomal dysfunction. Pigmentation is a well-recognized adverse effect of minocycline therapy (Eisen and Hakim 1998)[18].

CONCLUSION:

Based on this study it is concluded that long term use of minocycline may causes adverse effects on thyroid gland by changing its structure and function such as decrease in follicular area and increase in height of cells and nuclei which is suggestive of its hypofunction. So that one should be careful to prescribe minocycline for long term.

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