ORIGINAL PROF-1370

HYPERPLASTIC THYROID GLAND;
STEROID HAZARDS

DR. ANWAAR HUSSAIN
MBBS, M Phil (Sr. Demonstrator Anatomy)
Punjab Medical College Faisalabad

DR. SAIF-U-DIN
MBBS, M Phil (Associate professor Anatomy)
Punjab Medical College Faisalabad

DR. KHALID MEHMOOD AKHTAR
MBBS, M Phil (Associate Professor Anatomy)
Punjab Medical College Faisalabad

Dr. Nabeela Kaukab
MBBS, M Phil (Associate Professor Anatomy)
Punjab Medical College Faisalabad

Dr. Muhammad Ismail
MBBS, DMRD (APMO)
Punjab Medical College Faisalabad

Article Citation:
Hussain A, Saif-u-Din, Akhtar KM, Kaukab N, Ismail M. Hyperplastic thyroid gland; steroid hazards. Professional Med J Mar 2010;17(1):21-30.

ABSTRACT... Aim and Objective: The study was conducted to see the effects of dexamethasone (synthetic corticosteroid) on involution of hyperplastic thyroid gland in albino rats, and to evaluate its uses in thyroid diseases. **Study Design:** Comparative histological study done in Postgraduate Medical Institute Lahore in 1998. **Materials and methods:** 54 adult male rats were taken and divided at random into control group having 27 rats and experimental groups containing 27 rats. Control group was given normal diet along with 2 microgram of Potassium iodide for 21 days while experimental groups further subdivided into group A having 3 rats and group B and C containing 12 rats each. All the experimental groups were treated with Thiourea. Group A was sacrificed on day 22 after withdrawal of thiourea. Group B was given Potassium iodide after stoppage of thiourea on day 21 and were sacrificed on days 22, 26, 30 and 50 in 4 sub groups (B1- B4). Group C was injected Dexamethasone daily from day 22 to day 50 and sacrificed on same days in 4 subgroups (C1-C2) to study involution process. **Results:** Histologically experimental group A exhibited significant increase in width and length of follicular cells lining the small sized follicles having scanty colloid. The results of experimental subgroups B revealed early and complete involution whereas subgroups C showed significantly persistent hyperplastic changes in the form of tall follicular cells lining small empty follicles. **Conclusion:** Thus it was concluded that dexamehasone did retain hyperplastic changes during involution process, so steroid should consciously be used in thyroid diseases.

Key words: Dexamethasone, thyroid, involution, steroid and thyroid gland.

INTRODUCTION

The thyroid gland is brownish color highly vascular gland placed anteriorly in the lower neck. It is unsheathed by the pretrachial fascia consists of right and left lobes connected by a narrow median lobe isthmus. A conical pyramidal lobe often extend upward to the hyoid bone and some time muscular cord or fibrous band extends to the isthmus of the thyroid gland[1].

Histologically thyroid gland is covered by thin fibrous capsule which extends into the glandular parenchyma which divides each lobe into irregular shaped and sized lobules. The functional units of the thyroid gland are the follicles of variable sizes which are spherical or cyst like having colloid in the centre lined by single layered epithelium resting on basal lamina.

Article received on: 14/06/2008
Accepted for Publication: 16/10/2008
Received after proof reading: 07/12/2009
Correspondence Address:
Dr. Anwaar Hussain
MBBS, M Phil (Sr. Demonstrator Anatomy)
Punjab Medical College,
Faisalabad

The sufficient amount of hormone is stored in the form of colloid in the follicles is present enough for body need for up to 3 months[1]. The follicular cells vary from squamous to low cuboidal to coloumnar, depending on their level of activity[2]. Colloid consists of almost entirely by an iodinated glycoprotein, iodothyroglobulin.

This is an inactive form of active thyroid hormone triiodothyronin (T3) and tetraiodothyronin, thyroxin (T4).Thyroid follicles are surrounded by delicate connective tissue stroma containing dense plexuses of fenestrated blood capillaries Thyroid parenchyma also contain C cells (clear cells) located in the thyroid follicle resting on basal lamina not reaching to the lumen of the follicles and have pale looking cytoplasm, and they secrete thyrocalcitonin[1].

STEROID

Steroid are naturally occurring or synthetic fat soluble organic compounds having as a basis 17 carbon atoms arranged in four rings and include in these are sterols and bile acids, adrenal and sex hormone, certain natural drugs like digitalis, it is also the precursor of certain vitamins.

DEXAMETHASONE

Dexamethasone is the synthetic corticosteroid, a hormone released by zona fasciculata of adrenal gland. It is commonly used in certain autoimmune diseases and its release is enhanced in stressful condition and is mediated by mutual interaction of hypothalamic pituitary axis and immune mechanism[3].

PHARMAKOKINETICS

It is absorbed both orally and parentrally with the dose of 1.25mg/kg body weight. Steroid globulin reaches receptors site, and is translocated across cell membrane and acts on target gene located on DNA. Some effects of it are immediate and mediated by G protein phosphate complex which activate second messenger across the cytoplasm[4].

HYPERPLASIA OF THYROID GLAND

Hyperlasia is defined as an increase in the number of cells in an organ which contributes to increase in volume. Both structural and functional activity is under the control of thyroid stimulating hormone (TSH) released by pituitary gland. TSH stimulates synthesis of inositol triphosphate and causes increase in cytosolic Ca++ in follicular cells[5,6,7], this in turns stimulates H_2O_2[8].Thyroid stimulating hormone stimulate adenyl cyclase which in the presence of Ca++ converts ATP into cAMP which acts as intercellular messenger that stimulates enzyme protein kinase[9,10] responsible for hyperplastic changes.

Goiter is visible swelling in the neck found in iodine deficient areas. Hyperplasia can also be produced by antithyroid drugs like Thiourea. It inactivates peroxidase enzyme necessary for iodine activation but only occur when heme is oxidized[11,12].Histologically hyperplastic changes are visible both in the stroma and parenchyma. Stromal changes are thick capsule having congested interfollicular tissue[13,14] with invasion of inflammatory cells. Whereas tall follicular cells lining the small empty follicles[15,16] are the parenchymal changes.

IODINE AND THYROID GLAND

Normal blood level iodine ranges from 150 to 300mg/100ml. It is necessary for the iodination of thyroglobulin and is transported from basal side of follicular cells by a specific protein named sodium iodide symporter[17,18,19]. Some drugs like thiocyanate and perchlorates inhibits its absorption by attaching to receptors site. Iodide ions gets activated by peroxidase located at apical plasmalema[20] and attaches to the tyrosine residues of globulin and forms mono and di-iodotyrosin coupling of mono and di-iodotyrosin leads to formation of tri-iodithyronin (T3) and thyroxin (T4), this process can be blocked by thiourea and carbimazole.

AIMS AND OBJECTIVES

The present study is designed to:

- See the histological changes produced by Dexamethasone during involution of hyperplastic thyroid gland.
- Evaluate its clinical application in thyroid diseases.

MATERIALS AND METHODS

Total 54 adult male albino rats weighing 120-300 grams were taken from National Institute of Health Islamabad.

All the rats were healthy and kept in animal house of PGMI Lahore in standard cages for two weeks for acclimatization. They all were given normal diet and were divided at random into control and experimental groups.

GROUPING

CONTROL GROUPS

Control group comprised of 27 rats and was randomly divided into 3 subgroups. First group contain 3 rats sacrificed on day 22 along with Experimental group A. Second and 3rd groups contain 12 rats each sacrificed on days 22, 26, 30 and 50 along with experimental groups B and C

EXPERIMENTAL GROUPS

Experimental groups comprised of 27 rats and were given Thiourea, an antithyroid drug in the dose of 10 mg/ 100 gram bodyweight orally for 21 days, This group was divided at random into A, B and C.

GROUP A

3 rats were given thiourea at the dose of 10mg/ 100 gram body weight orally for 21 days and were sacrificed on day 22.

GROUP B

Total 12 rats were placed in this group were given thiourea 10 mg /100 gram body weight for 21 days along with 2 microgram Potassium Iodide (KI) intrapertonealy for iodine replacement. They were sacrificed on day 22, 26,30 and 50 in 4 subgroups B1-B4 respectively each comprising of 3 rats.

GROUP C

The rest of 12 were given Thiourea 10mg /100 gram body weight orally along with 2 microgram of potassium iodide for 21 days as replacement therapy. These were also injected 1.25 mg/kg body weight of dexamethasone I/M daily from day 22 to 50. They were sacrificed on day 22, 26, 30 and 50 in sub groups C1-C4 respectively, each containing 3 rats.

DRUGS

THIOUREA

It is the anti thyroid drug and marketed as Thiourea GPR prepared by BDH London, taken from the Lahore Scientific Store Lahore. It is white crystalline powder, soluble in water.

DOSE PREPARATION

The dose was prepared by dissolving 2 grams of TU in 50ml of distilled water and with the help of insulin syringe calibrated for one ml into 100 units so 0.25ml=25units=10mgTU.

DEXAMETHASONE

It is synthetic corticosteroid available in market. In this study DX used in the form of ampule of 1 ml containing 4 mg of dexamethasone prepared by Tabrose Pharmaceutical Karachi.

DOSE PREPARATION

The dose used was 1.25 mg / kg body weight per day by intramuscular injection in quadriceps muscles of rats. The dose was calculated with the help of insulin syringe, according to the dose 100 grams body weight required 0.125 mg dexamethasone, as one ampule of dexamethasone contains 1ml=4mg. i.e 0.03 ml=0.125mg from the ampule. Insulin syringe is calibrated for 100 units per ml so 3 units = 0.125mg per 100 grams body weight.

PROCEDURE

All the rats both control and experimental were sacrificed on specified days using ether anesthesia. A paramedian incision was made in the neck extending to upper larynx to expose thyroid gland. The thyroid lobes were fixed in 10% formaldehyde for 16 hours processed and dehydrated. Tissue was cut at 5 micron meter by rotary microtome. 10 slides of each group were prepared stained with H&E and examined by light microscopy. Students' t test and Duncan Multiple Range Test (DMRT) were applied for significant value.

PARAMETERS

HISTOLOGICAL PARAMETER

- Length and width of the follicular cells

- Colloid content of the follicles

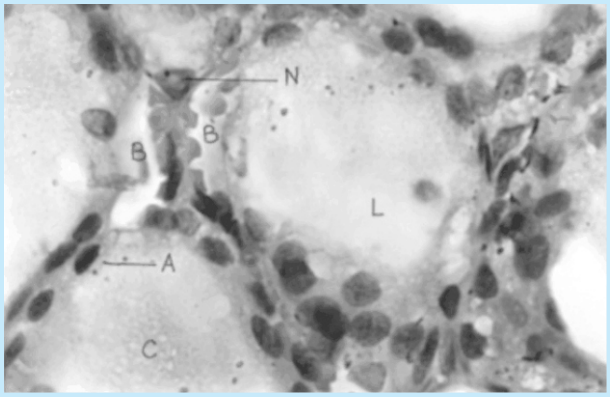
All the measurements were carried out in three random fields of microscope. In each field 10 follicles with 10 follicular cells at one specific marker point to another, those follicles were observed which were cut at right angle. Measurements were carried out with the help of an oculometer under 100x.

RESULTS

FOLLICULAR CELLS

In the control group follicles were lined by low cuboidal cells, some were squamous resting on basal lamina containing well defined nuclei, no visible detached cells found in the follicles (Fig.1).

Fig-1. Control Group

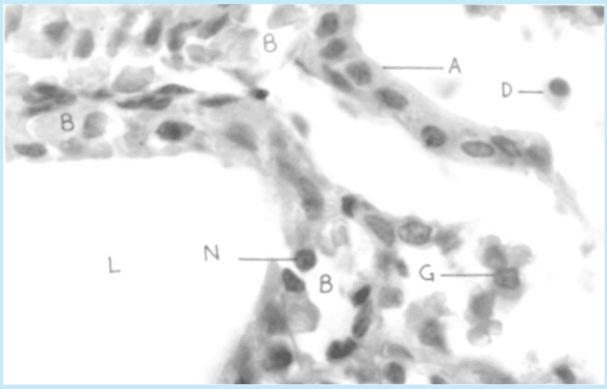


A: Flat follicular cells
B: Blood vessels
Mag: 2800x100
C: Colliod fully and partially filled
N: Well defined nucleus with nuclocolus

The mean width of the cells was 11.65+ 1.24 and height 8.9 ± .865 micrometer.

In the experimental group A epithelial cells having well defined boundaries resting on basal lamina. The cells were tall cuboidal or columnar in shape containing large nuclei with nucleoli (Fig. 2). These cells were lining the follicles and some were in the forms groups.(Fig.2) The parenchyma in the central field showed dispersed follicular cells, few detached cells also found. The width of the cells was significantly reduced (mean=8.13±0.84) compared to control whereas height was increased significantly (Table I), mean =10.22 ±1.03 compared to control.

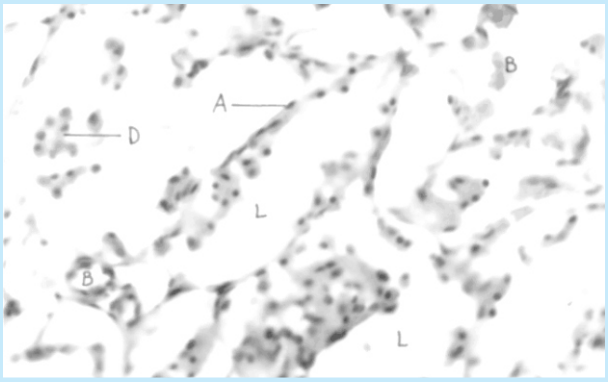
Fig-2. Experimental Group A



A: Tall cuboidal cells
B: Multiple blood vessels
N: Well defined nucleus with nucleoli
Mag. 2800x
D: Detached cells
L: lumen of empty follicles
G: Groups of follicular cells

All the follicles in experimental group B were lined by low cuboidal cells, few were flat and some were tall containing flat nuclei. Some cells were detached in the form of groups with visible nuclei (Fig.3).

Fig-3. Experimental Group B (B3)

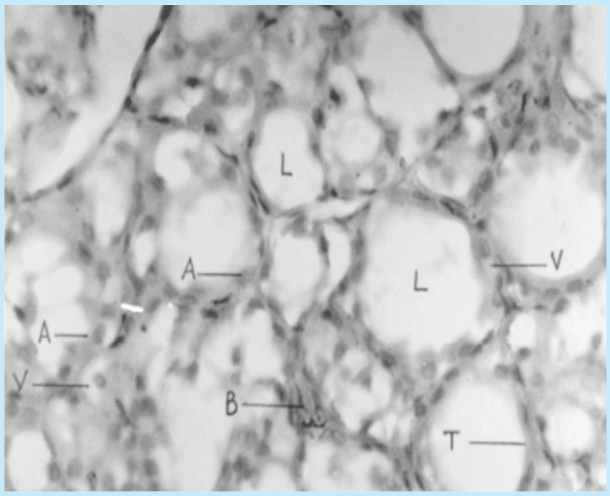


A: Low cuboidal cells
B: Numerous blood vessels
D: Detached follicular cells
L: irregular iumina of the follicles
Mag. 2800x

The mean width was significantly reduced as compared to control (P=.0002), mean height (7.289±.457) of the cells was significantly reduced (Table I) compared to control.

Experimental group C the epithelial cells were tall cuboidal to columnar in shape resting on basal lamina. These cells showed well defined large round nuclei containing visible neucleoli and cytoplasm was basophilic, some cells showed vacuolated empty spaces (fig.4).

Fig-4. Experimental Group C (C4)



A: High cuboidal cells
B: Well localized blood vessels
N: Lumina of the empty follicles
Mag. 2800x
M: Marginal colloid
T: Thick plasma lemma
V: Vacoulated spaces

The apical plasmalemma thickened and lumen of the follicles appeared uniform. The width of the cells was significantly reduced (P=0.0001) but the height did not showed significant change (Table I) compared to control.

On comparing the subgroups of both groups B and C the follicular cells were low cuboidal to columnar, the latter was predominant in all subgroups C. In subgroups B few detached cells were found but none in group C (Fig-3).

The width of follicular cells did not show any significant change in both groups B & C. But there was significant increase in cell height (mean= 9.48) in subgroups C as compared to group B (mean= 7.37). (Table II, Fig.4).

On comparing subgroups there was significant increase in the cell height on days 22,26, and 50 (C1,C2 and C4) in group C with respect to group B on same days (Table II). The follicular cells showed well defined nuclei with nucleoli and smoothly organized around follicles.(Fig 4).

COLLOID CONTENT OF THE FOLLICLES

The follicles were visible in the peripheral and central field of control group containing colloid which was mostly partially filled, mean number per field was 15.35±0.93. Some were empty and were mostly located in the central field mean was 7.96±4.451and very few was fully filled (mean=1.23±0.93).

Amount of colloid showed striking changes in the experimental group A, there was significant increase in empty follicles (Fig.2 & Table 1) compared to control. Partially filled follicles showed significant reduction only visible at the margins of the lumen (Fig. 4). Colloid content was visible in the follicles located close to the capsule whereas in the central field colloid was dispersed in the breaking lumina of the follicles (Fig.2).

In the experimental group B colloid was invariably found in the follicles, mean number of fully filled follicles was significantly increased (mean= 2.789+ .940).partially filled showed reduction whereas empty follicles were significantly increased (Table 1 & Fig.3) compared to control.The experimental group C showed insignificant change in the number of fully filled follicles, partially filled follicles showed significant reduction (P= 0.000). But empty follicles were significantly increased (Table 1, fig 4) compared to control.

On comparing subgroups there was significant increase in fully filled follicle in subgroups B as compared to groups C (mean =3.23) (Table 2, Fig 3). The subgroups B showed gradual increase in the colloid content as the days proceeded whereas in group C exhibited significant increase on day 50 as compared to day 26. Partially filled follicles showed significant reduction on day 22 (B1) compared to days 26 (B2),50(B4) in subgroups B . The study of subgroups C demarcated colloid content at the

margin of the follicles (Fig. 4). There was significant increase in the empty follicle in group C (mean= 23.64) as compared to group B. Within subgroups of C it was observed significant reduction of these follicles on day 50 compared to day 22, initially on day 22(C1) the follicles were larger devoid of colloid but as the days proceeded sub groups showed smaller follicles with scanty marginal colloid.

Table-I. Comparison of experimental groups A, B and C variables (Width, length of the follicular cells (Micro meter) and colloid content of the follicles)

Groups	Variable	Mean	SE. Mean	P Value
A (n=3)	Width	8.13	0.27	<0.001
	Length	10.22	0.33	0.009
B (n=12)	Width	9.39	0.17	<0.001
	Length	7.289	0.14	<0.001
C (n=12)	Width	9.209	0.11	<0.001
	Length	8.341	0.18	0.073
A (n=3)	Colloid fully filled	1.26	0.47	0.96
	Partially filled	3.38	0.73	<0.001
	Empty	30.1	3.3	<0.001
B (n=12)	Colloid fully filled	2.79	0.30	<0.001
	Partially filled	8.43	0.44	<0.001
	Empty	18.68	0.63	<0.001
C (n=12)	Colloid fully filled	2.08	0.21	0.031
	Partially filled	7.56	0.55	<0.001
	Empty	23.40	0.69	<0.001

All the value were calculated with respect to control

Table-II. DMRT between experimental groups B & C variable: (Width & length follicular cells (Micro meter) and colloid content of the follicles)						
Variable		**Days**				
	Groups	22	26	30	50	Mean
Width of follicular cell	B	8.55	9.74	10.39	9.05	9.42
		b	ab	a	ab	
	C	8.17	9.63	9.58	9.72	9.27
		b	ab	ab	ab	
Length of follicular cell	B	7.63	6.33	6.88	8.65	7.37
		def	f	ef	cd	
	C	10.23	9.15	7.85	10.71	9.48
		ab	bc	cdef	a	
Colloid fully filled	B	2.58	5.95	1.25	3.15	3.23
		bcd	a	cde	bc	
	C	2.54	0.40	1.08	2.49	1.62
		bcd	e	de	bcd	
Partially filled colloid	B	3.36	12.19	7.35	10.23	8.28
		fg	abc	def	bcd	
	C	8.76	3.22	10.33	2.93	6.31
		cde	fg	bcd	cdef	
Empty follicles	B	18.83	20.37	23.86	20.33	20.84
		cd	cd	bc	cd	
	C	32.77	22.11	17.86	21.85	23.64
		a	c	cd	c	

DISCUSSION

In the present study hyperplasia was produced by the administration of anti thyroid drug thiourea with the dose of 10 mg / 100 gm body weight daily for 21 days. The dose was 1/3 less than given by Schneider and Golden(1987)[21], while Mitsumuri etal., in 1994[22] used thiourea at the dose of 0.05mg%. The duration of treatment 21 days was the same as previously given by many researcher including Wollman etal.,(1990) and Tachiwaki etal., 1990[23,24].

FOLLICULAR CELLS

The intraluminal cellular casts found during involution on days 26 and 30 in subgroups Band C were also previously observed by Wollman etal., (1968)[23], Olein (1969)[25], Many etal.,(1983)[26]and Mahamound etal.,(1986)[27]. These cells were found to have well defined cell boundries with visible nuclei. In the present

study few cells were found late during involution (Fig.7).and thus differ Wollman etal., 1968[23], Bellshaw and Baker 1973[28]. The rat follicular cells have longer half life (Wollman etal.,1968[23] Smed and Wollman 1982)[13]. Thus produce the larger follicles during hyperplasia. However, recent evidence suggests that follicular cells that divide just before initiation of involution die earlier[29].

Significant increase in the follicular cell height in group A and Group B correspond with the observation made by Wollman etal., 1990[23] and Kano etal., 1990[30]. Tall cuboidal cells observed in groups C during involution with DX treatment supports the observations of Ramanchandran and Joseph(1993)[29]. These cells showed well defined oval nuclei with prominent nucleoli denoting increase in protein synthesis (Kameda and Akeda 1980, Tachiwachi etal., 1990)[31,24], (Fig4). The small sized follicles with hypetrophied cells lining narrow follicular lumina suggestive of hyperplasia[15]. Thick apical plasmalemma (Fig 6)in subgroups C might be due to tall microvilli as observed by Many etal.,1983[26].

COLLOID ACCUMULATION

In experimental groups A,B and C peripherally located follicles were larger and partially filled with colloid lined by low cuboidal cells showing normal activity. As the involution process progressed in group B colloid started accumulating and showed resemblance with the control[18] Slow accumulation can be reasoned by the delay in resynthesis of peroxidase inactivated by thiourea. This is justified by Rognoni etal, (1982)[32] who observed about low concentration of thyroid hormone on day 4 of involution while TSH level is high.

In group C the follicles remained empty under the influence of dexamethasone suggesting persistent hyperplasia (Fig 4). The changes occurring in subgroups C can be discussed on the basis of colloid accumulation, it would be evident that colloid readily get absorbed and follicles found empty lined by tall follicular cells. Further more most of the follicles were small in size denoting increased activity. Colloid in this group rapidly processed in the cells that might result in high level of T3 and T4 which is contrary to many researcher[33,34,35] Kasuga etal.,(1990)[36]. Metsonega etal., (1988)[37] described decreased in phagocytic activity of thyroid after dexamethasone and was contradicting to the observation made in the present study. The reason for this contradiction was due to fact that larger dose might be required to suppress the phagocytic activity.

CONCLUSION

It is concluded that dexamethasone did retain hyperplastic changes during involution process of thyroid gland and thus should be carefully used in thyroid diseases.

REFERENCES

1. Junqueira LC, Carneiro LC. **Basic histology.** 11th ed. New York: McGraw- Hill; 2005. p411-13.

2. Susan Standring, Harold Ellis, Jeremiah C and Andrew Williams Grays Anatomy 39th edn.560-561. Elsevier Churchill Livingstone Philadelphia USA 2005.

3. Spolsky RM, Romero LM, and Malissck AU. **How do glucocorticoid influence stress response ? integrating, permissive, suppressive, stimulatory and preparatory action.** Endocrinol., Res., 2000; 212:55-89s.

4. Christ M, Hasiroth K, Falkenstein E, and Wehling M. **Non genomic steroid action. Factor. Fantasy.** Vitam., Horm., 1999; (57): 325-375.

5. Corda D, Marcocci C, Kohn LD, Axelard J and Luini A. **Association of changes in cytosolic Ca++ and efflux induced by thyrotropin and stimulation by alpha 1 receptor in cultured rat thyroid cells.** Chem 1985; 260 : 9230-9236.

6. Field JB, Ealey PA, Marshall NJ, and Cockroft s. **T.S.H stimulates increase in inositol phosphate as well as cyclic A.M.P in F. R. T. L – 5 rat thyroid cell.** J. Biochem 1987; 247: 519- 524.

7. Laurant E, Mocket J, Vansandae J, Graff I Dumont JE. **Dual activation by throtropin op phospholipase C and Cyclic AMP cascade in human thyroid.** Cell. Mol. Endocrinol 1989; 52: 273-278.

8. Takasu N, Yanade T, and Shammizin Y. **Generation of H2O2 is regulated by cytoplasmic free Ca++ in cultured porcine thyroid cell.** Biochem. Bio physcic., 1987; 148:1527-1532.

9. Manley SW, Rose DS, Huxam GJ, Bourke JR. **Role of Ca++ in the secretomotor response of the thyrpoid; Effect of Ca++ ionophore A 23187 on radioiodine turn over membrane potential and fluid transport in cultured porcine Thyroid cell.** J. Endocrinol 1988; 116: 373-380.

10. Vansandae J, Respe E, Perret J. **Thyrotropin activates bothb cyclic AMP and PI O2 cascade in CHO cell expressing in human. C DNA of TSH receptor.** Mol. Cell. Endocrinol 1990:74:1-6.

11. Davidson B, Soodek M. **The irreversible inactivation of thyroid peroxidase by methyl mercaptoimadazole, tiouracil and propyl thiouracil in vitro and its relation in vivo findings.** Endocrinol 1978;128:3073-3080.

12. Engler H, Taurog A, Nakashing J. **Mechanism of inactivation of thyroid peroxidase by thiouryline grugs.** Biochem. Pharm 1982;31:3801-3806.

13. Smed S, Wollman S. **Capillary endothelial cells proliferation in adipose tissue pad on thyroid glend during feeding of thiouracil.** Endocrinol 1983;12(5): 1718-1722.

14. Ericson LW, and Wollman SH. **Increase in the rough endoplasmic reticulum in capillary endothelial cells and pericytes in hyperplastic thyroid gland.** Endocrinol 1980; (3): 732-738.

15. Valenta J, Florscheim WC, Sharma BS. **Acute effect of iodine on the stimulated rat thyroid gland.** Endocrinol 1982;111 (3) 1720-1727.

16. Handa RJ, and Chasson RB. **Comparative effects of three goitrogen treatments on white leg horn chicken.** Avian, Dis 1980; 24 (4): 916-929.

17. Eskandre J, Loo DD, Dai G, Leavy O, Wright EM, and Coresco M. **Thyroid sodium iodide symporter, mechanism, stichiometry and specificity.** J. Bio. Chem 1997; 277: 27230-27238.

18. Dai G, Leavy O and Corresco M. **Cloning and charactization of th thyroid iodide transporter.** Nature 1996; 379: 450-460.

19. Smanik PA, Liu Q, Furminges TI, Ryu K, Xing S, and Taurog S.M. **Cloning of sodium iodide symporter.** Bio. Biophy. Commu 1996; 226: 239-25.

20. Wollman SH, Androse G, Cannon GB, and Eagleton GB. **Production and involution of hyperplastic thyroid gland. In. Thyroid neoplasia.** S. Young And D.R Inman, Acedemic press, New York 1968; 201-208.

21. Schneider Bf, and Golden WL. **Acquisition of acoustic startle response in relation to growth and thyroid function in rat.** In,. J. Dev. Neurosec 1987; 5(2); 99-106.

22. Mitsumuri K, Shimmo T, Onodera H, Kano J, Takihashi M,and Kitaura K. **Synergic effect of phenobarbitone and thiourea in proliferative lesion of rat liver.** Cen. Leh 1994;81(1):45-52.

23. Wollman SH, Hervig JP, and Tachwachi. **Histological changes in tissue component in hyperplastic thyroid gland during its involution In the rat.** American. J. Ana 1990;189:35-44.

24. Tachiwaki O, Zeilig D, Wollman SH. **Ultra structural changes in thyroid epithelium during involution of hyperplastic thyroid.** Am. J. Ana 1990;89(1)45-56.

25. Olein E. **The fine structure of experimentally induced hyperplasia and colloid goiter in hamster,** 1969.

26. Many MC, Denef JF, Gathy P and haumount S. **Morphological and functional changes during hyperplasia and involution in C3H Mice, Evidence of folliculogenisis during involution.** Endocrinol., 1983; 112 (4): 1292-1302. Lab. Invest 21:336-346.

27. Mahmound I, Collin I and Hamount S. **Propphlective effect of vitamin E in the toxic effect of iodine in excess in hyperplastic thyroid Gland.** Ann. Endocrinol 1986;17:72.A.

28. Bellshaw BE, and Becker DV 1973. **Necrosis of follicular cells and discharge of thyroidal iodine induced by administration of Iodide in iodine deficient dogs.** J. Clin. Endocrinol 1973;36:456-476.

29. Rognoni J, Panel C, and Golstein J. **Negative effect of iodide in the survival of newly divided epithelial cells in abnormally Stimulated rat thyroid cells.** Cell. Tissue. Kinet 1986;19:449-453.

30. Kano J, Matsoaka C, Furuta K, Onodera H and Haekiwa A. **Tumor promoting effect of goitrogen in the rat thyroid.** Toxicol. Path 1990;18(2):299-346.

31. Joseph J, and ramanchandran AV. **Effects of exogenous dexamethasone and corticosteroid on weight gain, organ growth in Post hatched white-leghorn chicks.** Indian, J. Exp.Bio 1993;31(10); 858-860.

32. Kameda Y, and Akeda A. **Immunohistochemical reaction of C cell complexes in dogs after induced hypercalcemia, Anti-thyroid drug treatment and**

hypophsectomy. Cell, Tissue, Res 1980;208 (3): 417-432.

33. Brouselle E, Ducottet X, Martin C barbier Y, bornet H, and Noel G. **Rapid effectiveness of prednisone and thioniamide combine therapy in severe amiodarone. Induced thyrotoxicosis, a comparison of two groups with apparently normal throid..**, J Endo Invest 1989;12 (1)37-42.

34. Messer NT, and Ganjum VK. **Effect of dexamethasone administration on serum thyroid hormone concentration in Chemically normal horses** J. Ana. Vet. Med 1995;206(1):63-66.

35. Kasuga Y, Sugenoya A, Kobayashi S, Kaneco G, Mamuta H. **Clinical and immunological study on patients with graves disease preoperative treated With corticosteroid and iodide.** J. Pn. Surg 1990; 20(3)283-289.

36. Metsonega M, Eguchi M, Fukuda T, Tazuka H and Otsobu T. **The effect of cytokines, anti thyroid drugs and glucocirticoid on phagocytosis by the Thyroid cells Acta.** Endocrinol 1988;119(3):413-41.

LETTER TO EDITOR

(IN RESPONSE TO E-MAIL FROM M. IBRAHIM ibrahim_ap98@yahoo.com)

Dear Sir;

As the objective was to observe the number of “bicycle passenger injuries in children” in different type of injury groups. There is no need to apply any statistical test as no comparison or result about general population was required.

Irfan Dilber
Statistician
The Prof Med J