ORIGINAL ARTICLE

Protective Effect of Thyroxine on Minocycline Induced Thyroid Gland Damage

Naheed Gohar[1], Aisha Qamar[2], Asma Siddiqui[3], Tahira Assad[4], Sahal Salman[5]

[1]Department of Anatomy, Sir Syed College of Medical Sciences for Girls, [2]Department of Anatomy, Bahria University Medical and Dental College, [3]Student, [4]Department of Pharmacology, Karachi Institute of Medical Sciences, Malir Cantt, Karachi, [5]Student.

ABSTRACT

Background: Thyroxine has shown beneficial effects on intelligence, learning, and memory process in patients of congenital hypothyroidism. Minocycline has been used in clinical practice for various indications and reported to have anti-thyroid effects. This study was specifically designed to observe the role of thyroxine on minocycline induced damage to thyroid gland.

Methods: This experimental study was undertaken at Anatomy department of BMSI, JPMC, Karachi, for eight weeks, from October to November 2019. Thirty adult (10-12 months) male guinea pigs, weighing from 450-650 gm were obtained and divided into 3 groups. Group A served as control, group B was given Minocycline 0.02mg/gram/day once daily and group C was administered Minocycline in similar amount as group B along with thyroxine 0.5µg/gram/day for the same duration. Dosing was continued for 8 weeks, at the completion of which all the animals were sacrificed. Thyroid gland was processed and tissue sections were stained with Haematoxylin and Eosin for morphology.

Results: The absolute weight of thyroid gland was significantly increased ($p<0.001$) in minocycline treated group B animals compared to the control animals, whereas substantial decrease ($p<0.01$) in absolute weight of thyroid gland was witnessed in group C in comparison to group B animals. The follicular cells showed hypertrophy and shrinkage of colloid in the thyroid follicles. These changes were prevented when animals were co-administered with thyroxine and minocycline in Group C.

Conclusion: Concomitant administration of thyroxine with the antimicrobial drug minocycline showed protective effects of thyroxine on Minocycline induced damage to thyroid gland of animals.

Keywords: Thyroxine; Minocycline; Thyroid Gland; Guinea Pig; Protective Effect.

Corresponding Author:
Dr. Tahira Assad
Department of Pharmacology,
Karachi Institute of Medical Sciences,
Malir Cantt, Karachi, Pakistan.
Email: drtahiraassad@gmail.com
doi.org/10.36283/PJMD9-2/003

INTRODUCTION

The thyroid gland secretes two major hormones Tri-iodothyronine and Thyroxine, commonly known as T_3 and T_4 respectively with Thyroxine (T_4) being the most the active form (93%). Thyroxine is inconsistently absorbed from the gut on oral administration. Absorption is augmented in fasting condition and differs according to the contents of gastrointestinal tract[1].

Human experimental studies have described the advantages of thyroxine replacement treatment on the intelligence, learning and memory process in congenital hypothyroidism[2]. Thyroid hormone upsurges the metabolic rate of cells in the body. In the fetus and new born, it is important for the growth of all the body tissues including bones and brain. Thyroid hormone affects brain development by regulating gene expression[3]. In adults, they help to preserve brain function, metabolism and body temperature[4].

Minocycline belongs to the long acting tetracycline antibiotics. It has been used in the clinical practice for years[5]. It is prescribed for the treatment of acne, infectious diseases, and rheumatoid arthritis[6]. It exerts its anti-inflammatory effects by inhibiting neutrophil-mediated tissue injury, by preventing their migration and degranulation, and by suppressing the formation of free oxygen radicals. Minocycline inhibits the enzymes, which lead to inflammation, such as the inducible form of nitric oxide synthase, and interleukin-1B-converting enzyme[6]. Minocycline is was also found effective for the prevention and treatment of different types of dementia including Alzheimer's disease. It also prevents cardiovascular, renal, and nervous system diseases at the cellular level[7].

In addition to the benefits of Minocycline, it has numerous undesirable effects. One of the most important is its anti- thyroid effects. Studies have shown that it is a more effective inhibitor of iodide coupling than iodination. Dark pigmentation of thyroid gland has been witnessed in humans on long term minocycline treatment, known as "black thyroid syndrome"[8,9]. The pigment aggregates in follicular cells and colloid, interstitial fibrosis, and pyknotic nuclei suggesting epithelial damage[10]. Keeping these facts in mind, this study was aimed to evaluate the actions of minocycline on thyroid gland with possible protection conferred by thyroxine.

METHODS

This experimental study was performed in the Anatomy Department of Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi following ethical approval (ERC No:F,1-2/2019/BMSI-E.COMT/NG/JPMC. Thirty adult male guinea pigs weighing from 450-650 gram were obtained from the animal house of BMSI. All the animals were uniformly divided into three groups; Group A, Group B and group C, each containing 10 animals. Division is based on the treatment they will receive.

Group A served as control and group B received Minocycline 0.02mg/gm/day (Steifel Laboratories Pakistan Pvt., Ltd; Stiefel Laboratories Inc. Coral Gables, FL33134, USA) by nasogastric tube[11]. Group C animals were administered same dose of Minocycline as in group B, along with Thyroxine 0.5µg/gm/day[12] (GlaxoSmithKline Pakistan Ltd., RN 000374, MI 000017 and 000233) by the same method as employed in group B. The dose of thyroxine was based on the human dose of 50µg/day[7], if 50 µg is dose for an adult weighing 60kg, then it would be 0.5µg in average guinea pig of 600g dissolved in 2 ml of distilled water administered with the help of NG tube. Dosing was continued for a period of 8 weeks.

The animals were observed for one week before the start of experimental work for their general health, activity, behavior and diet as well as during the study period. They were sacrificed at the end of 8th week. The neck was opened by a midline incision extending from the chin to the sternum. Skin, fascia and muscles were carefully removed, and then infrahyoid muscles were retracted to expose the thyroid gland. The gland was removed from its site and washed with normal saline. It was weighed on Sartorius balance. Then it was preserved in 10% buffered formalin for 24 hours. Thyroid tissue was processed and embedded in paraffin. Four micron thick sections were cut on rotary microtome, placed in water bath at 42°C and then positioned on albuminized glass slides. Tissue was fixed and stained with Haematoxylin and Eosin[13]. All statistical calculations were performed by using computer software SPSS version 20. The results were assessed by paired student t-test, p-value<0.05 was considered significant.

RESULTS

Table 1 reveals the comparison of gross toxicities such as average weight variation, loss of hair, diarrhea, aggressive behavior, loss of activity and hematuria. Effect on activity was observed in the fourth week of treatment in Minocycline group in comparison to control group. Some loss of average weight was also observed throughout the experimental period that was more marked in the minocycline group but it was statistically insignificant as shown in Table 1.

The animals of Group A (control) stayed healthy and active during the course of the study period. Whereas, the animals of group B (Minocycline treated) became inactive with the passage of time. They appeared lethargic with sluggish response to the stimuli. Group C (Minocycline with Thyroxine treated) animals appeared healthy, active and responded quickly to the stimuli in comparison to group B (Table 1).

Table 1: Effect on Gross toxicities in animals of different groups.

Duration	Animal group	Weight variation (gm)	Loss of hair	Diarrhea	Aggressive behavior	Activity	Hematuria
1st week	Control	Nil	NIL	NIL	NIL	NIL	NIL
	minocycline	Nil	NIL	NIL	NIL	NIL	NIL
	Thyroxine plus minocycline	Nil	NIL	NIL	NIL	NIL	NIL
2nd week	Control	-15	NIL	NIL	NIL	NIL	NIL
	minocycline	-44	NIL	NIL	NIL	NIL	NIL
	Thyroxine plus minocycline	-22	NIL	NIL	NIL	NIL	NIL
3rd week	Control	-21	NIL	NIL	NIL	NIL	NIL
	Minocycline	-77	NIL	NIL	NIL	NIL	NIL
	Thyroxine plus minocycline	-17	NIL	NIL	NIL	NIL	NIL
4th week	Control	-24	NIL	NIL	NIL	Active	N IL
	Minocycline	-75	NIL	NIL	NIL	lethargic	NIL
	Thyroxine plus minocycline	-27	NIL	NIL	NIL	Active	NIL
5th week	Control	-27	NIL	NIL	NIL	Active	NIL
	Minocycline	-80	NIL	NIL	NIL	lethargic	NIL

 doi.org/10.36283/PJMD9-2/003

6th week	Control	-27	NIL	NIL	NIL	Active	NIL
	Minocycline	-81	NIL	NIL	NIL	lethargic	NIL
	Thyroxine plus minocycline	-26	NIL	NIL	NIL	Active	NIL
7th week	Control	-29	NIL	NIL	NIL	Active	NIL
	Minocycline	-81	NIL	NIL	NIL	Lethargic	NIL
	Thyroxine plus minocycline	-27	NIL	NIL	NIL	Active	NIL
8th week	Control	-29	NIL	NIL	NIL	Active	NIL
	Minocycline	-83	NIL	NIL	NIL	Lethargic	NIL
	Thyroxine plus minocycline	-27	NIL	NIL	NIL	Active	NIL

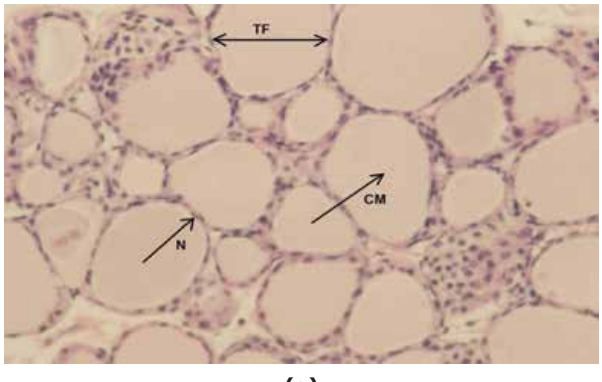
The absolute weight of thyroid gland was significantly increased ($p<0.001$) in minocycline treated group B animals compared to the control animals, whereas substantial decrease ($p<0.01$) in absolute weight of thyroid gland was witnessed in group C in comparison to group B animals (Table 2).

Table 2: Essential parameters vs. thyroid gland (g) and thyroid follicle (µm).

Parameter	Group-A	Group-B	Group-C
Treatment Given	Control	Minocycline	Minocycline+Thyroxine
Mean absolute weight of thyroid gland	63	118	94.7
Mean area of thyroid follicle	136.2	98.3	132.4
Mean height of thyroid follicle	1.06	6.98	1.05

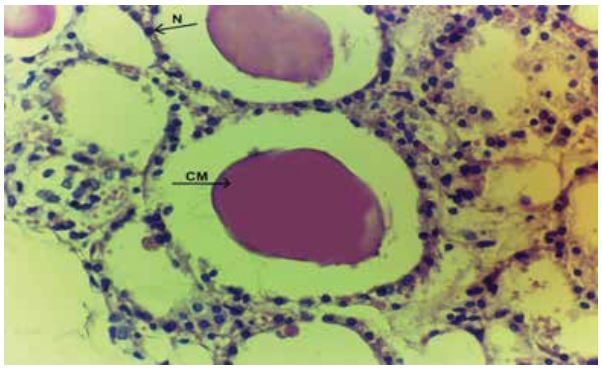
There was a highly noteworthy decrease ($p<0.001$) in mean area of thyroid follicles in Minocycline-treated group B animals, with a highly noteworthy increase ($p<0.001$) in height of follicle in group B animals in comparison to control. In case of Minocycline with Thyroxine treated animals (group C), there was noteworthy increase ($p<0.01$) in area of thyroid follicles and noteworthy decrease ($p<0.01$) in follicle height when compared with group B animals (Table 2).

The microscopic examination of H and E stained sections of thyroid gland of group A animals showed normal architecture. The thyroid follicles were roughly spherical, with simple squamous to cuboidal epithelium. Gel-like colloid was present in their lumina. The apical surface of these cells was in contact with the colloid whereas the basal surface of these cells was lying on the basal lamina (Figure 1a).



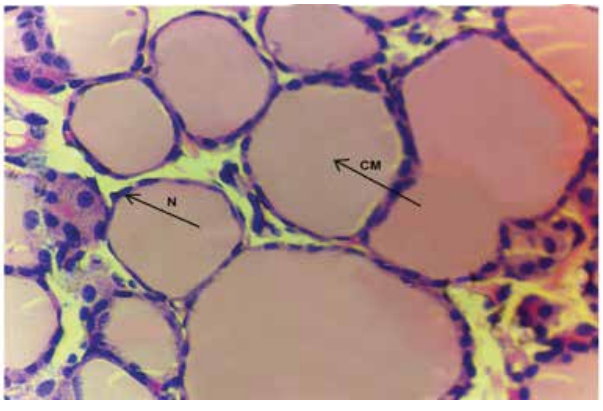
(a)

Figure 1(a): Haematoxylin and eosin stained, 4 µm thick section of thyroid gland from group A (control) guinea pig showing normal architecture of thyroid follicles (TF) with squamous to cuboidal epithelium and its nucleus (N). Thyroid follicles are filled with colloid material (CM). (Photomicrograph X400).



(b)

Figure 1(b): Hand E stained, 4-µm thick section of thyroid gland from minocycline treated group B guinea pig showing thyroid follicles with columnar epithelial cells, shrinkage of colloid material (CM) and rounded shaped nuclei (N). (PhotomicrographX400).



(c)

Figure 1(c): H and E stained 4-µm thick section of thyroid gland from minocycline with thyroxine treated group C guinea pig showing thyroid follicles with squamous to cuboidal epithelium with round to flattened nuclei (N) and follicles filled with colloid material (CM). (Photomicrograph X400).

The tissue sections from Minocycline-treated group B animals showed that most of the thyroid follicles had little or no colloid. The follicles had undergone shrinkage, and their lining epithelial cells were tall cuboidal or columnar in shape. They were also more basophilic as compared to the control (Figure 1b). The histological examination of Haematoxylin and Eosin stained thyroid tissue sections of group C animals, which were treated with Minocycline and Thyroxine, revealed that the architecture of thyroid follicles in group C animals was also normal as found in control group A animals (Figure 1c). Table 3 demonstrates the effect of various treatments on hemoglobin, RBC, WBC, and platelet count after 8 weeks of uninterrupted administration to animals. It can be seen clearly that there was insignificant effect of all the treatments on various blood parameters in comparison to the control group after 8 weeks of continuous administration.

Table 3: Effect of treatments on blood parameters after 8 weeks.

Group/Dosage	Hemoglobin (mg/dl)	RBC (x 10^6/ul)	WBC (x 10^3/ul)	Platelets (x 10^3/ul)
Control	10.38 ±1.11	3.53 ±0.37	3.82 ±0.12	4.21 ±0.17
Minocycline 0.02 mg/gram/day	10.70 ±0.83	3.74 ±0.31	3.89 ±0.66	4.23 ±0.12
Min ocycline plus thyroxine 0.02 mg/gram/day +0.5 µg/gram/day	10.93 ±0.88	3.10 ±0.01	3.77 ±0.31	4.16 ±0.08

n=10, Figures are stated as mean ± standard error of mean

 doi.org/10.36283/PJMD9-2/003

DISCUSSION

In this study, it was found that animals received Minocycline (group B) showed highly noteworthy increase in absolute weight of thyroid gland compared to the animals of control group A. This was possibly due to the hypertrophy of the follicular cells with deposition of pigment in them and within the colloid, which induced hypothyroidism. Similar effects were reported by another study[10]. In addition, the animals were ill looking and lethargic when compared to group-A control animals.

The animals of group C appeared active and healthy similar to control group. The absolute weight of thyroid gland in group C animals which received minocycline plus thyroxine treatment was reduced as compared to group B animals. This may be because thyroxine decreases cellular hypertrophy and inflammatory changes induced by minocycline leading to amelioration of minocycline induced hypothyroidism and its related changes[10].

Microscopic examination of thyroid gland in group B animals revealed that most of the thyroid follicles had no colloid and the follicles which contained colloid had undergone shrinkage. The epithelial cells seen were cuboidal or columnar and darkly stained. Minocycline has been shown to have anti-thyroid effect because of its strong inhibitory effect on iodide coupling, which is responsible for causing hypothyroidism[14]. These findings are in agreement with the findings of another study[15] which concluded that hypothyroidism leads to the disruption of thyroid follicular structure, the follicular cells become columnar and colloid diminishes markedly. The findings of this study that most of the follicles were devoid of colloid were similar to the findings observed by Tajima et al[16]. According to Pantanowitz, minocycline is stored in the colloid as well as follicular epithelial cells, due to oxidative interaction between the drug and the enzyme thyroid peroxidase in the thyroid tissue[10].

Microscopic examination of thyroid gland of group C animals, which received minocycline plus thyroxine, showed that most of the follicles were filled with colloid. Few follicles were empty or had scanty colloid. The follicular epithelial cells were cuboidal in shape. This could be explained by the fact that thyroxine attenuated minocycline-induced hypothyroidism which caused increase in the height of follicular cells and inhibited thyroglobulin synthesis[17].

In this study, the area of follicles in group B animals showed a highly noteworthy decrease in diameter as equated to the animals of control group A, which was in accordance with another study[18] which described presence of smaller thyroid follicles in minocycline-treated rats. Moreover, there was highly significant increase in height of follicular cells in group B animals as compared to the control animals. It was most likely due to the increased activity of the gland. This finding was similar to the study by Inuwa and William[15] that revealed significant increase in the height of follicular epithelial cells in hypothyroid rats. Bowles[18] also stated that minocycline directly affected thyroid gland in animals resulting in inhibition of thyroid hormone synthesis with subsequent increase in follicular cell height and goiter formation.

It was found that in animals of group C which received minocycline plus thyroxine treatment, there was noteworthy increase in the area of follicles as well as the height of follicular cells when compared to mean values in group B animals. This might be due to the exogenous administration of thyroxine, which leads to the lowering of plasma thyroid stimulating hormone level near to control group A[19]. This finding was also in agreement with the findings of Joffe et al. who showed that thyroxine administration resulted in reversing the height of follicular cells near to their normal height with restoration of colloid in the lumen of follicles in hypothyroidrats[20].

Thyroid gland is predisposed to interactions with extensive types of medicines and natural substances. These substances upset every characteristic of thyroid physiology and hormone pharmacology. It is therefore essential to identify these interactions in order to evade therapeutic failures, needless treatments or incorrect diagnosis. These interactions can cause different forms of thyroid abnormalities[21,22]. The antibiotic Minocycline is primarily used to treat acne vulgaris. Benitz et al. labeled minocycline induced black thyroid in 1967. Over the following decades, more than 125 cases were described in the literature. Even though the black pigmentation of the thyroid has been considered characteristic of chronic minocycline intake, other tetracyclines such as doxycycline may induce it in just 12-days. This pigmentation may also affect other tissues such as skin, sclera, bone, teeth, gingiva and nails. The insoluble black pigment occurs due to minocycline oxidation by thyroid peroxidase. Minocycline may also inhibit thyroid function and may have goitrogenic effects, which might be due to blocking of the peroxidase activity on the coupling reaction[23-25]. Thyroid cancer has also been reported by various Minocycline products. When Minocycline therapy is given over extended periods, signs of thyroid cancer should be observed.[26] Other than the antibiotics, minerals can also damage thyroid gland such as cadmium and mercury. Vitamin C has been found to be fundamental antioxidant and enzyme co-factor against metal toxicity[27].

CONCLUSION

Thyroxine ameliorates the damaging effects of Minocycline on thyroid gland. It is thus recommended that the concomitant use of Thyroxine with long-term Minocycline treatment should be suggested. This study can be extended to human subjects to evaluate the restorative effect of Thyroxine on patients receiving long-term Minocycline treatment.

ACKNOWLEDGEMENTS

We specifically thank staff and faculty of Anatomy department of Basic Medical Sciences Institute,

Jinnah Post Graduate Medical Center for their assistance and facilitation in experimental work.

CONFLICT OF INTEREST

There was no conflict of interest among the authors.

ETHICS APPROVAL

The study approval was sort from the Basic Medical Sciences Institute, Jinnah Post Graduate Medical Center (Ref No. F.1-2/2019/BMSI-E.Comt/NG/JP-MC).

AUTHORS' CONTRIBUTIONS

NG Conceived the idea, taken part in the synopsis and approval making, did the experimental work, literature survey, wrote-up of manuscript and the statistical analysis. AQ supervised the whole project, helped in data interpretation and critical analysis of the manuscript. AS helped in the data entry procedure as well as bibliography. TA critically analyzed the manuscript, checked the references and the article correspondence. SS helped in the literature survey, critically analysis of the manuscript and references checking.

REFERENCES

1. Roberts GW. Taking care of thyroxine clinical pharmacist. Aust Prescr. 2004; 27: 75-6.
2. Reid RE, Kim EM, Page D, Mara SMO, Hare EO. Thyroxine replacement in an animal model of congenital hypothyroidism. Physiol Behav. 2007; 91:299-303.
3. Darbra S, Balada F, Assumpcio M, Carbonell M, Garau A. Perinatal hypothyroidism effects on step-through passive avoidance task in rats. Physiol Behav. 2004; 82:497-501.
4. Saicic ZS, Mijalkovic DN, Nikolic AL, Blagojevic DP, Spasic MB. Effects of thyroxine on antioxidant defense system in the Liver of Rats of different age. Physiol Res. 2006; 55:561-8.
5. Fagan SC, Cronic LE, Hess DC. Minocycline development for acute Ischemic Stroke. Transl Stroke Res. 2011; 2:202-8.
6. Mannargudi B, McNally D, Reynolds W, Uetrecht J. Bioactivation of minocycline to reactive intermediates by myeloperoxidase, horseradish peroxidase, and hepatic microsomes: implications for minocycline-induced lupus and hepatitis. Drug Metab Dispos. 2009; 37 (9):1806-18.
7. Kwang HC, Park J, Wonkyung C, GeonGu D, Jeong K, Hwang SJ. Design of PH- independent extended release matrix tablets of minocycline hydrochloride for treatment of Dementia. Arch Pharmacal Res. 2009; 32(11):1593-8.
8. Shigematsu T, Matsumoto F, Imai T, Sakurai I, Shiba K, Kawaguchi Y, et al. Suppressive effects of minocycline on the pituitary-thyroid axis in humans. J Infect Chemother. 1995;1(2):116-21.
9. Taurog A, Dorris ML, Doerge DR. Minocycline and thyroid: Antithyroid effects of the drugs, and the role of thyroid peroxidase in minocycline induced Black pigmentation of the gland. Thyroid. 1996; 6(3):211-9.
10. Attwood HD, Dennett X. A black thyroid and minocycline treatment. Br Med J. 1976;2:1109-1110.
11. Silveira MG, Torok NJ, Gossard AA. Minocycline in the treatment of patients with primary sclerosing cholangitis. Am J Gastroenterol. 2009; 104:83-9.
12. Garg SK. Effect of oral administration of l-thyroxine (T 4) on growth performance, digestibility, and nutrient retention in Channa punctatus (Bloch) and Heteropneustes fossilis (Bloch). Fish Physiol Biochem. 2007;33(4):347-58.
13. Bancroft JD, Cook HC, Stirling RW. Manual of histological techniques and their diagnostic application. Churchill Livingstone. 1994; 27:43-44,136-8.
14. Bruins NA, Oswald JE, Morreau H, Kievit J, Pavel S, Smelt AH. Papillary thyroid carcinoma in a patient with sarcoidosis treated with minocycline. Neth J Med. 2007;65(5):185-7.
15. Inuwa I, Williams MA. Morphometric study on the uterine horn and thyroid glandin hypothyroid, and thyroxine treated hypothyroid rats. J Anat. 1996; 188:383-93.
16. Tajima K, Miyagawa JI, Nakajima H, Shimizu M, Katayama S, Mashita K. Morphological and biochemical studies on minocycline induced black thyroid in rats. Toxicol Appl Pharmacol. 1985; 81:393-400.
17. Davoren P. Modern management of thyroid replacement therapy. Aust Prescr. 2008;31(6).
18. Bowles, WH. Protection against minocycline pigment formation by ascorbic acid (vitamin C). J Esthet Dent. 1998; 10(4):182-6.
19. Rajab NM, Ukropina M, Cakic-Milosevic M. Histological and ultrastructural alterations of rat thyroid gland after short-term treatment with high doses of thyroid hormones. Saudi J Biol Sci. 2015;27 (6).
20. Joffe RT, Brimacombe M, Levitt AJ, Stagnaro-Green A. Treatment of clinical hypothyroidism with thyroxine and triiodothyronine. Psychosom. 2007; 48:379-84.
21. Rizzo LF, Mana DL, Serra HA. Drug-induced hypothyroidism. Medicina (Buenos Aires). 2017;77(5).
22. Rizzo L, Serra HA, Niepomniszcze H. Alterations of thyroid function induced by drugs. Rev Arg Med. 2000; 2: 67-78.
23. Rizzo L. Drug Induced Hypothyroidism. En: Niepomniszcze H, Novelli JL (eds) Hipotiroidismo. UNR Editora: Rosario, 2009, p. 215-23.
24. Miller BT, Lewis C, Bentz BG. Black thyroid resulting fromshort-term doxycycline use: case report, review of the literature, and discussion of implications. Head Neck. 2006; 28: 373-7.
25. Taurog A, Dorris ML, Doerge DR. Minocycline and the Thyroid: Antithyroid effects of the drug, and the role of thyroid peroxidase in minocycline-induced black pigmentation of the gland. Thyroid. 1996; 6: 211-9.
26. Minocycline-FDA Prescribing information, side effects and Use, https//www.drug.com.2019.
27. Khan R, Ali S, Mumtaz S, Andleeb S, Ulhaq M, Tahir HM, et al. Toxicological effects of toxic metals (cadmium and mercury) on blood and the thyroid gland and pharmacological intervention by vitamin C in rabbits. Environ Sci Pollut Res Int. 26(16):16727-16741.