Study of Serum FSH, LH and Prolactin in Female Albino Rats by Experimentally Creating Hypothyroidism

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Abstract

Objective: The current research was draft to contrast the variation in levels of Prolactin (PRL), Luteinizing Hormone (LH) and Follicle stimulating hormone (FSH) in female rodents by experimentally producing hypothyroidism.

Methods: The Animal House of Dow University of Health science was used for the purpose of doing this research which was almost completed in a period of 10 months. The total number of 30 rodents of Wistar Albino species were taken to use in this research. Only adult healthy female rodents weighing between 180-200 gm were included in the study and animals which were found to be slow and lethargic were excluded from the study. These rats were separated into two parts by dividing them into two groups of fifteen animals each. The primary group received the regular normal diet and routine tap water for six weeks and was labeled as the control group whereas the other group was forced to accept Carbimazole (Antithyroid drug) through feeding tube by mixing 0.02% / ml of regular drinking water for a period of six weeks. After six weeks all the rats were slaughtered. The cardiac puncture of animal was done in order to collect blood. Analysis of Follicle Stimulating Hormone, Luteinizing Hormone and Prolactin was carried out by using the ELISA kits on serum of animal which was centrifuged and separated.

Results: The result observed for control group showed a mean value ± standard deviation(SD) of 5.13 ± 1.39 mIU/ml for Follicle Stimulating Hormone. In the same group the mean value ± SD observed for Luteinizing Hormone was 8.38 ± 16.02 mIU/ml and for PRL was 0.40 ± 0.05 ng/ml respectively. The second group that is the hypothyroid group revealed a mean value ± (SD) of 5.89 ± 1.63 mIU/ml for Follicle Stimulating Hormone. For Luteinizing Hormones in hypothyroid group, the mean value ± SD turned out to be 9.30 ± 13.34 mIU/ml whereas the mean value ± SD observed for Prolactin in hypothyroid group was 0.62 ± 0.12 ng/ml. The information was entered on SPSS version 16. This information was analysed by applying an independent sample t-test in between the two groups to compare the levels of Follicle Stimulating Hormone, Luteinizing Hormone and PRL. The p-value was observed which turned out to be insignificant for Follicle Stimulating Hormone and Luteinizing Hormone but quite remarkable for Prolactin.

Conclusion: Hypothyroidism created by an antithyroid drug (Carbimazole) leads to a remarkable rise in the levels of PRL regardless of the changes in levels of gonadotropins which may lead to the sterility in females.

Keywords: Hypothyroidism, Antithyroid agents, Prolactin (PRL), Gonadotropins

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Introduction

One of the butterflies shaped endocrine gland is beneficial for producing and storing thyroid hormones namely thyroxine (T4) and tri-iodothyronine (T3). This gland is termed as thyroid gland which lies in the neck and produce hormones that play an essential part in reproduction. The disproportion in secretion of hormones is one amongst its commonest manifestations[1]. This imbalance may occur anytime in life. The most common cause of thyroid dysfunction in the female procreative age group is considered to be the autoimmunity of thyroid[2]. Various degree of gonadal dysfunction is seen in both males and females as significant relationship is

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found between the reproductive abnormalities in both the hypothyroidism and hyperthyroidism[3]. Hypothyroidism is outlined as a condition that truly ends up in reduce amount of secretion by thyroid gland resulting in less levels of thyroid hormones which may be because of either hypothalamic disorders or pituitary conditions[4]. In humans, the most well-known dysfunction and the most common disorder causing reproductive abnormalities is hypothyroidism. It may occur with a wide range of clinical manifestation that is from multisystem failure to asymptomatic individuals. In different animals even the commonest cause found in the impairment of reproductive functions is hypothyroidism[5]. The process behind this procreative dysfunction continues to be still unidentified[6]. Infertility and early menopause are found to be associated with poor functional ovarian reserve which results in the early onset of ovarian aging. The functional mechanism of this loss is not clearly understood but environmental and genetic factors found to play key role in disturbing the ovarian reserve. These factors influence the morphological structure of ovaries and also the hormones required for maturation in reproductive age group. Earlier studies have revealed the effect of different concentration of thyroid hormones on ovarian function. It was found that the exposure to thyroid hormone helps in the growth of pre-antral follicles in ovary whereas the menstrual irregularities are one of the commonest findings in hypothyroid women[7]. It may lead to impairment in ovulation due to inadequate stimulation of thyroid gland and may be one of the causes of infertility in females.In addition, if there is intra organ conversion of more biologically active form of T4 to T3, it leads to the regulation of negative feedback mechanism which in return causes the inhibition in release of TSH and TRH secretion[7].

Lack of thyroid hormones produces biological disorders in females which increases the chances of sterility during their reproductive age. Hypothalamic pituitary gonadal and thyroid axis were found to be interrelated as the concentration of thyroid hormones influence the development of gonads[8]. Moreover, the concentration of T3 and T4 are also regulated by a feedback mechanism in vertebrates as pituitary gland is concerned with Thyroid stimulating hormone (TSH) secretion and even the hormone releasing thyrotropin (TRH)[9]. Different pituitary hormones like growth hormone, TSH or PRL work in co-ordination with FSH and LH and helps in the growth of the follicles[10]. Lack in secretion of thyroid hormones may lead to higher secretion of prolactin which would also impede ovulation due to insufficient gonadotropin releasing hormone (GnRH) secretion so the levels of FSH and LH are also altered in hypothyroid females. Therefore, the aim of the present study was to check the levels of gonadotrophic hormone, luteinizing hormone and PRL in artificially elicited hypothyroid female rodents.

Subject and Methods

The commencement of research was done after approval was received from different committees like Local University Research Committee, Institutional Review Board (IRB), Funding Committee Board of Advance Studies and Research (B.A.S.R) of DUHS. The research took about 10 months and was conducted at the animal house of Dow University of health sciences. It was an experimental study which started with the observation of rodents, ten days before the beginning of the experiment. Animals weighing between 180-200g were included in the study and the rest were excluded. The animals were housed in an experimental room for continuous monitoring of their health and fitness. Assurance was ascertained also for every rodent to be of albino species and an adult female of Wistar breed. They were tracked for 10 days at the start to rule out any pathology. Any signs in the change of behaviour of animal like sluggishness or idleness are carefully noticed and also a steady decrease in weight was deemed unfit for the experiment and was substituted. The animals were provided with regular normal diet and ordinary water for drinking. A safe parameter has been taken to be the constant weight gain of an animal.

There were 30 animals picked altogether for the project and were separated into 2 categories.

These animals of different groups were kept in two separate cages. The groups were labelled as control and hypothyroid group respectively. Each of the respective group consists of 15 albino rats. All the animals were kept on a standard healthy diet and regular ordinary water throughout the experiment. The diet was measured and administered regularly. The leftover diet was also carefully measured the next day to have an idea about the feeding habits of the animals during the experiment. The animals were ascertained rigorously and were well taken care of throughout the experiment.

A mixture of antithyroid drug Neomercazole was prepared which was given in 0.02% per ml through mouth for 6 weeks.

One of the batches was only kept on ordinary water and regulardiet for the whole experiment which lasted about 6 weeks. This batch was labelled as the control group. The health and the fitness of the animals was carefully monitored throughout an experiment however at the end of 5th week, one of the sample from control group got expired which then was not included in the study. On the other hand, the other group taken as hypothyroid group was given Neomercazole daily through oral feeding tube at a dose of 0.02% in the gut of experimental rats for 6 weeks. Complete ingestion of the prescribed dose by animal is ascertained with no wastage[11]. The animals were weighed regularly throughout an experiment, first at the onset of experiment then on weekly basis until the end of 6 weeks. After the completion of six weeks, all the animals of two groups were slaughtered. The animals were operated under general anaesthesia. At first an incision in midline was given and the sternum of animal was dissected in order to expose the heart with the aid of a scissor. Then a syringe was inserted in the heart of an animal for drawing of blood. Almost 3cc blood was withdrawn through the cardiac puncture and was kept in yellow gel tubes. Centrifugation of blood for 10 to 15 minutes was carried out by centrifugation system for the separation of serum from blood. The serum was finally moved into the aliquots and was tagged for identification. Once the samples were prepared these were immediately taken to Dow Diagnostic Research and Reference Laboratory / DUHS and were kept in cold storage. ELISA kits were used on these samples for hormonal essays of FSH, LH and PRL levels.

The data of hormonal essays was collected after performing the test on the samples and then the information was entered on the worksheet of SPSS.16. To compare the means of groups, Independent sample T-Test was applied and the groups were compared. $P < 0.05$ was taken significant

Results

In control group the mean ± Standard Deviation (SD) recorded for FSH was 5.13 ± 1.39 mIU/ml and LH was 8.38 ± 16.02 mIU/ml as shown in Table 1. When the mean ± SD of FSH was calculated in hypothyroid group, the result depicted was 5.89 ± 1.63 mIU/ml whereas for LH in the same group showed a value of 9.30 ± 13.34 mIU/ml as shown in Table 1. respectively.

The result observed for mean ± SD for PRL in control group was 0.40 ± 0.05 ng/ml whereas in hypothyroid group, it depicted a value of 0.62 ± 0.12 ng/ml shown in Table 1. On comparing the results the p value was analysed for PRL, FSH and LH in between the two groups. The p value of serum FSH observed to be insignificant (p-value + 0.40). For LH the P =0.27 which was also concluded insignificant however the PRL statistical analysis revealed a significant finding with $p < 0.05$ as shown in Table 2.

Discussion

Numerous explorations have additionally exhibit the impact of glandular disease on endocrine functions. The combined effect of thyroid and gonadal axis is of prime importance in standard breeding. Literature indicates that decline in functioning of thyroid leads to decrease gonadotropins however increase in PRL may be the probable cause of cycles without ovulation[12]. Rise in PRL can cause disruption to follicle maturation and corpus luteum

function that may affect the adequate secretion of GnRH in hypothalamus[13]. The levels of FSH and LH are also altered which is due to lack of proper production of ovarian response[14]. Disruption in thyroid hormones can cause ovulation disorders and infertility[15]. In gonadal axis serum PRL is one among the foremost pituitary hormones having an essential role in causing infertility. Hyperprolactinemia is one amongst the most important explanation of anovulatory cycles that results in procreative disorders.

Table 1. Levels of Serum Prolactin, LH & FSH in Control and Hypothyroid group

Groups	No of animals n = 29	Serum FSH (mIU/ml)	Serum LH (mIU/ml)	Serum Prolactin (ng/ml)
		Mean ± S.D	Mean ± S.D	Mean ± S.D
Control	14	5.13 ± 1.39	8.38 ± 16.02	0.40 ± 0.05
Hypo-thyroid	15	5.89 ±1.63	9.30 ± 13.34	0.62 ± 0.12

S.D is the Standard Deviation

Table 2. Statistical Analysis of Serum Prolactin, LH & FSH in Between Groups showing the p-value

Comparison	Serum FSH P value	Serum LH P value	Serum Prolactin P value
Control with hypothyroid	0.40	0.38	0.00*

*P value < 0.05 is significant

Different studies have provided the evidences of association between hypothalamic pituitary thyroid and ovarian axis. This relationship is because of the existence of the unique receptors for thyroid hormone in ovary which may affect the oestrogen and reproductive functions showing the close relationship between two endocrine axis. Most of the ovulatory disorders are due to the dysfunction in HPO axis[16]. The study done by Krassas G.E. is strongly suggestive of long-term hypothyroidism as the cause of hyperprolactinemia which may strongly disrupt the functions of ovary. These functions can derange from disruption in secretion of progesterone from corpus luteum to oligomenorhea when the prolactin is mildly elevated and ammenorhea in case of high levels of PRL in circulation[17]. This study is supported by Goswami. B et al indicating an important link between a rise in PRL and cycles with no ovulation as they had discovered an irregularity in menstruation and anovulation in subjects with raised PRL[18].

In the current study different hormonal assays were performed on rat blood and a remarkable rise in PRL was obtained in hypothyroid group which is in agreement with the results obtained by Happon who also observe a rise in serum PRL with insignificant variations in gonadotropins[19]. In rats, the rise of PRL results in reduce response of pituitary to GnRH thus impedes the secretion of LH. Sun J et al also didn't find any effect on distribution of GnRH12 which is in accordance with the study done by us.

Tohei A et al. is additionally affirmative of the rise in serum PRL level in glandular disease particularly hypothyroidism. Furthermore, he mentioned the connection of oestrogen which is a steroid hormone and PRL to explain the conflicts in his studies concerning rise in PRL in hypothyroidism. In his study he additionally cited the details of hypothyroidism which revealed a rise in the levels of vasoactive intestinal peptides (VIP). This VIP behaves like a liberating element for releasing PRL in anterior hypophysis gland[20]. Dopamine is also detected to possess an effect on inhibiting hypothalamus and is therefore playing its role in controlling the secretion of PRL. Besides this restrictive dopamine playing its key role on secretion of PRL, it is additionally controlled by thyrotropin releasing hormone (TRH), vasoactive peptides (VIP), and other neuropeptides. These all-contributing elements facilitate the release of PRL releasing factor which helps in the secretion of PRL[21-22].

In the current study analysis of secretion level of hormones FSH and LH revealed no important difference in between the control and hypothyroid group. This result is in mutual concurrence with the research done by Armada Dias et al, whose work also exhibited no significant change in investigation of hormones. In his study he simply discovered a rise in PRL with no major distinction in gonadotro-

pic hormones and gonadotropin. These secretion changes conjointly as a consequence leads to unbalance of secretion in hypothyroidism that may cause infertility. Ultimately not each one of the hormones are essentially unstable, however just some variation in PRL is additionally enough to produce problems in getting pregnant[23]. The insignificant findings of the present study may also encourage us to think that probably the FSH and LH are biologically inactive which is in accordance to a study done previously[24].

This ends up in an interpretation that despite of average gonadotropins, the follicles of ovaries were not fully grown in hypothyroid group because of the probability of immediate consequences of thyroid hormones on the development of follicles in ovaries without influencing remarkably the yield by ovaries of sexual steroid hormones. Thus, in order to draw a final conclusion, it is highly recommended that the other contributing factors like oestrogen, progesterone and the VIP should be analysed to further elaborate the inconsistency in relation to the secretion of gonadotropin hormones FSH and LH which was the limitation of this study and has to be explored further.

Conclusion

Hypothyroidism created by an antithyroid drug (Carbimazole) leads to a remarkable rise in the levels of PRL regardless of the changes in levels of gonadotropins which may lead to the sterility in females

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