

Effect of Kisspeptin on Serum Prolactin and Gonadotropins Levels in Balb-c Mice with Induced Chronic Stress

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ABSTRACT

Objective: To determine and compare effects of kisspeptin on serum prolactin, luteinizing hormone and follicle stimulating hormone levels in Balb-c mice with and without exposure to chronic restraint stress.

Study Design: An animal experimental study.

Place and Duration of Study: Shifa College of Medicine / Shifa International Hospital Islamabad, in collaboration with National Institute of Health, Islamabad and Centre for Research in Experimental and Applied Medicine Laboratory, Army Medical College, Rawalpindi, from April 2014 to June 2015.

Methodology: Mice divided into three groups, each containing 30 mice. Control group (group A) received intraperitoneal injection of saline, group B was administered with intraperitoneal injection of saline and restrained stress, and group C was administered with both stress and kisspeptin 100 ng daily for four weeks. Restraint stress was applied to groups B and C for three hours per day by immobilising individual mice in wire-mesh restrainers. At the end of four weeks blood sampling was done. Serum luteinizing hormones (LH), serum follicular stimulating hormone (FSH) and serum prolactin (PRL) were analysed by ELISA.

Results: Serum prolactin level increased in group B (stressed) and group C (stressed + kisspeptin treated) as compared to control group; and decreased in group C as compared to group B. Serum LH and FSH in group B was decreased as compared to control, and it was increased in group C as compared to control and group B.

Conclusion: Administration of kisspeptin increases level of gonadotropins and reduces stress-induced hyperprolactinemia, which may improve fertility despite stress in animal.

Key Words: Kisspeptin, Prolactin, Gonadotropins and infertility.

INTRODUCTION

Stress is a condition which threatens homeostatic equilibrium of human body.¹ Stress can be either acute or chronic in nature. Acute stress lasts for few minutes to hours, and chronic stress persists for several hours, days, weeks or months.² The stress responses are necessary for survival and become harmful when these persist for a prolonged period of time. Acute stress is adaptive, and it involves multiple mediators, neurotransmitters, neuropeptides and hormones, while chronic stress is harmful and makes the individual vulnerable to various diseases. When body faces chronic or intense stress, stressors constantly prevent body system to return to the equilibrium.³

Long term stress can induce changes in multiple hormonal systems that can disrupt the stability of physiological systems like reproduction with negative impact on fertility. Stress hormones interact with hormones that influence fertility directly, such as

gonadotropin releasing hormone (GnRH), prolactin (PRL), luteinizing hormones (LH) and follicular stimulating hormone (FSH).⁴ Prolactin, one of stress hormones that is controlled by means of dopamine, is involved in different biological functions like lactation, reproduction, osmoregulation, and immunoregulation.^{5,6} Prolactin negatively modulates secretion of gonadal hormones including luteinizing hormone and follicle-stimulating hormone.⁷

During pregnancy, postpartum, and lactation, high levels of PRL inhibits LH secretion by inhibitory action of PRL on hypothalamic GnRH neurons.⁸ Kisspeptin, a peptide hormone discovered by scientists who belong to Hershey, Pennsylvania, home of the famous "Hershey Chocolate Kiss."^{9,10} Kisspeptin is major regulator of hypothalamic-pituitary-gonadal axis, leading to pubertal onset. GnRH neurons have kisspeptin receptor on them which releases luteinizing hormone and follicles stimulating hormone from GnRH neurons.¹⁰ Hyperprolactinemia and stress in mice reduced GnRH, gonadotropins secretion and diminished kisspeptin expression. Kisspeptin administration restored gonadotropin secretion and ovarian cyclicity suggesting that kisspeptin neurons play a role in hyperprolactinemic anovulation.^{11,12}

Hypothalamic pituitary gonadal axis is affected by acute stress which results in transient suppression of gonadotropin secretion. In stressful conditions, there

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may be alterations of hypothalamic kisspeptin system. So application of different forms of stress have obvious effect on kisspeptin system by changing level of kisspeptin mRNA levels and disturbing pulsatile release of LH in female rats.^{11,13}

This study was designed to analyse the effects of kisspeptin on serum prolactin and gonadotrophic hormones in chronic stress.

METHODOLOGY

This study was approved by Institutional Review Board and Ethical Committee of Shifa College of Medicine and by Research Evaluation Unit of College of Physicians and Surgeons Pakistan. Ninety female adult 6-8 weeks old, BALB-c mice having weight 25 ±5 grams were divided into three equal groups. The mice were kept in a room which was properly ventilated and daily photo period of 12 hours light-dark was ensured along with maintenance of room temperature 23 ±5°C. Mice of the control group were placed in cages made of steel (1 x 1.5 feet). Mice of other groups were also placed in similar cages, but restraint stress was applied to these groups for three consecutive hours by immobilising individual mice in wire-mesh restrainers. Mice of control group A received daily intraperitoneal injections of 100 ng normal saline for four weeks. Group B includes mice exposed to stress in a wire-mesh restrainer for three consecutive hours every day for four weeks.^{14,15} These mice received daily intraperitoneal injections of 100 ng normal saline before stress. All mice of group C were exposed to stress in a mesh wire restrainer for three consecutive hours every day for four weeks. The stress was administered each day at random times to prevent habituation to the procedure.¹⁶ These mice received daily intraperitoneal injections of 100 ng kisspeptin before stress. At the end of four weeks, intracardiac blood sampling was done. Serum was separated, stored at -70°C till further analyses. Serum LH, serum FSH, and serum prolactin were analysed by ELISA. Data was analysed by using SPSS version 17. In first step, we apply Shapiro-Wilk normality test to check the normality of the serum prolactin (ng/ml), serum LH (mIU/ml), and serum FSH (ng/ml).

Median and Interquartile Range was calculated. Statistical significance among control (A), Stress (B) and Stress + Kisspeptin (C) groups were determined by applying Kruskal-Wallis test. Statistical significance between two groups were determined by applying

Table II: Comparison of serum prolactin, serum LH and serum FSH among A, B and C groups by Mann-Whitney test.

Groups	Serum Prolactin (ng/ml)	Serum LH (mIU/ml)	Serum FSH (ng/ml)
Control (A)			
Stress(B)	<0.001	.044	.012
Stress + Kisspeptin (C)	.002	.074	.103
Stress(B)			
Stress + Kisspeptin (C)	.034	<0.001	<0.001

P-value was obtained by Mann-Whitney test at (p <0.05) significant.

Mann-Whitney test. The p-value less than 0.05 was considered significant.

RESULTS

Median serum level of prolactin in control group A was 4.53 (3.22) ng/ml; in group B which was exposed to restrain stress, was 9.99 (5.61) ng/ml; and in group C which was treated with kisspeptin after stress, was 8.29 (4.05) ng/ml, which was statistically significant ($p < 0.001$). Median serum level of prolactin in group B was also raised in comparison with control group, which was statistically significant ($p < 0.001$). While comparing group B with group C, median serum level of prolactin was decreased significantly in group C ($p = 0.034$).

Median serum level of luteinizing hormone in control group A was 22.90 (30.10) mIU/ml, in group B was 13.69 (18.55) mIU/ml, and 30.72 (34.78) mIU/ml, in group C which was statistically significant ($p = 0.001$). Median serum level of LH in group B was significantly ($p = 0.044$) decreased as compared to control group A. Median serum level of LH was found to be increased in group C as compared to control group A ($p = 0.074$) which was not significant. While comparing group B with group C, serum LH level was significantly raised in group C ($p < 0.001$). Median serum level of follicular stimulating hormone in control group A was 83.48 (33.50) ng/ml; in group B, it was found to be 69.34 (46.70) ng/ml; and in group C, it was 95.06 (28.70) ng/ml, which was statistically significant ($p < 0.001$). It was significantly decreased in group B ($p = 0.012$) and increased in group C as compared to control group A ($p = 0.103$); but increase was not significant. In comparison of group B with group C, serum FSH was found to be significantly raised in group C ($p < 0.001$).

DISCUSSION

Results of the current study showed that administration of exogenous kisspeptin in mice induced with chronic

Table I: Comparison of serum prolactin, FSH, and LH in group A, B and C by Kruskal-Wallis test.

Variables	Group A (Control) Median (IQR)	Group B (Stress) Median (IQR)	Group C (Stress + Kisspeptin) Median (IQR)	Kruskal-Wallis Test (Chi-Square)	p-value
Serum Prolactin (ng/ml)	4.53(3.22)	9.99 (5.61)	8.29 (4.05)	23.501	<0.001
Serum LH (mIU/ml)	22.90(30.10)	13.69(18.55)	30.72(34.78)	13.515	0.001
Serum FSH (ng/ml)	83.48 (33.50)	69.34(46.70)	95.06(28.70)	16.484	<0.001

IQR: Interquartile range. P-value was obtained by Kruskal-Wallis test, (p <0.05) was significant.

stress through immobilisation, resulted in an increase in concentration of both serum luteinizing and follicle stimulating hormone. This is supported by a study conducted by Tovar *et al.* which showed that systemic intravenous injection of kisspeptin-10 elicited, significant rise in LH secretion in male rats with metabolic stress like fasting.¹⁷ Calley and Dhillon, conducted a study in which they administered kisspeptin-10 to healthy human male volunteers resulted in significant increase in LH release and a smaller increase in FSH release. Similarly, they took healthy female volunteers and administered them both kisspeptin-10 and kisspeptin-54 which acutely stimulated a gonadotropins secretion, with much more potency for stimulation of LH than FSH.¹⁸ Castellano *et al.* took 30 days old male and female rats and divided in two groups. One group was subjected to metabolic stress by depriving them food for 72 hours with only access to tap water. One nmol of kisspeptin-10 per rat was administered by intracerebroventricular route. Animals injected with vehicle like NaCl served as controls. Results showed that kisspeptin was effective in inducing LH secretion significantly in fasted animals as compared to controls.¹⁹ The results of their study are consistent with the result of this study.

There is considerable evidence in rats and humans that prolactin inhibits LH secretion, and this is likely to be the cause of infertility by elevated prolactin. Present study showed that administration of kisspeptin decreased concentration of prolactin, which was increased due to stress but this decrease in prolactin level was not so significant. Studies regarding correlation between kisspeptin and prolactin have revealed varying results and no such studies have been directed to determine effect of kisspeptin on raised level of serum prolactin. In the study by Sonigo *et al.*, 31 mice were treated with PRL over 28 days and then observed for recovery of cycles. An additional group received PRL and daily intraperitoneal injections of 100 ng. Continuous infusion of prolactin subcutaneously in female mice resulting in hyperprolactinemia along with reduction in circulating level of GnRH, LH, and FSH. Kisspeptin immunoreactivity was also reduced in hypothalamus in hyperprolactinemic mouse model. Administration of intraperitoneal injections of kisspeptin once daily into hyperprolactinemic mice for 20 days increased pituitary LH and FSH and circulating GnRH.²⁰ So the study by Sonigo *et al.* showed that hyperprolactinemia due to exogenous administration of prolactin in mice induces hypogonadotropic anovulation and that peripheral kisspeptin administration restores GnRH, gonadotropins secretion, and ovarian cyclicity. Study results of Sonigo *et al.* are different from present study in a way that hyperprolactinemia in the former study is induced by exogenous administration of prolactin rather than hyperprolactinemia induced by chronic restrain stress, and showed that kisspeptin administration reverses

anovulation by restoring the level of GnRH, LH, and FSH but this study did not show whether kisspeptin administration decreased raised prolactin or not.

CONCLUSION

On the basis of results obtained in this study, it is concluded that chronic stress causes an increase in level of serum prolactin and decrease in levels of serum luteinizing and follicle stimulating hormones. Administration of kisspeptin leads to significant increase in stress-induced decrease in serum luteinizing and follicular stimulating hormone levels and reduces hyperprolactinemia.

Disclosure: This is dissertation-based study.

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