Biochemical Changes in Female Infertility: Highlights on Leptin, Adiponectin, Visfatin, and Resistin

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ABSTRACT

Introduction: Infertility is one of the major health concerns among women of reproductive age group. Food habits and sedentary life style has lead to obesity among young women. With obesity, the incidence of infertility is on the raise in the last decade. Imbalance of sex hormones like FSH, LH, prolactin, estrogen, progesterone and testosterone contributes to the female infertility. Obesity also plays a significant role on reproductive system contributing to female infertility. The adipokines secreted from adipose tissue also to be evaluated to understand its role on infertility. Adipokines, like adiponectin, leptin, resistin and visfatin secreted from adipocytes, influence the reproductive organs via various mechanisms. So it is essential to evaluate the adipokines and sex hormones for better understanding of infertility and hence help in the treatment of infertility.

Aim: To estimate the levels of fasting blood sugar, triglyceride, total cholesterol, HDL, LDL, VLDL, insulin, insulin resistance, FSH, LH, testosterone, prolactin, leptin, adiponectin, visfatin and resistin among infertile women and compare with fertile women of reproductive age group.

Materials and methods: Fifty women with unexplained infertility were included as cases and fifty fertile women in reproductive age were included as controls.

Results: BMI, waist/hip ratio, insulin, insulin resistance was significantly high in cases than controls. FSH, LH, prolactin, testosterone was high in cases compared to controls. Adiponectin was low in cases than controls, whereas leptin, visfatin and resistin was high in cases than controls.

Conclusion: Sex hormones have influence on adipokines indicating role of adipokines in infertility.

Keywords: Adiponectin, FSH, Infertility, Leptin, Obesity, Prolactin, Resistin, Testosterone, Visfatin.

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INTRODUCTION

The incidence of infertility is one of the major emerging health concerns among the women of reproductive age and is always associated with obesity. Infertility is failure to conceive after 12 months of unprotected intercourse. The prevalence of infertility is around 9–10% in India. Obesity and metabolic dearrangements have an effect on functioning of female reproduction system. Central type of obesity in women is associated with polycystic ovarian syndrome (PCOS). PCOS is common cause of infertility and is characterized by a novulatory cycle and hyperandrogenemia. They present with an irregular menstrual cycle, an increased miscarriage, and a low pregnancy rate. Various factors like polycystic ovaries, hyperandrogenemia, dyslipidemia, insulin resistance, hyperinsulinemia, and obesity are highlighted as the causes of infertility.[1] The insulin and insulin-like growth factors have effects on reproductive events like steroidogenesis, folliculogenesis, and ovulation physiology. Insulin resistance plays a key role in PCOS, and hence, insulin-sensitizing drugs are used widely to induce ovulation and improve fertility in women.[2]

FSH promotes maturation of preovulatory follicle by initiating granulose cell aromatase activity and enzymes involved in progesterone biosynthesis. It also induces receptor of LH on granulose cell, thereby inducing ovulation and development of corpus luteum in response to mid-cycle LH surge.[3]

The mid-cycle LH surge triggers ovulation and convert residual follicle to corpus luteum. LH is necessary to maintain luteal function for the first two weeks of pregnancy and further maintained by hCG. Hyperprolactinemia is associated with ovulatory disturbances and luteal phase defects, thus affecting infertility.[4] Increased rate of FSH/LH inversion, PCOS, and septate uterus is associated with elevated testosterone.[5]

White adipose tissue is a multifunctional organ. Apart from energy-storing function, it also serves as an endocrine organ

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secreting adipokines. Adipokines are adiponectin, leptin, resistin, visfatin, ghrelin, omentin and chemerin.[1] Adiponectin is a beneficial adipokine, having insulin-sensitizing, antiatherogenic, and anti-inflammatory action. It increases sensitivity of peripheral tissue to insulin.[6] Leptin plays a role in hypothalamus, pituitary ovary, oocyte and embryo level. Leptin, a novel mediator of appetite, promotes GnRH pulsatility by hypothalamus and hence secretion of FSH and LH by pituitary.[7] Resistin is increased in diet-induced obesity linking obesity and diabetes.[9] An overview of resistin gene was found in adipocytes from PCOS, suggesting that resistin may play a role in local pathogenesis of infertility.[9] Visfatin is positively associated with obesity in healthy women of reproductive age, and PCOS is associated metabolic and hormonal disturbances.[10]

MATERIALS AND METHODS

One hundred women, aged 20–30, were selected for the study. The study was conducted by the Department of Biochemistry with Department of Obstetrics and Gynecology, Dr BR Ambedkar

Medical College, Bengaluru. Fifty women with complaints of infertility were considered for the study. Fifty normal healthy volunteer women were considered as controls.

Inclusion criteria for cases are infertile women according to WHO ICMART.[11] Partners have normal semen analysis. Exclusion criteria for cases include women under hormonal replacement treatment, diabetics, hyperandrogenism-like prolactinoma, galactorrhea, congenital adrenal hyperplasia, Cushing syndrome, and ovarian tumor. Fifty normal healthy volunteer women with regular ovulatory cycles, normal menstrual history, nonlactating, and last pregnancy (with live birth) 2 years back are considered as controls.

Sample Collection

A detailed history was taken. Weight, height, and BMI were assessed. Skinfold thickness was measured using Harpenden calipers. 5 mL of fasting venous blood sample was collected on 3rd day of menstrual cycle from the study group. Serum was separated and analyzed for fasting blood sugar, triglycerides, total cholesterol, and HDL in the BS 300 auto analyser. Serum is stored at −20°C for further analysis. Insulin, FSH, LH, prolactin, testosterone, insulin, leptin, resistin, visfatin, and adiponectin were estimated by ELISA method using Alere Elisa Reader and Washer. VLDL is calculated by formula, TGL/5. LDL is calculated by Friedewald formula T Chol − (HDL + VLDL). Insulin resistance index was calculated by homeostasis model assessment of insulin resistance (HOMA − IR) by fasting insulin mU/L × fasting glucose mmol/L 22.5[12].

Results

Table 1 shows BMI and W/H ratio is high in cases compared with controls, whereas skinfold thickness does not vary between cases and controls. Table 2 shows that though nondiabetics are considered for the study, FBS is high, but not significant in cases compared with controls. Total cholesterol and triglycerides are high in cases than in controls. HDL-Chol is significantly low is cases than in controls. VLDL and LDL did not vary between cases and controls. Table 3 shows FSH is high in cases, but does not vary significantly. LH, prolactin, and testosterone are significantly high in cases than

in controls. Insulin and insulin resistance are high in cases than in controls.

Table 4 shows leptin is mildly increased in cases than in controls. Adiponectin is low in cases and do not show significant changes. Visfatin and resistin is significantly high in cases than in control.

Discussion

Our study showed nonsignificant raise in FSH in cases. FSH helps in oogenesis, follicle development, and gametogenesis. FSH levels in obese PCOS are less than in nonobese PCOS, causing hypogondadism contributing to the cause of infertility. Significant high levels of LH, prolactin, and testosterone are also seen in obese PCOS, which tends to suppress the ovulatory cycle, by inhibiting the secretion of FSH.[13]

Prolactin inhibits the production and secretion of adiponectin by primary adipocytes in lean lactating women.[14] LH:FSH ratio in patients with PCOS showed inverse correlation with adiponectin.[15] Our study also suggest increases in prolactin, LH, and testosterone in cases compared with controls.

The sex hormone binding globulin (SHBG), growth hormone, and insulin-like growth factor binding proteins are decreased in infertility. The hypothalamopituatary-gonadal axis deteriorates, explaining impaired ovulatory function.[16] The hyperinsulinemia leads to reduced SHBG levels, increased production of testosterone levels in theca cells of the ovary, increase LH secretion without affecting FSH levels in obese women.[17] Insulin resistance and resulting hyperinsulinemia contribute to PCOS.[18]

Leptin is a 16 kDa, nonglycosylated polypeptide with 146 amino acid. It is the hormonal product of ob-gene, predominantly expressed in adipose tissue. Higher concentration of leptin is detected in obese than in thin individual inhibiting appetite and reducing food intake. Also, it has effect on reproducing organ influencing fertility. Leptin helps in gonadal function, embryo development, and embryo-endometrial interactions facilitating implantation. Leptin increases the releases of NO from adrenergic interneurons, which then induces GnRH release from GnRH neurons by activating both guanylate cyclase and cyclooxygenase-1.[8]

Table 1: Demographic and anthropometric parameters in cases and controls

Variables	Cases	Controls	Total	p value
Age (years)	25.64 ± 3.58	26.00 ± 4.75	25.82 ± 4.19	0.669
BMI (kg/m^2)	28.79 ± 3.85	26.33 ± 3.72	27.56 ± 3.96	0.002*
W/H Ratio	0.93 ± 0.09	0.88 ± 0.07	0.90 ± 0.08	0.002*
Skin fold	29.68 ± 5.10	28.38 ± 4.14	29.03 ± 4.67	0.165

*Strongly suggestive

Table 2: Levels of FBS and lipid profile in cases and controls

Variables	Cases	Controls	Total	p value
Fasting blood sugar (mg/dL)	89.30 ± 10.71	86.18 ± 9.50	87.74 ± 10.19	0.127
Total cholesterol (mg/dL)	156.62 ± 63.74	136.44 ± 44.47	146.53 ± 55.61	0.069$^+$
TGL (mg/dL)	136.75 ± 11.95	132.12 ± 8.89	34.43 ± 10.74	0.030*
HDL (mg/dL)	27.29 ± 8.91	32.84 ± 15.48	30.06 ± 12.87	0.030*
VLDL (mg/dL)	123.57 ± 52.54	112.92 ± 44.16	118.24 ± 48.59	0.275
LDL (mg/dL)	110.63 ± 43.64	112.92 ± 44.16	111.77 ± 43.70	0.795

$^+$Suggestive significance
*Moderately significant

Table 3: Levels of sex hormones and insulin in cases and controls

	Cases	Controls	Total	p value
FSH mIU/mL	10.51 ± 18.99	8.40 ± 6.45	9.45 ± 14.15	0.458
LH mIU/mL	11.98 ± 7.14	7.04 ± 5.18	9.51 ± 6.69	<0.001**
Prolactin ng/mL	22.86 ± 13.46	17.89 ± 10.95	20.38 ± 12.46	0.046*
Testosterone ng/mL	1.55 ± 3.22	0.67 ± 0.40	1.11 ± 2.32	0.059+
Insulin µIU/mL	19.13 ± 29.49	9.81 ± 14.43	14.47 ± 23.57	0.048*
Insulin resistance	3.10 ± 3.55	1.79 ± 1.80	2.44 ± 2.88	0.022*

**Strongly suggestive
+Suggestive significance
*Moderately significant

Table 4: Levels of adipokines in cases and controls

Variables	Cases	Controls	Total	p value
Leptin ng/mL	10.80 ± 5.31	7.07 ± 12.31	9.24 ± 9.56	0.093+
Adiponectin ng/mL	11.51 ± 6.97	12.89 ± 6.62	12.20 ± 6.80	0.313
Visfatin ng/mL	12.30 ± 10.24	7.84 ± 5.87	10.07 ± 8.60	0.009*
Resistin ng/mL	7.70 ± 4.00	5.95 ± 2.54	6.83 ± 3.45	0.010*

*Strongly suggestive
+Suggestive significance

Leptin is high in obese patients with PCOS, associating with central obesity and not directly related to PCOS.[19] Our study also showed a mild increase in leptin in cases compared with controls. Leptin receptors are present in syncytiotrophoblast at the maternal interface implicating a potential autocrine or paracrine effect of leptin on placental function.[20] Stimulated treated females had elevated levels of LH, increased ovarian, uterine weight, and stimulated aspects of ovarian and uterine histology. High leptin levels may signal the attainment of the successful reproduction.[21] Low leptin levels are detected in hypothalamic amenorrheic women with eating disorder and normal weight, suggesting that hypoleptinemia alone, independent of fat mass, may be linked to hypothalamic amenohorrea.[22] Decreased LH and FSH response to GnRH were associated with increased adiposity and hypoleptinemia.[23] Leptin-deficient mice exhibit low LH and partial development of reproductive organs, on treatment inducing pubertal development and maturity of reproductive organs, increasing LH, and restoring fertility explaining the importance of leptin signaling in female reproduction.[27]

Resistin is cysteine-rich, 94 amino acid polypeptide, and adipokine, a self-explaining hormone (resisting insulin) secreted from scrotal cell and macrophages of adipose tissue. It activates suppressor of cytokine signaling-3, an anti-inflammatory mediator, which suppress insulin signaling in several tissues. Resistin mRNA is also found in hypothalamopituatary axis, rat testis, and bovine ovaries promoting steroidogenesis and granulose cell proliferation.[1] The association of resistin gene polymorphism in PCOS women insulin high BMI suggests that resistin might be related to adiposity. On treatment with insulin sensitizers, serum resistin levels reduces significantly in obese PCOS.[24].

Visfatin is a recent protein, having 52 kDa, also known as pre-B cell colony-enhancing factor. Visfatin is expressed from adipocytes, lymphocytes, bone marrow, muscle trophoblasts, and fetal membranes. Visfatin is present more in visceral fat than in subcutaneous fat and hence named as visfatin.[1,24] Visfatin shares antidiabetic effects of insulin-mimetic properties, mediated by a distinct binding site on the insulin receptor.[25] Visfatin induces

proinflammatory markers like WBC, C-reactive protein, and endothelial dysfunction in PCOS cases independent of obesity and insulin resistance.[9] Our study also showed a significant increase in resistin and visfatin in cases compared with controls.

Adiponectin is a 30-kDa protein secreted by adipocytes, and muscle and liver cells, and expressed during adipogenesis in adipocytes.[28] Three major forms of adiponectin are identified: a trimeric low-molecular-weight form, a hexameric medium-molecular-weight form, and a multimeric high-molecular-weight form. The major functions of adiponectin include increase in insulin sensitivity by stimulating glucose uptake in the liver and muscle, reducing hepatic gluconeogenesis and stimulating fatty acid β-oxidation in the skeletal muscle. Consequently, adiponectin reduces triglyceride accumulation and augments insulin sensitivity.[29] These have the typical seven-transmembrane structure but differ from G protein-coupled receptors in that the C-terminal portion is extracellular whereas the N-terminal is cytoplasmic. The main intracellular signaling pathway is through AMP-activated kinase, but several other pathways are also involved. Adiponectin receptors have been demonstrated in reproductive tissues, including ovary, placenta, and endometrium.[30] Adiponectin induces expression of cluster of proteins associated with process of ovulation, inducing cyclooxygenase-2, prostaglandin E2, and vascular endothelial growth factor. Adiponectin induces ovarian gene expression and steroidogenesis to maintain ovaries. Our study also showed a decrease in adiponectin cases compared with controls. Decreased levels are seen in obesity, which plays an important role in the pathogenesis of PCOS.[6,26]

It appears that leptin and adiponectin act in an autocrine/paracrine fashion in the placenta, playing an important role in the maternal–fetal interface and contributing to glucose metabolism and fetal development. Leptin plays a role in the regulation of menstrual cycles and suggested that obese individuals exhibit both leptin resistance and decreased serum adiponectin levels. A progressive increase in adiposity throughout life seems to influence the relationship between leptin and adiponectin in women.[31]

We conclude that adiponectin is negatively correlated with sex hormones and insulin resistance, whereas leptin, visfatin, and resistin are positively correlated with sex hormones and insulin resistance. All these factors are interrelated with each other and contribute to the cause of infertility. The study demarcates the role of adipokines and its influence on reproductive hormones in female infertility. And in future, adiponectin, resistin, leptin, and visfatin can be taken as adipose tissue function test, and their estimation will help in the treatment of infertility. This will help in reducing the prevalence of infertility.

Acknowledgment

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