Original Article

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Serum Adiponectin Levels in Insulin Resistant Infertile Women

Biochemistry Section

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ABSTRACT

Introduction: Adiponectin, a beneficial adipocytokine plays a crucial role that regulates insulin sensitivity and also has a role in the ovulation process. Visceral adiposity adversely affects the reproductive hormone production and ovulation that impacts fertility in reproductive females. Adiponectin may be considered as the vital link between central obesity and insulin resistance that regulates ovulation to occur.

Aim: To compare the levels of Adiponectin in insulin resistant and non-insulin resistant infertile women with ovulatory dysfunction and to correlate Adiponectin levels with hormones such as Luteinising Hormone (LH), Follicle-Stimulating Hormone (FSH), LH/FSH ratio, Thyroid Stimulating Hormone (TSH), insulin and variables like Waist Circumference (WC) and Waist-Hip Ratio (WHR).

Materials and Methods: The present cross-sectional study was conducted in the Department of Obstetrics and Gynaecology, SRM Medical College and Research centre, Tamil Nadu, India. The study included 88 married women of reproductive age group between 20 to 40 years attending the infertility clinic. All the participants had a complete physical examination with measurements of weight (Kg), height (m), WC (cm) and HC (cm) to calculate the BMI and WHR. After overnight fasting, blood samples were collected on second or third day of menstrual cycle for estimating LH, FSH, TSH, insulin and Adiponectin levels. Based on HOMA-IR calculation the individuals were categorised as Insulin resistant Group (A) n=48 with HOMA-IR ≥2 and non-Insulin resistant Group (B) n=40 with HOMA-IR <2. Serum Adiponectin levels were measured by Human Adiponectin ELISA kit (Biovendor). Statistical analysis was done with Student's t-test and Pearson correlation analysis.

Results: The Adiponectin levels in insulin-resistant women were significantly decreased (3.4±1.3) µg/mL compared to non-IR women (5.2±2.0) µg/mL, p <0.05. A significant negative correlation was observed between Adiponectin with WC, insulin levels and LH/FSH.

Conclusion: The present study clearly highlighted the fact that infertile women with insulin resistance have decreased adiponectin levels that subsequently affects the ovulatory process. Adiponectin is one of the important factors that influence ovulation and implantation of fertilised ovum. Thus, improvement in insulin resistance increases the chance of ovulation in reproductive females.

Keywords: Insulin sensitivity, Luteinising hormone, Waist circumference

INTRODUCTION

In developing countries, infertility remains as women's social stigma. Infertility is defined as not being able to conceive after 1 year of unprotected sexual activity. Various biological factors, social factors and stress play a significant role in lowering the fertility rate [1].

Female infertility occurs due to ovulatory dysfunction, tubal problems, endometriosis and also due to unexplained reasons. The effects of current lifestyle variables had received the new attention which includes body weight, body composition, physical activity and nutrients that when altered affects the reproductive life [2].

The reproductive period is maintained by the reciprocal regulation of energy metabolism and hormone secretion. Adipose tissue is the storage form of energy in the body. It regulates indirectly multiple hormonal signals and plays an important role in modulating insulin action. Adipose tissue is considered as an endocrine gland that secretes bioactive cytokines and adipokines including adiponectin, leptin and resistin.

Adiponectin is a 30kDa protein produced by the mature adipocytes mainly by the visceral adipose tissue. Its plasma concentration is paradoxically lower in obese individuals than non-obese. Adiponectin is considered to influence insulin-sensitising actions [3]. In fact, adiponectin favours insulin-stimulated tyrosine phosphorylation of insulin receptors which when impaired results in insulin resistance. Adipocytes stored with excess fatty acids inhibit Adiponectin gene transcription [4]. Insulin Resistance is a state characterised by an impaired metabolic response to both endogenous and exogenous insulin [5].

Evidence suggested that visceral fat accumulation results in dysregulated expression of the adipokines, resulting in the insulin resistant state [6]. Adiponectin R1 and R2 receptors are expressed largely in the female reproductive tissues such as ovary, endometrium, placenta and oviduct [7]. The Adiponectin modulates central reproductive hormonal axis which was substantiated by its role in inhibiting LH and Gonadotrophins Releasing Hormone (GnRH) release. Studies have demonstrated the important role of Adiponectin signalling in uterine receptivity in infertile females with implantation failure and pregnancy loss [8].

Furthermore, it has been reported that insulin resistant state has been associated with a reduction in adiponectin concentration [9]. Under physiological conditions, the signalling pathways of insulin, leptin and adiponectin direct the sex hormones in ovarian activity and reproduction [10].

Thus, the hypothesises of the present study is to prove that increased insulin levels together with reduced adiponectin levels contributes to impaired ovarian function and study was carried out with an aim to compare the Adiponectin level in insulin and non-insulin resistant infertile women and to correlate with LH, FSH and LH/FSH ratio, TSH and the adiposity indicators WC and WHR.

MATERIALS AND METHODS

This observational cross-sectional study was conducted in 88 infertile women attending the infertility clinic at SRM Medical College and Research centre, Tamil Nadu, India. Institutional Ethics Review Committee reviewed and approved the study protocols (IEC: No: 963/IEC/2016) before the commencement of the study. The

purpose of the study was explained and written informed consent was obtained from all the participants.

Inclusion Criteria

The present observational study included 88 infertile married women in the age group of 20 to 40 years with complaints of irregular menstrual cycles or ovulatory dysfunction (ultrasound finding) [11] or PCOS (according to Rotterdam's criteria) [12]. Out of these 88 women, 69 women presented with primary infertility and 19 with secondary infertility.

Participants were further categorised based on HOMA-IR or elevated fasting insulin levels as insulin resistant group (A) n=48 and Non-insulin resistant group (B) n=40.

Exclusion Criteria

The participants with major systemic illness, uterine or fallopian anatomical defects, tubal lesions, hypertension and women with infertile male partners were excluded from the study. Participants with sexually transmitted diseases were also excluded.

A detailed medical and menstrual history was obtained based on the questionnaire format and preliminary clinical examination as carried out by the gynaecologist. Weight (Kg), height (metres), waist circumference (cm) and hip circumference (cm) were measured. The anthropometrics indices BMI and waist-hip circumference ratio were calculated. The Standard Consensus statement for Indian population was considered as the cut-off for BMI [5].

SAMPLE COLLECTION AND ANALYSIS

By random sampling method, overnight fasting venous samples were collected from the participants on second or third day of menstrual cycle. Fasting plasma glucose was estimated in Beckman Coulter AU 400 (Hexokinase method). Serum TSH, FSH, LH and prolactin were assayed on the day of sample collection in automated Hormone analysers based on the method Chemiluminescence with standard reagent kits. Insulin was assayed in Bio-Rad ELISA using Calbiotech 96 wells ELISA kit. The remaining samples were stored in -20^{0}C deep freezer until further analysis. Serum Adiponectin levels were assayed in Bio-Rad ELISA reader using Biovendor 96 wells ELISA kit (normal reference range: 4-12 µg/mL). The markers of insulin resistance such as elevated fasting insulin levels (> 15µIU/mL) and HOMA-IR was calculated with (Fasting insulin X FPG)/405 [13].

STATISTICAL ANALYSIS

Statistical analysis was performed with SPSS version 17.0. Student's t-test was used to compare the biochemical parameters between the groups. Pearson's correlation coefficient was used to correlate adiponectin with the biochemical parameters. For all the statistical analysis, p-value <0.05 was considered statistically significant.

RESULTS

The data of 88 infertile female participants with a disturbance in ovulation were analysed. The mean age of infertile women was 28±6.48 years. Insulin resistance was assessed in the participants with impaired ovulation based on HOMA-IR calculation; out of 88 participants, 55% were insulin resistant and 45% were non-insulin resistant infertile women. About 34% of women presented as PCOS cases with Insulin resistance and 14% infertile women presented as PCOS cases without insulin resistance. The anthropometric and biochemical parameters compared between the groups [Table/Fig-1]. The mean Adiponectin levels were 3.4±1.3 and 5.2±2.0 µg/mL in insulin resistant and non-insulin resistant groups respectively.

As per [Table/Fig-2], serum Adiponectin was correlated with variables in the insulin-resistant group. Pearson's correlation analysis revealed a statistically significant negative correlation with WC, insulin levels and LH/FSH ratio.

Characteristics	Insulin resistant participants (n=48)	Non-Insulin resistant participants (n=40)	p-value
Age (year)	30.27±5.0	26.36±3.55	<0.001*
Duration of infertility(year)	6.2±3.68	3.06±2.56	<0.001*
WC (cm)	87.85±3.19	84.87±2.8	<0.001*
HC (cm)	101.45±2.79	101.66±2.75	0.72 (NS)
WHR	0.87±0.03	0.83±0.02	<0.001*
TSH mIU/L	4.97±1.781	2.09±2.47	<0.05
LH mIU/mL	12.47±5.05	8.4±5.48	<0.0005**
FSH mIU/mL	4.59±3.51	9.07±6.44	<0.001*
LH/FSH	2.57±0.90	1.12±0.79	<0.005*
Insulin (µIU/mL)	14.7±6.23	9.10±5.68	<0.0001**
Adiponectin (µg/mL)	3.4±1.3	5.2±2.0	<0.001*

[Table/Fig-1]: Anthropometric and Biochemical characteristics of Insulin resistant and non-insulin resistant infertile women.
Values are expressed in Mean±Standard Deviation; p-value <0.05 is considered significant. NS-Not significant **Highly significant *Significant

Adiponectin	Insulin resistant infertile group (correlation coefficient value)	p-value
Waist circumference (cm)	- 0.51	0.017
WHR	- 0.27	0.24
LHmIU/mL	-0.39	0.16
FSHmIU/mL	-0.30	0.23
LH/FSH	-0.51	0.017
TSHmIU/L	- 0.38	0.19
Insulin (µIU/mL)	-0.57	0.007
Correlation	**Negative**	**Positive**
Small	- 0.3 to – 0.1	0.1 to 0.3
Medium	- 0.5 to – 0.3	0.3 to 0.5
Strong	- 1.0 to – 0.5	0.5 to 1.0

[Table/Fig-2]: Correlation of Adiponectin with waist circumference, WHR, insulin, LH, FSH, LH/FSH ratio in Insulin resistant group.

DISCUSSION

Adiponectin, considered as a beneficial adipocytokine is secreted by the adipose tissue and it has an insulin-sensitising effect and anti-inflammatory action.

Adiponectin and Visceral Obesity

Adiponectin is the protein product of *APM 1* gene transcript located at chromosomes 3q27. It is closely located near to the locus responsible for Diabetes mellitus Type 2 and adiposity [14]. Adipocytes with increased storage levels of free fatty acids interfere with Adiponectin gene transcription, thereby; Adiponectin levels are markedly reduced in visceral obesity [4]. In the present study, a negative correlation was observed between adiponectin and WC, which demonstrates that adiponectin levels are reduced in individuals with visceral adiposity.

Insulin and Visceral Obesity

Participants with increased WC had increased insulin levels and were insulin resistant. A similar observation was reported that visceral fat accumulation reflected by WC measurement contributes to the development of insulin resistance [15,16]. The impact of visceral obesity mainly attributes to impaired-neuroendocrine and ovarian function affecting the ovulatory process.

Adiponectin and Insulin Resistance

Adiponectin levels in insulin-resistant individuals had a significant negative correlation with serum insulin levels thereby peripheral insulin sensitivity is compromised. Insulin-sensitising effect of Adiponectin is contributed by its intracellular signalling action that enhances insulin

stimulated tyrosine phosphorylation of insulin receptors [4]. The implication of cross-talk between the two pathways is contributed by adiponectin which causes activation of IRS 1/2 which sensitises the insulin action in insulin-responsive tissues [11].

Adiponectin and Thyroid Status

Insulin resistant infertile group showed mildly increased TSH levels compared to the non-insulin resistant infertile women. The finding is supported by a study that showed decreased Adiponectin levels in hypothyroid individuals. The role of thyroid status influencing adipocytokines with respect to adiponectin levels is still not clear. The fact explained was that thyroid dysfunction alters metabolism of adipose tissue contributing to oxidative stress and insulin-resistant state that can down-regulate adiponectin levels [17,18].

Disturbance in Ovulatory Process

In hyperinsulinemia, there is increased production of androgens that converts to oestrogen which in turn causes increased LH. An excess LH leads to premature differentiation of granulosa cells or early arrest of follicular growth and that ends up with anovulation [19]. The present study, the insulin resistant participants had increased LH levels and increased LH/FSH ratio. Thus visceral adiposity leads to insulin resistant state and also contributes to reduced secretion of adiponectin creating an environment that impairs the ovulatory process.

The present study showed a significant negative correlation between serum adiponectin and insulin, WC, LH/FSH. Studies have reported that decrease in BMI is accompanied by an increase in Adiponectin levels with improvement in insulin resistant state and increased the possibility of improved fertility rate [20,21].

This study evaluated that decreased adiponectin levels in insulin-resistant infertile women contribute to ovarian dysfunction, which is evident by the increased LH levels and altered LH/FSH ratio. Thus the production of adiponectin is impaired in visceral adiposity, it is considered as an important link that leads to impaired insulin peripheral action and thereby ovulatory process is impaired resulting in infertility.

LIMITATION

The limitation of this present study was small sample size.

CONCLUSION

The study concludes that serum Adiponectin concentration was decreased in insulin-resistant infertile women with visceral adiposity that contributes to ovarian dysfunction and results in infertility. Lifestyle changes and weight reduction can improve insulin sensitivity and successfully increases the fertility rate.

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