

ORIGINAL ARTICLE

A Study to Correlate the Homocysteine Level in Follicular Fluid With Oocyte Number and Quality in Iraqi Women Undergo ICSI

Estabraq AR. Al-Wasiti¹, Maysoon Mahmood Hussein², Basheer Anwer Baker³, Enas Jasim Kadim⁴

¹ AL-Nahrain University / , College of Medicine / Department of Clinical Biochemistry, 10011-alkadhymia/ Baghdad /Iraq

² Ministry of Health and environment of Iraq,10011- alharthia / Baghdad, Iraq.

³ Ministry of Health and environment of Iraq, 36001-Street 3rd square building / Kirkuk general hospital/ Iraq

⁴ Kamal Al-Samurai Hospital for Infertility Treatment and IVF, / 10011Baghdad / Iraq

ABSTRACT

Introduction: Hyperhomocysteinemia may affect the reproductive system and it has been linked to poor oocytes quality, congenital abnormalities, miscarriages, hypertension, low birth weight and toxicity of the endothelium, the less follicular fluid (FF) homocysteine the better oocyte quality and better clinical pregnancy. **Methods:** Forty-five women, who were subjected to intracytoplasmic sperm injection (ICSI), were enrolled in this work, their ages ranged between 18-42 years. Blood samples were taken before ovulation induction to measure serum estrogen before ovulation induction. Blood samples and follicular fluid were collected on day 11 of the menstrual cycle (the day of oocytes pickup), the samples were centrifuged and stored at -40C° till the day of measuring. **Results:** Statistically, the result revealed an insignificant difference in Homocysteine level between conceived and non-conceived women ($p > 0.05$). Homocysteine concentration of both follicular fluid and serum is inversely associated with the total oocytes number ($p \leq 0.05$) and those in the Metaphase II ($p \leq 0.05$). Homocysteine level in both Serum and follicular is negatively correlated with S.E2 before ICSI ($p = 0.028$), ($p = 0.022$) respectively. A positive correlation between the concentration of serum and follicular fluid Homocysteine ($p = 0.000$) was noticed. **Conclusion:** Higher concentration of serum and follicular fluid homocysteine negatively affects the growth and maturation of oocytes, which might subsequently result in a lower implantation rate.

Keywords: Homocystein, Follicle development, CSI (intracytoplasmic sperm injection), Oocytes number Metaphase II (MII)

Corresponding Author:

Estabraq AR. Al-Wasiti, Ph.D.

Email: dr.estabraq.alwasiti@colmed-alnahrain.edu.iq

Tel: +9647902386699

INTRODUCTION

Infertility has been defined as the disease of the couple reproductive systems characterized as the couple's failure to conceive after one year of regular non protected sex. This definition has been agreed upon it by the International Committee for Monitoring of Assisted Reproductive Technology and World Health Organization (WHO) (1). According to the later, 50 million couples suffer from infertility worldwide (WHO, 2013).

Female infertility can be labeled as the failure to conceive, preserve a conception, or convey a pregnancy to living

birth (WHO, 2013). Fifty percent of infertility cases are attributed to male factors; indeed 7% of males have health issues that impair their fertility ability (2). These either due to problems in the pituitary hypothalamic axis or due to pathologies in the sperm production or its transport or coitus (3).

Assisted Reproductive Techniques (ARTs) can be pronounced as approaches used to attain pregnancy in infertile couple by totally or partly non-natural ways. Intracytoplasmic Sperm Injection (ICSI) is an advanced ARTs technique in which the zona pellucida and the lemma are bypassed by direct injection of a sperm into ovum (4).

The success of ICSI depend on several factors such as the quality of the oocytes and their number which are collected for the procedure (4). Both depend on the protocol used for ovulation stimulation aiming to attain

advanced fertilization and pregnancy rates irrespective of sperms quality (4).

A differing number of research roughly maintains the spotlight on the oocytes inner setting and paracrine factors that are concealed by the oocytes for controlling the maturation procedure and these factors typically has a protein-like nature and thus might be established in the follicular fluid (FF) (5).

Homocysteine is non-essential amino acid containing sulfur that is formed from metabolism of the essential amino acid methionine which can be reconverted to methionine by re-methylation pathway. Hyperhomocysteinemia might jeopardize the reproductive process at either the preconception stage such as poor egg and sperm qualities or at post conception level due to congenital malformation, loss, maternal hypertension and neonatal little birth weight. Hyperhomocysteinemia delivers its adverse effects via two potential mechanisms. First, Oxidation of Homomcystein propagates toxic free radicals to vascular endothelium; second, it favors pro-thrombotic milieu by impairing the delicate balance between the pro- and anti thrombotic pathways.

It has been shown previously that the homocysteine concentration in follicular fluid improves fertilization and increases the rate of clinical pregnancy (6). Additionally, its level can be utilized as predictive indicator for embryo quality oocyte quality and fertilization rate, in patients undergoing ART (7).

The current research was carried to determine homocysteine levels in the follicular and serum of women who underwent ICSI procedure and to identify the correlation between the concentration of homocysteine and both total oocytes number and those in Metaphase II (MII).

MATERIALS AND METHODS

Subjects

An observational potential research has been directed in 2015-2016 from August first to April third at Baghdad, Iraq in the hospital of Kamal Al-Samurai for infertility treatment and IVF. The initial study population included three hundred women who were interviewed. Final study population included one hundred fifty, the other 150 patients were excluded from the study due to lack of inclusion criteria as shown in the below table.

The females were questioned by means of a survey including the subsequent data: past examination, infertility type, infertility duration, occurrence of additional illnesses including renal, thyroid and Polycystic Ovarian Disease (PCOS).

Only 45 females aging between (18-42) years who underwent IVF/ICSI procedure were selected for the ultimate consequences due to the problems that happened in the potential research that were as follows and were considered enough to exclude patients from the study:

- Blood-contaminated FF (N=60)
- Females who possess hyperstimulation syndrome (N=10)
- The females whose counterparts suffer from lacking sperms in the testicle biopsy (N=10)
- Females who were not collaborating or declined to contribute to this research (N=20)
- Others reasons (N= 5)

Inclusion criteria: An infertility of Male factor, Tubal – factor, unknown origin

Exclusion criteria: polycystic ovarian syndrome, Endometriosis, Diminished ovarian reserve, Females with renal illness, Females with thyroid illness (hypo, hyper).

This work was in agreement with Declaration of Helsinki of the “Ethical Principles for Medical Research Involving Human Subjects”, and was verified by the Ethical Committee at Al-Nahrain University, Baghdad, Iraq. The research aims and procedures were explained to the participants and verbal or written consent were obtained from the participant.

Methods

Patients who were registered in this research were subjected to two kinds of protocols of IVF/ICSI cycle and there count is attributed as: Long protocol, (N=7), short protocol, (N=38). Serum and follicular fluid was attained from the on oocyte pickup day. Serum and FF homocysteine concentration was determined by a fully automated instrument; the RX imola® autoanalyser (UK), using its costume kit (HY 4036, UK).

Statistical analysis

The study statistics were examined by means of the commercially-available Statistical Package for Social Sciences (SPSS) software version 23 (SPSS Inc., Chicago, Ill., USA). Numeric data were presented as mean± standard error (SE), on the other hand, nominal variables were shown as number and percentage.

Independent samples were tested using the t-test for comparison of the numeric variables mean between any two groups. Chi square (Pearson’s correlation coefficient) was employed for the evaluation of the association among numerical variables. P-value < 0.05 was regarded statistically significant.

RESULT

The total number of oocyte ($r = -0.676$, $p \leq 0.05$), and those in the metaphase II fluid ($r = -0.582$, $p \leq 0.05$)

were inversely associated with the concentration homocysteine in serum and follicular respectively as can be seen in figures 1 and 2 respectively.

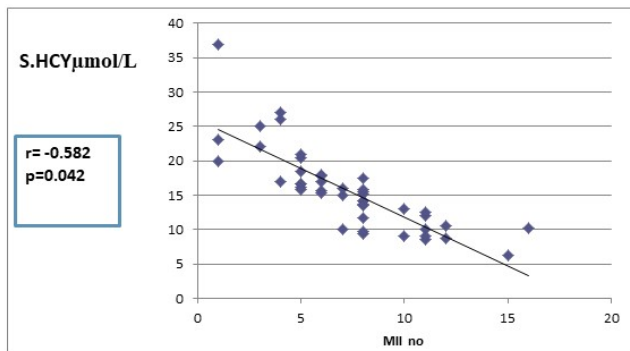


Figure 1: Correlation between serum homocystin level and number MII, the diagram shows clear inverse relation between them. Higher level of homocystin is associated with lower number of MII.

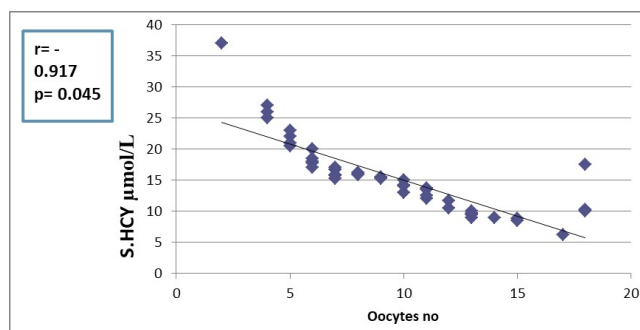


Figure 2: The correlation between serum homocystin level and the number of oocytes. It is clear that higher level of serum homocystin was associated with smaller number of oocytes.

There was no statistically difference in the serum and follicular fluid concentration of Homocysteine between conceived and non-conceived women ($p > 0.05$) although there is a trend toward higher level in non-conceived group as detailed in table I.

Table I Comparison of mean follicular and serum homocysteine in between conceived and nonconceived women group

Parameter	Nonconceived N=33	conceived N=12	P value	interpretation
	Mean±SE	Mean±SE		
S. HCY μmol/L	14.47±0.99	14.04±1.0	0.765	Not significant
F. HCY μmol/L	10.92±0.88	10.11±0.93	0.534	Not significant

Higher concentration of homocysteine in the both follicular fluid and serum was associated with lower implantation rate ($r = -0.485, p \leq 0.05$), ($r = -0.289, p \leq 0.05$) respectively as clarified in table II.

Table II : Correlation of serum and ff homocysteine with implantation rate in all study cases

Parameter	Implantation rate		interpretation
	r	p	
S. HCY (μmol/L)	-0.289	0.045	significant
F. HCY (μmol/L)	-0.485	0.040	significant

DISCUSSION

In this study we tried to find if the level of serum and follicular fluid homocysteine would influence oocyte number and their metaphase II and sequentially fertilization. The discovery of the thrombophilic etiology of recurrent abortion exemplified by the antiphospholipid syndrome (APLS) brought Homocysteine to the field of infertility and abortion. Homocysteine has been implicated in infertility through its oxidative and thrombophilic effects. Free radicals produced by Homocysteine lead to endothelial injury with subsequent predisposition to thrombosis (8). Homocysteine increases the expression of tissue factor which increase coagulability (8).

In this work, despite the presence of an inverse correlation between Homocysteine and oocyte number and quality, this correlation has not been reflected at the level of fertilization.

Homocysteine (Hcy) has been proven to cause direct damage to endothelial cells in vitro, which can lead to thrombogenesis and arteriosclerosis. Furthermore, Hcy causes the expression of tissue factor (TF) in vitro, which is the initiator of blood coagulation in vivo (8). Inherited thrombophilia is projected to serve key-role in the pathogenesis of recurrent IVF failures and subgroups with unexplained fertility, according to certain research. (9, 10).

CONCLUSION

Homocysteine has a deleterious impact on the growth and maturation of oocytes. Higher serum and FF homocysteine are associated with poor implantation rates. Accordingly, homocysteine might be used as a predictive marker for IVF and ICSI outcomes in the future after further validation by other studies.

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