Al-Kufa University Journal for Biology / VOL.12 / NO.2 / Year: 2020
Print ISSN: 2073-8854 Online ISSN: 2311-6544

Evaluation trace minerals and oxidative status in follicular fluid for unexplained infertile female undergoing Intra-cytoplasmic sperm injection program.

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Abstract:
Objective: To evaluate the level of zinc and 8-OHdg in follicular fluid and determine the correlation of both with Intracytoplasmic sperm injection outcome.**Aim:** The aim of this study is. To evaluate the Level of zinc and oxidative stress biomarker 8-OHdg in follicular fluid for unexplained female and compare with male factor. Study the correlation between zinc and 8-OHdg with ICSI outcome. **Material and method:** eighty eight subfertile couples were involved in this study. They divided into two groups. First group male factor and second group unexplained female factor. Both were interred in ICSI program. **Result**: The study showed that the mean follicular fluid level of zinc in pregnant group (17.47) and non-pregnant group (16.62). However, not different level of zinc in follicular fluid in both group unexplained female factor and male factor (16.66) and (16.97), respectively. The level of zinc significantly with No. of the oocyte (p=0.004), MII (p=0.033), No. of 2PN (p=0.035), Fertilization rate (p=0.001), Embryo G1 (p=0.044) and Embryo G2 (p=0.026). No significant statistical difference between level of 8- OHdg in follicular fluid in two groups regarding the cause of infertility unexplained female factor and male factor mean (2.20) and (2.25), respectively. But significant with pregnant status in pregnant group mean (1.37) and non pregnant group mean (2.54). The level of 8-OHdg non-significantly with ICSIoutcome. **Conclusion**: zinc level is statically significant and positively correlated with ICSI outcome, as the number of oocytes, mature oocyte (MII), fertilized oocytes 2PN, fertility rate, embryo G1 and embryo G2.The level of 8-OHdg in follicular fluid statically non- significant with ICSI outcome and not found a correlation between zinc and 8-OHdg.
Key word: *zinc, 8-OHdg and ICSIoutcome.*

Introduction:-

Zinc essential trace minerals important to humane live it is absorbed by the gastrointestinal system by the processes of exogenous zinc and excretion of endogenous zinc also by gastrointestinal system all cells in the body contain zinc and it's a vital nutrient for growth and development. Zinc is single trace mineral intracellular required for a number of cellular live processes, including cell proliferation, defense against free radicals, reproduction and immune function (Włostowski, 1992) (Ferguson et al., 2003). Zinc plays critical roles in membrane stabilization, nucleic acid metabolism, protein synthesis, growth of tissue and cofactor more than 80 metalloenzymes Included in DNA transcription the latter is important for germ cell development (Vasto S, 2008). The Zinc is important role to metalloprotein cofactor that is tightly regulated cell activity (Berg and Shi, 1996; Finney, 2003; Maret, 2013) high affinity binding in catalytic active or inactive sites of metalloenzymes of protein synthesis in regulation of transcription and translation and

play crucial role in cell cycle in mammalian oocyte. Zinc influx is important during maturation at16 h before fertilization and zinc efflux is important in embryo formation post 2 h following fertilization (Kim et al., 2010 and Krauchunas et al., 2013).

Zinc plays a vital role in reproductive health areas including oocyte production, maintaining proper follicular fluid levels and regulation hormone. Zn is an important trace mineral used by the body to keep the hormone stability level of estrogen, progesterone and testosterone. Zn plays role in ovulation and the menstrual cycle, low zinc level effect on pregnancy and hormonal imbalances can cause ovarian function defect, menstrual irregularity and anovulation cycle. Decrease in zinc, lead to severe defects in oocyte maturation, quality of oocyte, this deficiency can affect in fertilization and pre-implantation of oocyte development. Zinc important in cell division after oocyte, fertilization by sperm and for the placental formation .

Zinc also very important role in anti-apoptotic (Chimienti et al., 2003) and antioxidant (Zago and Oteiza, 2001). Zinc act against the oxidation by binding sulfhydryl groups in proteins and by control binding sites for lipid, proteins and DNA (Bray and Bettger, 1990) Antioxidant effects of zinc found to oxidative damage of lipid, protein and DNA (Oteiza et al., 1995; Bagchi et al., 1998). These occur by two mechanisms firstly in the early apoptotic pathway zinc may inhibit the protease enzyme in programme cell death, secondly zinc may suppress endonucleases these enzyme calcium and magnesium-dependent which cause apoptosis and DNA fragmentation (Perry et al., 1997)

Truong- Tran et al., 2000; Chimienti et al., 2003). But excessively high level of zinc may be toxic and can lead to apoptosis and necrosis to the cell. Zinc is one of important essential mineral for co-factor about 300 enzymes and 2000 transcription factors for several proteins, important mediator of cellular signaling (Roshanravan, and Alizadeh, 2015) (Jurowski and Szewczyk, 2014) provides stability to cell membranes (Foster and Chu, 2014) (Jansen and Rosenkranz, 2012) and Zinc act a proper functioning antioxidant defense system by protecting the cells against the effect oxidative damage. The most important causing damage to the basic structures of cell (proteins, DNA and membrane lipids) is the hydroxyl radical. The hydroxyl peroxide radical can be produced by the various mechanisms, especially these produce By the Fenton reaction (these diffuses into the DNA strands of the nucleus) the interaction of hydrogen peroxide with the DNA base strand of the nucleus, such as guanine, leads to formation of C8-hydroxyguanine (8-OHGua) or, the 8-hydroxy-2 -deoxy -guanosine (8-OH-dG) the (8-OH-dG) is a biomarker of oxidative stress induce the-DNA strand damage (Kasai H, 1997).

The Oocyte quality is effective with 8-hydroxy-2'-deoxyguanosine (8-OHdG) biomarker levels in granulose cells. When elevated give Poor oocyte quality these leads to bad embryo development and effects on ICSI outcome. Cumulus cells derived from undifferentiated granulose cells. The cumulus surrounds the oocyte and made the extracellular matrix (Zhou and Kimata, 2001) Cumulus cells provide support maturation and developing the oocyte .Cumulus cells can produce antioxidants like superoxide dismutase (SOD), these suggested protection to oocyte from Reactive Oxygen Species (ROS) damage(Huang and Wells, 2010) Higher level of (SOD) in cumulus cells is associated with ICSI outcome succes (Matos et al., 2009) and when increased levels of the 8-OHdG in cumulus cells lead to lower rates of oocyte fertilization and lower embryo quality (Seino et al., 2002).

Study design:-

This is a planned as a prospective cohort study, members were recorded (N=88) infertile couples undergoing intracytoplasmic sperm injection ICSI procedure who are referred from different Iraq governorates to the fertility center in A1 -Sadder Medical City in AL- Najaf Al-Ashraf/ Iraq. Under management of Urology department in AL-Kufa medical college.

MATERIALS AND METHODS:-

Eighty eight subfertile couples were included in this study and all of the couples were involved in inter in intracytoplasmic sperm injection (ICSI) program throughout the period between November 2018 and February 2019.

Female and male partners of both groups had been assessed by urologists and gynecologist confide on history and physical examination, hormonal analysis on cycle day two which include (estrogen, luteinizing hormone, follicle stimulating hormone and prolactin), trans vaginal ultrasound (TVUS) and semen analysis for male partners the normal semen parameters according to WHO criteria 1999. The specific protocol used and pick up after this take about 1 milliliters from follicular fluid was get from the first retrieved follicle to keep away from contamination of flush medium and blood, after this put in plane tube and centrifuged about 15 minutes at 3000 RPM and keep at - 20◦C in freezer before the analysis and after this use to evaluate trace minerals (zinc, copper and calcium) and oxidative stress biomarker (glutathione and 8-OHdg).

Result:-

Eighty eight subfertile couples were included in this study and divided to two groups:
Group I In which male factor.
Group II In which unexplained female factor.

Table (1) show Correlation between zinc concentration in follicular fluid with ICSI outcome for study patient and hormonal profile, the zinc level significantly effect on number of oocyte(0.004) ,mature metaphase II Oocyte (0.033), number of two pro nuclear (0.035) and significant strongly positive correlation with fertility rate (0.0001).The level of zinc in follicular fluid significant correlation with good embryo quality GI(0.044) and GII (0.026), but non-significant with total number of embryo (0.39), number of embryo transfer (0.105) and cleavage rate (0.904). The zinc concentration on follicular fluid statically not significant with hormonal level E2 (0.425), FSH (0.962), LH (0.100).

Table (1) correlation between zinc and ICSI outcome parameters:

ICSI Outcomes parameters	Correlation Coefficient (r) n=88	P-Value
NO. of OOCYTE	0.306**	0.004
MII	0.228 *	0.033
No. of 2PN	0.225 *	0.035
Fertilization Rate	0.934 **	0.000
No. of Embryo	0.221 *	0.39
No. of Transfer Embryo	0.174	0.105
Embryo G1	0.215 *	0.044
Embryo G2	0.238 *	0.026
Cleavage Rate	0.013	0.904
E2	0.0860	0.425
FSH	0.005	0.962
LH	0.176	0.100

Values are presented as mean and stander deviation statically significant ($p<0.05$).

Table (2) show Correlation between the level of 8-OHdg in follicular fluid and ICSI outcome parameter for study patient and hormonal profile, the level of 8-OHdg No significant correlation was observed between 8-OHdg and ICSI outcome parameter such as, number of oocyte (0.877), number of two pro nuclear (0.986), fertility rate (0.578), total number of embryo (0.881), number of embryo transfer (0.065), good embryo quality GI(0.324) and GII (0.475), cleavage rate (0.374), The 8-OHdg concentration on follicular fluid statically not significant with hormonal level E2 (0.619), FSH (0.184), LH (0.312).

Table (2) The correlations between8-OHdg and ICSI outcome parameters:

ICSI Outcome	Correlation (r) n=88	P-Value
NO. of OOCYTE	-0.017	0.877
MII	0.002	0.986
No. of 2PN	-0.002	0.988
Fertilization Rate	0.06	0.578
No. of Embryo	-0.016	0.881
No. of Transfer Embryo	-0.198	0.065
Embryo G1	0.106	0.324
Embryo G2	-0.077	0.475
Cleavage Rate	-0.096	0.374
E2	-0.054	0.619
FSH	-0.143	0.184
LH	0.109	0.312

Values are presented as mean and stander deviation statically significant ($p<0.05$).

Tablet (3) show the Comparison between pregnant and non –pregnant group with zinc and 8-OHdg in follicular fluid ,the concentration of OHdg statisticaly significant the level of OHdg in non pregnant groups elevated and decrease level in pregnant groups.The level of zinc in both groupstaticaly not significant.

Tablet (3) Comparison between of pregnant state with zinc and 8-OHdg.

Biochemical variation	non–pregnant =65		pregnant =23		P-value
	Mean	Std.Dev.	Mean	Std.Dev.	
8-Ohdg	2.54	1.17	1.37	0.51	0.000
Zn	16.62	3.00	17.47	2.32	0.425

Values are presented as mean and stander deviation statically significant ($p<0.05$).

Tablet (4) show the Comparison between cause of subfertility with zinc and 8-OHdg in follicular fluid, the level of zinc and 8-OHdg not significant with cause of subfertility.

Tablet (4) Comparison between of cause subfertility with zinc and 8-OHdg.

Biochemical variation	Female Unexplained N=35		Male Factor N=53		P-Value
	Mean	SD.	Mean	SD.	
8-Ohdg	2.20	1.06	2.25	1.23	0.788
Zn	16.66	3.20	16.97	2.62	0.828

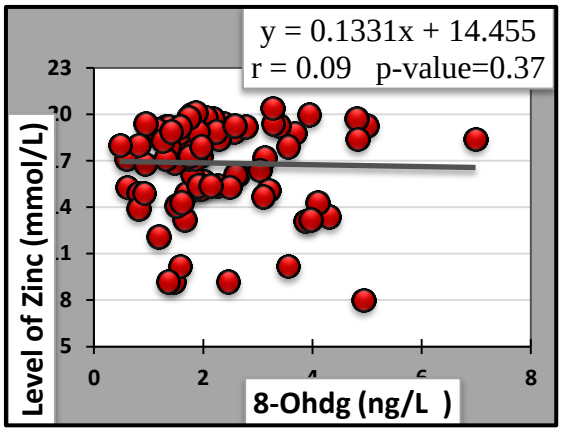

Figure: (---) correlation between level of zinc and 8-OHdg in follicular fluid for study patients.
In this figure show no relation between level of zinc and 8-OHdg in follicular fluid in the study group.

DISCUSSION:-

Our study reveals that zinc concentration in follicular fluid was no significantly different in pregnant and non-pregnant women for both pregnant and non-pregnant group. These observances are agreement with the results circumstance by (Yan Sun et al., 2017). Zinc is an essential trace mineral most important vital role in oocyte production, cell division after oocyte ovulation, fertilization by sperm and for the placental formation any change in level of zinc in the follicular fluid may reflect on the oocyte development and quality, fertilization and early development of embryo (Oktem and Urman, 2010) in our study statistically positive correlations between Zn concentrations in follicular fluid and the fertilization rate in IVF patients and a positive correlation found between follicular fluid Zn and the No. of oocytes, MII, 2PN these agreement with reported by (Tolunay et al., 2016) and agreement by other study positive correlation with M1, number of oocyte and No. of embryo transfer, these Studies have demonstrated the Zinc concentration affects not only the number of oocytes but also their quality of oocyte and Fertilization rate (%). The only definitive measure of quality to oocyte its ability to form a blastocyst, and MII metaphase II follow in vitro maturation (Ferguson et al., 2003). Furthermore, the present study highlight the positive efficacy between quality of embryo, grade of embryo and role of zinc antioxidant (Zago and Oteiza, 2001) and anti-apoptotic (Chimienti et al., 2003) mechanism by inhibit the protease enzyme and endonucleases these enzyme cause apoptosis by lipid ,protein and DNA fragmentation (Perry et al., 1997; Truong-Tran et al., 2000) also act co-factor for several enzymes to transcription and repair DNA (Roshanravan and Alizadeh, 2015) (Jurowski and Szewczyk, 2014). This feature is important at the first stage of human life, during and after fertilization and at the beginning embryonic development these agreement with the results stated by (Jaroudi et al., 2009; Me´ne´zo et al., 2007) and (Wells et al. .,2005). Also, the Current study exposes that there is negative correlation between 8-OHdg and number of oocyte, 2PN, and fertility rate. Some authors suggested that elevated oxidative stress effect on development the oocyte, quality of the oocyte, quality of embryos and

fertilization rate (Du Plessis et al., 2008). 8-OHdg is a sensitive indicator of DNA damage due to oxidative stress. Increase level 8-OHdg in follicular fluid in women significantly associated with high rates of degenerate oocytes and effect on oocyte quality (Tamura et al., 2008) while the other authors that not related to oocyte quality (Jozwik et al., 1999).

According to (Seino et al., 2002), high concentrations of 8-OHdG in follicular fluid in subfertile women undergoing ICSI are negatively correlated with fertilization rate and quality of embryos. Supporting these determinations, (Tamura et al., 2008), shows that higher 8-OHdG concentrations in the FF of women undergoing ICSI are correlated with higher rates of oocytes degenerated. These agreements with our study. Comment the same results, that higher 8-OHdG concentrations in the FF related with poor Intracytoplasmic sperm injection outcome (Berker et al., 2009; Rizzo et al., 2012; Takahashi et al., 2013) also, several studies reported the relationship between defect in follicular development, poor quality of oocyte and fertilization, development of embryonic and lower female fertility due to elevated 8-OHdG concentrations in the FF (Barygina et al., 2013; Sofi *et al*., 2013; Becatti *et al*., 2016).

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