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Effect of the relationship between vitamin C and serum ferritin on fertility.

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Abstract:

Eighty Eight subfertile women and fourty fertile women (control group) enrolled in this study. The subfertile women subdivided into two subgroups according to the cause of subfertility: first group are women with polycystic ovary syndrome (PCOS) including 45 women. Second group are women with unexplained subfertility including 43 women. Their age range (16-40) yr. At day 3 and day 13 of menstrual cycle estimation of serum level serum ferritin and vitamin C. The results show significant increase in serum ferritin value in women with PCOS at day 3 and day 13 of menstrual cycle. Serum ferritin is lower in subfertile women with unexplaiened subfertility but this decrement is not significant. No significant changes in vitamin C level.

Introduction:

ubfertility is defined as the inability to conceive after a certain period of time, the length of which vary **[1**]. Twelve months is the lower reference limit for Time to Pregnancy (TTP) by the World Health Organization **[2]**. Subfertility affects about 10-15% of couples and is a medical concern for 2.7 million women of reproductive age in U.S and some studies suggest a range of lifetime prevalence of infertility percentage range from 6.6 to 32.6% [**3**]. In both men and women the fertility process is complex. Even under ideal circumstances, the probability that a woman will get pregnant during a single menstrual cycle is only about 30% and when conception does occur, only 50-60% of pregnancies advance beyond the 20th week [4],[5]. About a third of subfertility problems are due to female causes and another third are due to male causes. In the remaining cases, subfertility affects both partners or the cause is unclear (unexplained). It is equally important for both partners to be tested at the same time [6].

Iron is an element of crucial importance to living cell and exists in a range of oxidation states, the most common being ferrous (Fe^{+2}) and ferric (Fe^{+3}) forms. Iron can be associated with proteins; bind to oxygen (O^2), transfer electrons and mediate catalytic reactions. Enzymes of the citric acid cycle (succinate dehydrogenase and aconitase) are iron-dependent. Iron is a critical component of heme in hemoglobin (Hb), myoglobin, cytochromes as well as iron–sulfur complexes of the electron transport chain [7]. Iron is also required for activity of ribonucleoside reductase, the rate-limiting enzyme of the first metabolic reaction committed to DNA ruplication. Therefore, iron plays an important role in metabolic processes including O^2 transport, electron transport, oxidative phosphorylation and energy production, xenobiotic metabolism, DNA ruplication, cell growth, apoptosis, gene regulation and inflammation [8],[9]. It is also a necessary cofactor for the synthesis of neurotransmitters, dopamine, norepinephrine and serotonin [10]. Although iron comprises only 0.008% of the body's mass (approximately 6 g for 75-kg for adult male), we cannot live without this important element in our bodies. In child bearing age women, the average daily iron absorption is about twice that in men, largely because of gestation, lactation periods and the blood loss during menstruation (around 20 mg iron/period) [11]. Up to 12% of all women of child-bearing age have an iron deficiency. Estimations of iron status before pregnancy can help to avoid deficiency which is a common condition during pregnancy. Serum ferritin is the most reliable test for body's iron stores [12]. A small clinical trial testing the efficacy of an iron-containing supplement among women who had unsuccessfully tried to become pregnant documented a higher pregnancy rate in the treatment group [13]. Dietary iron presents in food in two forms as heme and non-heme iron. Although plant materials contain only non-heme iron, animal products contain both heme and nonheme iron, (and the body needs a balanced diet with both heme and non-heme iron).

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Vitamin C **(ascorbic acid) is a simple sugar and** plays an important physiological role **within cells** as a reducing agent, antioxidants, free radical scavenger and enzyme cofactor. **It is involved in the hydroxylation of proline to hydroxyproline which is necessary for the formation of collagen [14]. Humans along with a few other animals (e.g. primates and the guinea-pig) are unable to synthesize ascorbic acid from glucose [15]. Vitamin C is present in all fresh fruit and vegetables and easily leached out from vegetables when they are placed in water and it is also oxidized to dehydro-ascorbic acid during cooking or exposure to copper or alkalis [16].** A recent study by Al-Katib and Al-Kaabi [17] showing that vitamin C act as promoting factor in productions of estrogen and progesterone that cause increasing the endometrial thickness in subfertile women especially in those poorly nutrition.

Materials and Methods

The subfertile women enrolled in this study were selected randomly from fertility center in the gynecological and obstetrical teaching hospital in Kerbala city and from multiple gynecology/obstetrics privet clinics during the period from July 2012- October 2013. The obtained total number for this study about 128 which divided into 2 groups: subfertile women (that account 88) and fertile or control women (that account 40). The subfertile women subdivided into two subgroups according to the cause of subfertility: polycystic ovary syndrome (45 women) and unexplained cause (43 women) .All of them at reproductive age (16-40) years and their body mass index (BMI) ranged from 20-45 kg/m^2.

Equipments and Kits:

Height and weight measurement apparatus: The height of the patient is measured by using a tape measure. The weight is measured by using the manual and weight scale apparatus. Measurement of height and weight for each patient was done to calculate their body mass index

Blood Samples collection: Ten milliliters of venous blood was drawn using a disposable needle and plastic syringes from each woman. The blood sample was left at room temperature about 10 minutes for complete clotting, centrifuged at 3000 round per minute (RPM) for 5 minutes, and then the serum was separated and transported into new disposable plain tubes and kept frozen for future analysis.

Chemical kits:

By using different enzyme techniques for determination the concentrations of the following:

1. **Serum vitamin C** (manual preparation kit): serum ascorbate was measured by a method described by Gowenlock *et al*, (1988)[18]. The reference value for serum vitamin C was 0.5-1.5 mg/dl.
2. **Serum Ferritin** (Accu-Bind, ELISA Microwells, Ferritin Product, USA). Approximate reference ranges for serum ferritin were (20-300 ng/ml) for males and (20-150 ng/ml) for females [19].

Methods: History taken from each women including her parity ,type of subfertility (primary or secondary) and its duration, menstrual history(date, duration, frequency, regularity and amount of bleeding) , past obstetrical and gynecological history like ectopic pregnancy, ovarian cyst, miscarriages. Evaluation of her investigations, like hormonal assay, ultrasound, hystrosalpingiograph, and semen analysis for her husband. If suspected PCOS, in addition to menstrual irregularity, ask about gaining weight and hirsutism. Nutritional history, good or poor nutrition through asking her about quality, quantity and frequency of fruit, vegetables, meats and dairy products, and their availability, to be ingested. At day 3 and day 13 of menstrual cycle estimation of serum level of ferritin and vitamin C were done.

Statistical Analysis: Type of study is case-control study .Statistical analysis was done by using SPSS (statistical package for social sciences) version 20 in which we use chi square test for categorical data and independent sample T-test and ANOVA (analysis of variance) with LSD for measurement data. We set P value <0.05 as significant.

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Results:

The relation between the levels of serum ferritin and vitamin C in fertile and subfertile women at day 3 of MC:

Table (1) show significantly higher serum feritin in women with polycystic ovarian syndrome .Serum ferritin is lower in subfertile women withunexplaiened infertility but this decrement is non significant. No significant difference between the groups (P>0.05) in the levels of vitamin C for fertile and subfertile women in day 3 of MC**.**

Table (1): Serum levels of ferritin and vitamin C for fertile and subfertile women at day 3 of MC (mean±SD).

Variable	Fertile women (n=40)	Subfertile women (n=88)	
		Unexplained cause (n=43)	PCOS (n=45)
S. ferritin (ng/dl)	14.80±11.23	12.47±11.58	19.92±10.87*
Vitamin C (mg/dl)	2.24±2.1	2.82±1.38	1.56±0.82

*significant difference (P >0.05) in the variables between all groups.

The relation between the serum levels of ferritin and vitamin C in fertile and subfertile women at day 13 of MC:

Table (2) shows that there is no significant difference in vitamin C level between all groups. Serum ferritin is significantly increase (P<0.05) in PCOS. Serum ferritin is lower in subfertile women with unexplaiened infertility but this decrement is non significant.

Table (2)Serum levels of ferritin and vitamin C for fertile and subfertile women at day 13 of MC (mean±SD).

Variable	Fertile women (n=40)	Subfertile women (n=88)	
		Unexplained cause (n=43)	PCOS (n=45)
S. ferritin (ng/dl)	16.45±15.32	13.91±8.63	27.48±17.09*
Vitamin C (mg/dl)	2.819±1.59	3.3922±2.13	3.4144±2.35

*significant difference (P >0.05) in the variables between all groups.

The serum levels of Vit. C in fertile and subfertile women in those having serum ferritin level less than normal and in those having normal value of serum ferritin level at day 13 of MC:

The results of table(3) show no significant differences between these variables at level of significance (P >0.05).

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Table (3): Serum levels of Vit. C in fertile and subfertile women in those having serum ferritin level less than normal and in those having normal value of serum ferritin level at day 13 of MC:

Variable	Fertile women (n=40)	Subfertile women (n=88)	
		Unexplained cause (n=43)	PCOS (n=45)
Vit C in women with s. ferritin less than normal (mg/dl)	3.03±1.78	3.55±2.23	2.35±1.77
Vit C in women with normal s. ferritin (mg/dl)	2.26±0.75	2.83±1.82	3.72±2.53

No significant difference (P >0.05)

Discussion: The results of statistical analysis shown that serum ferritin level less than normal value which range between (20-159 ng/ml) [19] in all groups at day 3 of M.C even there is no significant difference when compared between fertile and subfertile group, but at day 13 of M.C. the serum ferrtin level slightly increased and the difference in value between the 2 periods due to blood loss that lead to decrease in iron storage (ferritin) during M.C. and this significant increases in serum ferritin day 13 of M.C. more clear in PCOS as compared with fertile groups and this may be related to lower activity of ovary in PCOS and oligomenorrhea. The presence of transferrin and its receptor in granulosa cells and oocytes has been documented . More recently, it has been reported that granulosa cells can synthesize transferrin, which may be translocated to the oocytes. Although it is possible that transferrin and transferrin receptor are redundant in the ovary or do not play an important role in local iron metabolism, it has been suggested that these proteins are essential for ovum development and are required to support the increased iron demand of the developing follicle [20]. This result agrees with a study done by Sharifi [21] shows that overweight and obese cases with PCOS had higher ferritin levels than BMI matched controls and found that oligomenorrhea and less blood loss in PCOS subjects might be the best explanation for their higher ferritin levels. A similar result has been reported by another two studies suggested that increased body iron stores, expressed as increased serum ferritin concentrations, are present in women with PCOS [22],[23]. Sathiyanarayanan (2014)[24] studies show that women who do not get sufficient amounts of iron may suffer anovulation (lack of ovulation) and possibly poor egg health, which can inhibit pregnancy at a rate 60% higher than those with sufficient iron stores in their blood. When the blood does not get enough iron, anemia, or an insufficient number of red blood cells, may develop. Since the insufficiency of red blood cells that deliver oxygen to all of the body ´s tissue and organs including the ovaries and uterus may cause the eggs stored in the ovaries to weaken over time and become unviable. Also, the subfertile women may have iron deficiency that associated with low serum ferritin before they get try for pregnancy as explained by researchers [25] who found that up to 12 percent of all women of child-bearing age have an

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iron deficiency and the principle cause of iron deficiency anemia (IDA) in premenopausal women is blood lost during menses.

Regarding vitamin C, the results shown no significant difference in serum level of this vitamin at both day 3 and day 13 and hasn't any relation with serum ferrtin level and this agree with [26],[12] found no evidence of interaction of vitamin C intake with iron supplement use. Also, this result disagrees with [27] they found that treating iron deficiency anemic patients with 500 mg of vitamin C twice daily resulted in an increase in average haemoglobin level by 8%, increase serum iron concentration by 17%, transferrin saturation to 23% and a decrease in total iron binding capacity by 7%. They concluded that vitamin C was more effective for increasing iron status than iron supplements this is due to the role of vitamin C in ovarian function and its deficiency may have a role in ovarian dysfunction and this agree with researchers [28]. They explained that Vitamin C improves hormone levels and increases fertility in women with luteal phase defect.

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