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RESEARCH ARTICLE

Effect of Ferritin, Magnesium and Cobalt on Total Viable Count of Candida Spp. in Normal Pregnant and In-Vitro Fertilized Women

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ABSTRACT

Background: A correlation existed between diminished levels of vital mineral elements crucial for antioxidant activity in the bodies of pregnant women and an elevated susceptibility to preeclampsia. **Objective:** To assess the effect of salivary mineral elements on the salivary total viable count Candida spin pregnant, in vitro fertilization pregnant women and control. **Materials and Methods:** A total of 85 subjects were included in this study, first group consist of (30) normal pregnant women, second group consist of (30) In vitro fertilization (IVF), and third group consist of (25) non-pregnant women, the samples were collected from different clinics located in Baghdad, Iraq. Vitik-2 system was used in this study in order to diagnose the Candida spp. isolates, and Perkin-Elmer (USA) Atomic Absorption Spectrophotometer model 305B was used to measure the traces elements. **Results:** The Frequency of *Candida* spp in each pregnant group had a high proportion of *Candida Albicans* (84%) while in IVF group found a high proportion of *Candida Tropicalis* (90%). The count of *Candida* between the three groups were higher IVF groups ($9.767 * 10^3$ CFU/ml) followed by pregnant ($4.833 * 10^3$ CFU/ml) while the control shows the lower value ($3.520 * 10^3$CFU/ml) with significant difference (P-Value $\leq$ 0.000). The three traces elements were measured 114.767 μg/dL for iron (Fe) in IVF group, and for pregnant it was 93.200 μg/dL but for control group it is 70.000 μg/dL, all the results were compared statistically with a significant difference (P-Value $\leq$ 0.000), for t(Co), the highest mean value is found in the control group, while in pregnant group is lower than control group (0.138 μmol/dL), it is the lowest in IVF group with a value of (0.093 μg/dL) with no significant differences between the groups (P-Value $\leq$ 0.066), as for (Mg), the control group holds the highest value, followed by pregnant group while the lowest value of magnesium is found in IVF group significant statistical value between the groups ((P-Value $\leq$ 0.000). **Conclusions:** Traces elements are different between the three groups; Iron is in highest level in IVF group, while cobalt and magnesium appears in highest level in control group.

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1. Introduction

Pregnancy is characterized by significant physiological, social and emotional changes which can impact on maternal and fetal health and well-being across multiple domains [14]. There is comprehensive evidence that anxiety, depression, and stress in pregnancy are risk factors for adverse maternal and fetal outcomes ranging from preterm birth and low birth weight to adverse neuro developmental outcomes in infants and children [6]. Pregnancy is associated with several structural and functional changes that influence the processes of drug absorption, distribution, metabolism, and excretion, besides these pregnancy-related changes attributable to alterations in maternal blood flow, increased fluid retention, and the effect of hormones (such as vasopressin, progesterone, relaxin, estrogen, and angiotensin II), transmembrane transporter function, expression, and regulation also significantly influencing the pharmacokinetics of many drugs during pregnancy [4, 26].

Much of the success of IVF relies on women undergoing multiple embryo transfers and oocyte retrievals. However, multiple embryo transfers and oocyte retrievals can be cost prohibitive and emotionally and physically burdensome resulting in reported treatment attrition rates of up to 35–50%, While some factors associated with lower success of IVF treatment, such as advanced female age, are not modifiable, there is growing interest in the impact of modifiable factors, such as diet, on treatment outcomes [16].

A few fungi have developed a commensal relationship with humans and are part of the indigenous microbial flora (e.g., various species of Candida, especially Candida albicans). Candida species are present in the oral cavity of up to 75% of the population. In healthy individuals this colonization generally remains benign. However, mildly immunocompromised individuals can frequently suffer from recalcitrant infections of the oral cavity. These oral infections with Candida species are termed “oral candidiasis” [18].

Minerals and trace elements (MTEs) are inorganic micronutrients found in a variety of plant and animal foods. The functions of mineral elements include being a structural component of a vitamin (Zinc (Zn) and magnesium (Mg) and Iron (Fe), catalytic components of numerous enzymes, are also structural components of other important proteins. Deficiencies in iodine, iron, and zinc, have the largest negative impact on the public health [13]. Minerals are key components of complex enzyme systems responsible for antioxidant protection of the organism. This feature seems to be particularly important during pregnancy, which is associated with a higher frequency of oxidative reactions. There was a relationship between reduced levels of mineral elements essential for antioxidant activity in the body of pregnant women and an increased risk of developing preeclampsia [7].

This research aims to assess variations in salivary mineral element levels and investigate how these fluctuations may impact the overall count of Candida spp.

2. Materials and methods

2.1. The human sample

This study had been started after gaining the protocol approval, the ethical and the scientific committee approval at College of Dentistry/University of Baghdad. A total (85) subjects were enrolled in this study. They were admitted to (Elwea Maternity, Al-Jadiriah

Private, Kamal Al-Samarrai Specialist, Albinouk private Hospitals, College of dentistry university of Baghdad under follow up and control participant choose randomly), the study was carried out from 15 November to 25 of February, 2022. The subjects were divided in to three groups: first group consist of (30) normal pregnant women with age range (25–40) years, and second group consist of (30) Invitro fertilization pregnant women their age range between (25–40), and third group consist of (25) non-pregnant women their age range between (25–40) was chosen to be the control group who were matching the study group.

2.2. Inclusion criteria

- Pregnant women with age range between (25–40) years old.
- Second trimester of pregnancy.
- First pregnancy.
- The pregnant should seize any oral supplement prior sampling only keep folic acid.

2.3. Exclusion criteria

- Pregnant women with signs and symptoms of any systemic disease.
- Have history of chronic disease like diabetes mellitus, hypertension or heart diseases.
- History of smoking or alcohol drinking.

2.4. Ethical approval

All the subjects received detailed information concerning the nature of the study and the procedures involved, and their informed consent was obtained on a form approved by ethical committee of College of Dentistry in University of Baghdad.

2.5. Saliva samples collection

All participants were instructed not to eat or drink (except water) at least 1 hour prior to donation of saliva; the subject should sit in a relaxed position. Saliva was collected between 8–9 am. Whole un-stimulated saliva was collected into cups. After collecting the salivary sample from each patient, the tubes were placed in a cool box with ice to transfer them to the laboratory to be cultured within less than an hour, then 0.1 ml would be taken from the salivary sample by micropipette for the serial dilution tubes using PBS.

2.6. Phosphate Buffer Solution (PBS) preparation

2.6.1. Preparation of solutions and culture media

This isotonic solution used serial dilution was prepared by adding 8 g (NaCl), 1.21 g (K2HPO4) and 0.24 g (K2HPO4) to 1000 ml deionized water, mixed well using magnetic stirrer for dissolving the powder [3], then sterilized by autoclaving at 15 pound/inch2 pressure (121°C) for 15 minutes. After cooling, PBS solution used by 9.9 mL in each disposable tube and then add 0.1 mL from saliva.

2.7. Sabouraud Dextrose Agar (SDA)

This medium is selective for the isolation and cultivation of fungus and was prepared, sterilized and stored according to the manufacturer's instructions; 65 g were suspended in

1000 ml of D.W. Sterilization was done by autoclaving at 121°C at 15 psi for 15 minutes, left to cool till 45–50°C then poured into Petri dishes and left at room temperature to cool then store in refrigerator till use. (Add chloramphenicol 0.05 g/L)

2.8. Sterilization methods

The media sterilized in an autoclave for fifteen min at temperature of 12°C and 15 pound/inch2 pressure. All cleaned glass tools were sterilized in a hot air oven for 1 hour at 180°C. The laboratory's benches and floor were cleaned with a bleaching antiseptic agent.

2.9. Culturing method

Saliva was placed on a vortex machine to be homogenized for 120 second. Then serial dilution was done Tenfold steps, 0.1 ml was withdrawn by micropipette from (10^{-3} to 10^{-5}). A microbiological spreader was used to inoculate the 0.1 mL on (SDA) and agar sabouraud from each dilution. An anaerobic jar was used to incubate the MSBA agar for 48 hours, at 37°C, second incubation done at 37°C, for 24 hours without the jar, followed by aerobic incubation for 24 hrs; at 37°C. Sabouraud chloramphenicol agar was incubated aerobically for 45 hrs, then samples were placed in the centrifuge at 3000 rpm for 15 minute then the superannuant collected and stored in the freezer.

2.10. Vitek system 2

Vitik-2 system was used in this study in order to diagnose the Candida spp. isolates which included several steps as follows:

1. Preparation of fungus suspension A sterile swab was used to transfer a sufficient number of C. albicans, colonies of a pure culture and separately suspended in 3 ml of sterile saline in clear plastic test tube. The turbidity was adjusted up to 2.0 O.D.
2. Inoculation of identification card Identification card was inoculated with Candida spp. Isolates suspension using an integrated vacuum apparatus. A test tube containing the Candida suspension was placed into a special rack (cassette) and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassette can accommodate up to 10 tests or up to 15 tests.
3. Card sealing and incubation Inoculated card was passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types are incubated on-line at 35.5 ± 1.0°C. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15 minutes intervals during the entire incubation period.

2.11. Measurement of mineral elements

Perkin-Elmer (USA) Atomic Absorption Spectrophotometer model 305B fitted with Nitrous oxide acetylene burner head. Hollow cathode lamps were used as radiation emission source for (Ferrous Iron (Fe)/Magnesium (Mg)/Cobalt (Co)). Absorption was measured in a Fuel-rich flame to obtain maximum sensitivity.

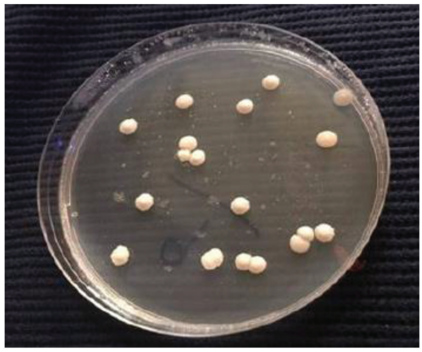

Fig. 1. Colony of *Candida* spp. on sabouraud dextrose agar.

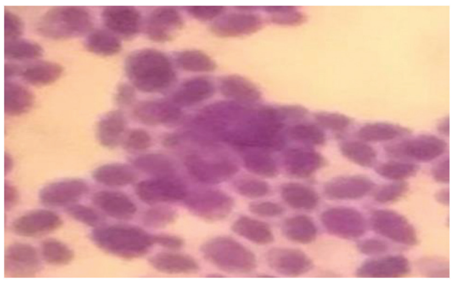

Fig. 2. Gram's stain for *Candida* spp. cells (1000X magnification).

3. Results

3.1. Identification of Candida spp

3.1.1. Colony morphology

On SDA plates, colony of *Candida spp. appeared* smooth, creamy in color with distinguished yeast smell. They were about 3-4 mm in diameter and 2 days later they were developed into high convex, off-white large colonies as shown in Fig. 1.

3.2. Microscopic examination

Under light microscope, *Candida* spp. appear as rounded or oval yeast cells and were Gram positive when Gram staining was performed, as shown in Fig. 2.

3.3. Germ tube formation

Under light microscope, the formation of germ tubes was observed which it is one of *Candida* Albicans characteristics, Fig. 3.

3.4. Frequency of Candida spp. among groups (Pregnant, IVF and Control)

The results shown in Table 1 demonstrate that the Frequency of *Candida* spp in each pregnant group had a high proportion of *Candida Albicans* (84%) while in IVF group found a high proportion of *Candida Tropicalis* (90%). The control group reported *Candida Albicans* 4%.

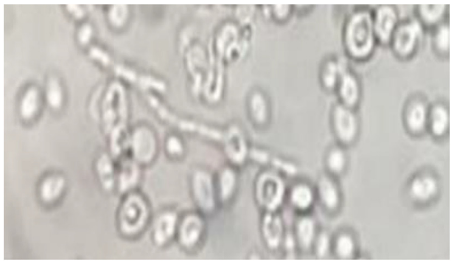

Fig. 3. Germ tubes of *Candida* spp. (40X magnification).

Table 1. Frequency of Candida spp. among the three groups (Pregnant, IVF and Control).

	Frequency of Candida spp. (%)	
Groups	*Candida Albicans* %	*Candida Tropicals* %
Pregnant	24(84%)	4(14%)
IVF	3(10%)	27(90%)
Control	1(4%)	—

Table 2. Descriptive and statistical test of *Candida* among groups X 10^3.

Groups	Mean	SD	SE	Minimum	Maximum	F	P value
Pregnant	4.833	3.896	0.711	1.000	19.000	14.579	**0.000**
IVF	9.767	6.420	1.172	2.000	25.000		
Control	3.520	2.044	0.409	1.000	7.000		

Table 3. Multiple comparisons of *Candida* using Games-Howell.

Groups		Mean difference	P value
Pregnant	IVF	4.933	**0.002**
	Control	6.247	**0.000**
IVF	Control	1.313	0.256

3.5. Viable count of Candida spp. among the three groups (Pregnant, IVF and Control)

Results in Table 2 shows that the data of viable count of *Candida* between the three groups and its statistics such as Mean, Standard Deviation (SD) and its Standard Error (SE), *Candida* counts are higher IVF groups ($9.767*10^3$ CFU/ml) followed by pregnant ($4.833*10^3$ CFU/ml) while the control shows the lower value ($3.520*10^3$ CFU/ml) with significant difference (P-Value $\leq$ 0.000).

Table 3 shows that the mean differences between the three groups, Multiple Comparisons of *Candida* was carried out using Games-Howell, however, the multiple pair wise comparison indicates the significant difference between each group when compared with other with (P-Value $\leq$ 0.000) and P-Value $\leq$ 0.000) respectively except between IVF and control which does not shows any significance (P-Value = 0.256).

Table 4. The descriptive and statistical test of trace elements among the three groups (Pregnant, IVF, and Control).

		Mean (μg/dL)	±SD	±SE	Minimum	Maximum	F	P-value
Fe	Pregnant	93.200	16.171	3.234	69.000	121.000	62.048	**0.000**
	IVF	114.767	16.546	3.021	87.000	140.000		
	Control	70.000	13.965	2.550	47.000	110.000		
Co	Pregnant	0.138	0.017	0.003	0.110	0.170	2.830	0.066
	IVF	0.093	0.025	0.005	0.012	0.140		
	Control	0.142	0.159	0.032	0.070	0.900		
Mg	Pregnant	1.518	0.170	0.034	1.280	1.930	82.813	**0.000**
	IVF	1.285	0.127	0.023	1.060	1.520		
	Control	1.831	0.192	0.035	1.480	2.200		

3.6. Description of traces elements (Fe, Cobalt and Mg) among the three groups (Pregnant, IVF and Control)

Three traces elements were measured and the mean values are listed in Table 4, the findings were; 114.767 μg/dL for iron (Fe) in IVF group, and for pregnant it was 93.200 μg/dL but for control group it is 70.000 μg/dL, all the results were compared statistically with a significant difference (P-Value $\leq$ 0.000), as it is shown in Table 4. As for the Cobalt (Co), the highest mean value is found in the control group with a value (0.142 μg/dL), while the value in pregnant group is lower than control group (0.138 μmol/dL), and the value of cobalt is the lowest in IVF group with a value of (0.093 μg/dL) with no significant differences between the groups (P-Value $\leq$ 0.066), regarding magnesium (Mg), the statistical analysis of MCP test shows that the control group holds the highest value (1.83 μg/dL), followed by pregnant group with a value of (1.518 μg/dL), while the lowest value of magnesium is found in IVF group with a value (1.28 μg/dL) with a significant statistical value between the groups ((P-Value $\leq$ 0.000), as depicted in Table 4.

4. Discussion

C. *albican* has lower frequency than C. *tropicalis* in IVF. The mixed biofilms formed with C. *tropicalis* indicated that C. *tropicalis* was able to limit the growth of C. *albicans*. Furthermore, the number of viable C. *albicans* cells in monotypic biofilms was significantly different from those in mixed biofilms, where C. *albicans* was reduced in the presence of C. *tropicalis* due to exhibited a significant decrease in metabolic activity in interaction group. Interestingly, decrease in the growth of C. *albicans* in presence of C. *tropicalis* could be attributed to an antagonistic relation between these two species. Therefore, C. *tropicalis* by reducing C. *albicans* virulence profile may limit the ability of this pathogenic fungus to cause infection. This agreed with previous study that evaluated the biofilm life cycle between *C. albicans* and *C. tropicalis* which proved the higher biofilm production of *C. tropicalis* [5].

Concerning *Candida* spp., results presented the data of Candida viable count between the three groups with higher counts in IVF groups than Pregnant, while the control shows the lower value. There could be several reasons why *Candida* spp. counts higher in the IVF group compared to pregnant group. Regarding hormonal changes, IVF involves hormonal stimulation to induce ovulation and prepare the uterus for implantation. These changes can affect the oral environment, making it more favorable for the growth of organism and this may differ between IVF and natural pregnancies [25].

IVF treatment may affect the immune system, leading to changes in immune response and susceptibility to oral infections that contribute to higher *Candida* spp counts in the IVF group. IVF treatment can be associated with higher levels of stress and emotional strain which linked to changes in oral health and an increased risk of oral infections. Additionally, lifestyle factors such as diet and oral hygiene practices may differ between the IVF and pregnant groups, potentially impacting their colonization [24]. Moreover, pregnancy is associated with immune system adaptations to accommodate the developing fetus. These adaptations can result in a slight suppression of the immune response, which can make pregnant individuals more susceptible to infections, including C. *albicans* [21].

Iron presence in saliva is one of a variety of nutritional factors has been associated in the pathogenesis of oral candidiasis, it is the most common fungal infection, caused by an overgrowth of opportunistic fungus *Candida* spp. in immunodeficiency hosts. Incidence of Iron deficiency anemia associated with pregnant women is more susceptible to oral candidiasis and C. *albicans* is the most frequent species in the oral cavity [21, 28]. The recorded data verified non-significant positive correlation between salivary Fe and *Candida* in pregnant, IVF and control groups. The previous studies showed that salivary Fe was negative correlation with serum Fe. These explain the increased growth of *candida* in T2 due to the presence of excess amount of Fe in saliva which is essential for *candida* proliferation [1, 20].

The results conveyed non-significance negative correlation between cobalt and *Candida* in pregnant, IVF and control groups. Cobalt is a necessary component of vitamin B12. As such, cobalt has no known nutritional function, except as a component of vitamin B12, so when we refer to the Co status, we are really referring to the vitamin B12 status [11]. It was found that patients with heavy *Candida* colonization had low levels of cobalt. In this category of susceptible patients, it seems that lower Vitamin B12 and consequently cobalt values may facilitate epithelial invasion by hyphae of Candida and contribute to heavy colonization [11, 27].

The data showed no significance negative correlation between Mg and *Candida* in pregnant group while IVF group shows significance negative correlation. This mineral is needed for *Candida* to survive as do all living organism. this manifestation could be elaborated due to Mg impact on immune system recognition toward *Candida* (through reducing hyphal damage, enhanced β-glucan exposure and altered vacuole homeostasis) that increase their level accordingly [12, 15]. Mg considered as a decreasing factor in growth rates of *Candidaalbicans* because of the effect on the germination tube configuration to prevent formation and effect on the divisions and growth [2]. The average levels iron (Fe) levels during pregnancy showed comprehensive statistical analysis that confirmed a highly significant variation across all groups with highest results obtained for IVF compared to pregnant (Table 4).

Studies observed that ferritin is presented in saliva with significantly higher levels compared to serum; moreover, it was noted that salivary ferritin serves as an indicator of iron deficiency anemia [10]. Consequently, it was suggested that iron plays a substantial role in pregnancy and in vitro fertilization (IVF). Notably, in the IVF group, ferritin levels were found to be elevated, possibly due to saliva conserves iron in the form of ferritin owing to its iron-dependent enzymatic function. Another potential explanation could be the endocytosis and excretion of ferritin by the salivary ducts [22]. Moreover, the rise in salivary ferritin levels may be attributed to the existence of high-molecular-weight iron-binding proteins and the uptake of ferritin from the intercalated ducts of the parotid gland. In addition, the increases in salivary ferritin levels could be linked to higher oxidative stress levels in the IVF group, which might have an impact on trace element levels [10, 22].

Cobalt is an essential trace element that accumulates more in women than men at similar exposure levels which may be related to higher metabolic iron loss especially during pregnancy [9]. Lower serum vitamin B12 levels are associated with a lower clinical pregnancy rate in IVFis well known that the core component of cobalamin in vitamin B12 is the corrin ring, which houses a central cobalt ion [17] so that depletion of vitamin B12 is concomitant with Cobalt reduction during pregnancy (with higher significance in IVF). Accordingly, it can be concluded that the cobalt level is more expected to be reduced in pregnant especially those with IVF than control.

The results of the Mg among the groups were evaluated. The IVF group exhibits the lowest magnesium value whereas the control group records the highest level. Notably, there exists a noteworthy statistical significance between the groups ((P-Value $\leq$ 0.000), Table 4. This could be justified that during pregnancy, a woman's body undergoes significant hormonal changes, including increased levels of hormones like estrogen which can impact the body's mineral balance, including magnesium. Magnesium metabolism is accurately controlled by estrogen through enhancing magnesium utilization and uptake by soft tissue and bone while level of plasma magnesium is decreased. So that it can be postulated that salivary magnesium reflects low serum value. This explains the increased demand of Mg during pregnancy that may lead to changes in magnesium levels in various bodily fluids, including saliva [8].

The magnesium value showed a lower concentration in the pregnant women saliva because of the association between magnesium and alkaline phosphates that the latter increases in the saliva of pregnant women [23]. Interestingly, the salivary magnesium level was lower in IVF compared to pregnant since IVF procedures can be emotionally and physically taxing, potentially leading to stress and anxiety that affect magnesium levels in the body [19].

5. Conclusions

- ✓ Traces elements play very important role in human daily activity
- ✓ Traces elements are different between the three groups; Iron is in highest level in IVF group, while cobalt and magnesium appears in highest level in control group.
- ✓ The variation in the level of the traces between the three groups (pregnant, IVF and control) could be related to hormones, medication, genetic, and medications

Authors contributions

The concept and study design was by AM, all the lab work and statistical analysis were carried out by LA, the critical revision were done by AM. Acquisition of data analysis, and the drafting of the manuscript was done by LA and AM.

Authors declaration

No conflicts of interest

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