FIBROBLAST GROWTH FACTOR-21 as a potent blood based biomarker in missed miscarriage

By

Israa Oueed Abdullah kadum[*1]; Dr. Israa Hashim Abdil Kareem[*2]

[*1] M.B.Ch. B, F.I.C.O.G/Consultant in Obstetrics and Gynecology/ Obstetrics and Gynecology Department/Salahaddin General Hospital; [*2]M.B.Ch. B, F.I.C.O.G/ Consultant in Obstetrics and Gynecology

Abstract:

Background: Missed miscarriage is very common complication, which affects about 15% of all clinically recognized pregnancies. The lack of prompt diagnostic biomarkers of missed miscarriage poses a great challenge for clinical implementation. Some authors reported that serum levels of fibroblast growth factor-21(FGF-21**)** may serve as a potential biomarker for the early recognition of missed miscarriage. **Aim of study:** To evaluate the role of FGF-21 as a biomarker for the missed miscarriage. **Patients and Methods:** A case control study that was carried out in the Department of Obstetrics and Gynecology during a period (January – September)2021. It included 100 pregnant women between 7 - 13 weeks of gestation with singleton pregnancy. They were divided into two groups: Case group included 50 pregnant women who presented with vaginal bleeding for the prior 12 hours and diagnosed as missed miscarriage and control group included 50 pregnant women with viable fetuses, matched with the other groups in age and gestational age. Four ml of venous peripheral blood was taken from each woman to test for FGF-21 biomarker. **Results:** In this study, women with missed miscarriage had significantly higher mean of fibroblast growth factor-21 compared with controls. FGF-21 level > 93.32 pg/ml is a predictor for missed miscarriage. Statistically significant weak negative correlation was detected between FGF-21 level and age. **Conclusion :** FGF-21biomarker represents a non-invasive, early, fast and excellent predictor of missed miscarriage which can be used if confirmed by further studies.

Keywords: Missed miscarriage, biomarkers, fibroblast growth factor-21: FGF-21, pregnancy, Iraq.

Introduction

Miscarriage is the spontaneous loss of a pregnancy before 12 weeks (early miscarriage) or from 12 to 24 weeks (late miscarriage) of gestation. Miscarriage can have considerable physiological and psychological implications for the patient[(1)]. There is a spectrum of terms including threatened abortion, inevitable or missed

miscarriage that describes different states of pregnancy loss. It is estimated that as many as 26% of all pregnancies end in miscarriage and up to 10% of clinically recognized pregnancies[2]. The most common cause of pregnancy loss in 1st trimester is chromosomal abnormalities.

Fibroblast growth factor 21(FGF-21) is an endocrine hormone that derived from the liver and exerts pleiotropic effects on the body to maintain overall metabolic homeostasis. The FGF-21 has diverse roles in development and signaling across a wide array of tissues. In addition to their vital roles in regulating cell growth, altered FGF function may contribute to diseases ranging from the cancer to bone disorders. The FGF-21 is part of the endocrine fibroblast growth factor subfamily that consists of three members, FGF-19, 21 and 23 all with hormone like actions[3]. FGF-21 shows its actions through acting on fibroblast growth factor receptors (FGFR) 1-4, despite most actions are mediated through FGFR-1[4]. The functional FGF-21 receptor complex consists of FGFR and its co-receptor β klotho (KLB), with both being essential for FGF-21 signaling[5]. The binding of FGF-21 to the receptor complex lead to monomeric dimerization of FGFR and auto-phosphorylation of FGFR tyrosine residues. This leads to the phosphorylation of tyrosine residues in the docking protein FGFR substrate-2 (FRS2).

Serum level of FGF-21 increases during the pregnancy period in humans[6]. Intriguingly, glucose transport genes parallelly increased with FGF-21 expression in the human placenta[7], indicating a potential role of FGF-21 in placental glucose metabolism. Importantly, abnormal high level of circulating levels of FABP4 and FGF-21 during pregnancy are commonly observed in patients with GDM, and gestational hypertension[8], which are all known risk factors for the onset of spontaneous miscarriage. However, the association between alterations in circulating levels of FABP4 and FGF-21 and the onset of MM still remains elusive[9].

Aim of study

To evaluate the role of fibroblast growth factor 21 as a biomarker for the missed miscarriage.

Patients and methods:

This is a case control study that was conducted in the Department of Obstetrics and Gynecology at Salahaddin General Hospital / Salahaddin Province during a period of eight months from (January – September) 2021.The study included 100 pregnant women between 7 - 13 weeks of gestation with singleton pregnancy attended the Outpatient Clinic, emergency or labor room. They were informed about the nature of the study and verbal consent was obtained from them. The data were collected on questionnaire which was designed for the study. Gestational age was calculated at the time of presentation according to first day of last menstrual period and confirmed by transvaginal ultrasound examination.

The women included in the study were divided into two groups.

- **Case group:** Included 50 pregnant women who presented with vaginal bleeding for the prior 12 hours and diagnosed as missed miscarriage.
- **Control group:** Includes 50 pregnant women with viable fetuses, matched with the other groups in age and gestational age.

Diagnosis of missed miscarriage was confirmed following the standard criteria based on transvaginal ultrasonography checking, which is defined as an oversized mean sac diameter of empty gestation sac (≥ 25mm) or embryo greater than 7 mm without the cardiac activity[(10)].

Exclusion criteria: Abortion type other than missed miscarriage; Presence of local gynecological disease (Uterine fibroids); Abortion in later pregnancy stage and Pelvic tumor.

Data collection tools: A questionnaire was applied to all enrolled pregnant women to collect the needed information. It includes questions to gather the following information: Age; Obstetrical history (Parity, last menstrual cycle, and gestational age); History of previous abortions; Chronic medical diseases; Body Mass Index (BMI): Calculated by weight in (kilograms) divided by the square of height in (meters). Weight and height are measured by the same scale for all the subjects. BMI = Weight (Kg) / Square height (m^2) [(11)].

FGF-21 test: A four ml of blood was drawn from the volar surface of the forearm from all pregnant women at presentation to test for FGF-21 levels. The FGF-21 kits principles were used enzyme-linked immune-sorbent assay based on biotin double antibody sandwich technology. Assay range: 5 – 1500 pg/ml.

Statistical analysis: The data analyzed using Statistical Package for Social Sciences (SPSS) version 26. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Independent t-test (two tailed) was used to compare the continuous variables between study groups. Pearson's correlation test (r) was used to assess correlation between FGF-21 level and certain parameters. Receiver operating characteristic (ROC) curve analysis was used for prediction of FGF-21 level as diagnostic of missed miscarriage. A level of P – value less than 0.05 was considered significant.

Results: The total number of study patients was 100. They were divided into two groups: Case group included 50 women diagnosed with missed miscarriage and control group included 50 women with pregnancies of normal births.

The distribution of study groups by general information is shown in figure and table (1). Study participants' age was ranging from 19 – 35 years with a mean of 26.48 years and a standard deviation (SD) of ± 4.3 years.

The highest proportion of case group was aged < 25 years (40%); while 38% of controls were aged between 25 – 29 years.

In this study, 68% of case group were obese and 52% of controls were overweighed; most patients in case and control groups were multiparous (88% and 86% respectively).

Regarding previous abortions, 42% of case group had more than one previous abortions; while 44% of controls had no previous abortions.

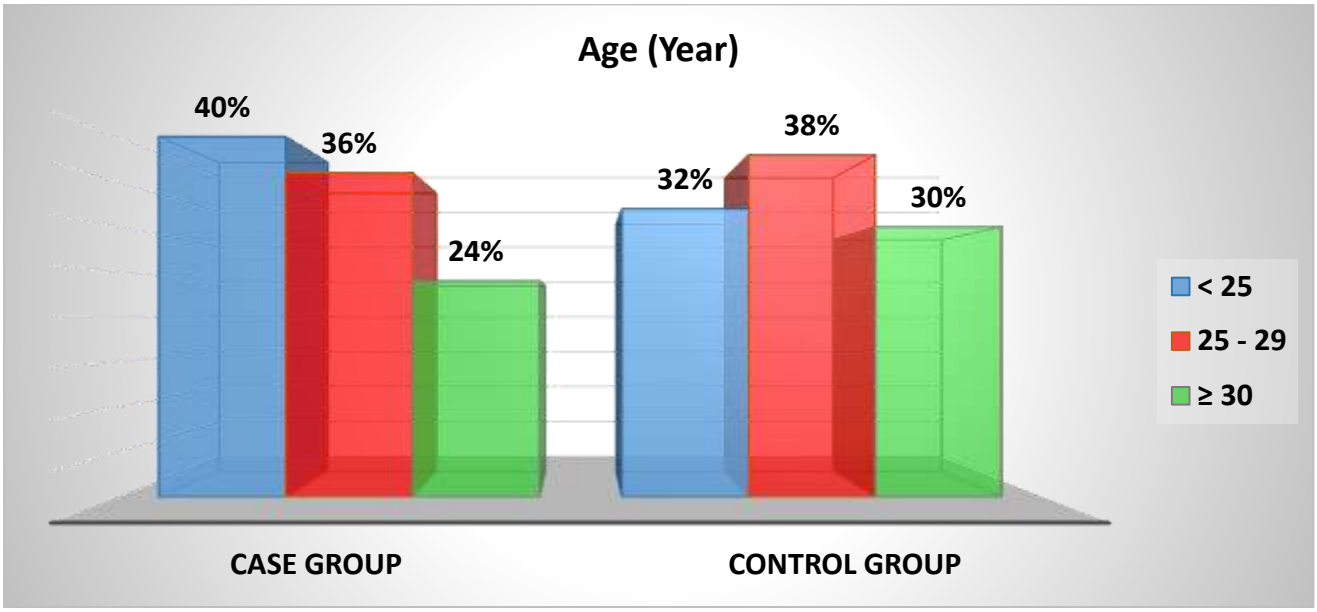

Figure (1): Distribution of study groups by age

Table (1): Distribution of the study groups by certain clinical characteristics

Clinical Characteristics	Study Groups		Total (%) n= 100
	Case (%) n= 50	Control (%) n= 50	
BMI level			
Normal	3 (6.0)	1 (2.0)	4 (4.0)
Overweight	13 (26.0)	26 (52.0)	39 (39.0)
Obese	34 (68.0)	23 (46.0)	57 (57.0)
			Parity
Nulliparous	6 (12.0)	7 (14.0)	13 (13.0)
Multiparous	44 (88.0)	43 (86.0)	87 (87.0)
Previous abortion			
No	14 (28.0)	22 (44.0)	36 (36.0)

one	15 (30.0)	15 (30.0)	30 (30.0)
More than one	21 (42.0)	13 (26.0)	34 (34.0)

The comparison between case group and controls by certain characteristics showed that mean of BMI was significantly higher in case group than controls (31.46 versus 29.66 kg/m^2, P= 0.012). There were no statistically significant differences (P ≥ 0.05) between the two groups in all other characteristics (Table-2).

Table (2): Comparison between study groups by certain characteristics

Characteristics	Study Group		P - Value
	Case Mean ± SD	**Control Mean ± SD**	
Age (Years)	25.78 ± 4.3	27.18 ± 4.1	**0.1**
BMI (kg/m^2)	31.46 ± 4.2	29.66 ± 2.7	**0.012**
GA (Weeks)	8.36 ± 1.5	7.88 ± 1.6	**0.124**
Parity	2.7 ± 2.1	2.34 ± 1.4	**0.323**
Previous abortion	1.24 ± 0.98	0.88 ± 0.93	**0.064**

In the present study, there was a statistically significant difference in FGF-21 levels between the study groups. Women with missed miscarriage had significantly higher mean of FGF-21 compared with controls (167.85 pg/ml versus 68.5 pg/ml, P= 0.001) (Table-3 and Figure-2).

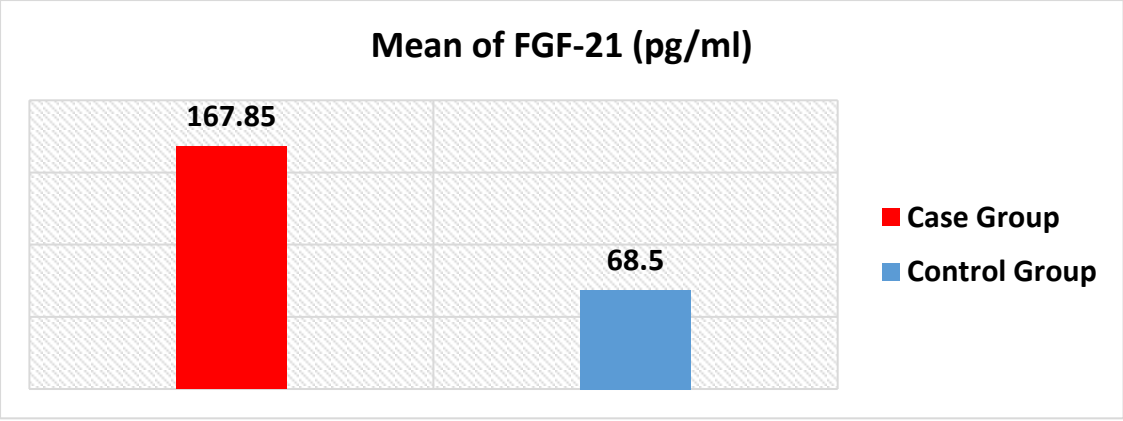

Figure (2): Mean of FGF-21 in study groups

Table (3): Comparison in mean level of FGF-21 between study groups

FGF-21 (pg/ml)	Study groups		P - Value
	Case Mean ± SD	Control Mean ± SD	
	167.85 ± 26.6	68.5 ± 16.6	**0.001**

Receiver operating characteristic (ROC) curve analysis was constructed for FGF-21 level as a predictor for missed miscarriage. The cut point of the FGF-21 level was 93.32 pg/ml. Hence, FGF-21 level > 93.32 pg/ml is a predictor for missed miscarriage, as a large significant area under the curve (AUC= 99.2%) indicating a significant association between the higher level of FGF-21 level and having missed miscarriage. FGF-21 level was 96% sensitive, 96% specific, and 96% accurate in diagnosis of missed miscarriage (Table -4) and (Figure -3).

Table (4): Diagnostic accuracy of FGF-21 level as a predictor for missed miscarriage

FGF-21 level (pg/ml)	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
	93.32	96%	96%	96%	96%	96%

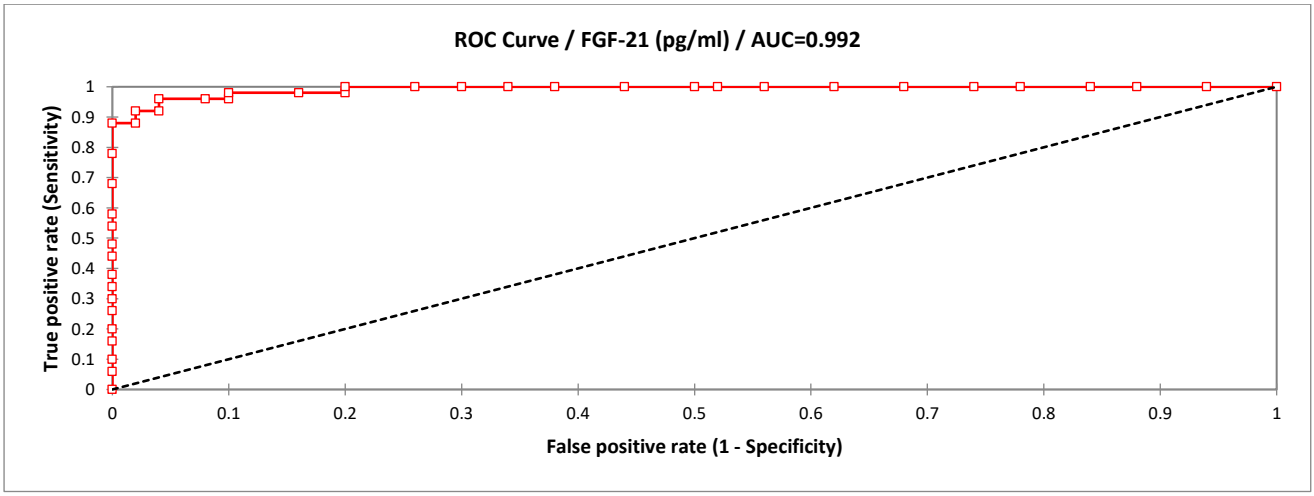

Figure (3): ROC curve for FGF-21 level in predicting of missed miscarriage

Correlation between FGF-21 level and certain parameters is shown in table (3.5) and figure (3.4). Statistically significant weak negative correlation was detected between FGF-21 level and age (r= - 0.217, P= 0.03).No significant correlation detected between FGF-21 level and all other parameters (P ≥ 0.05).

Table (5): Correlation between FGF-21 level and certain parameters

Parameter	FGF-21 (pg/ml)	
	r	P - Value
Age (Years)	- 0.217	0.03
BMI (kg/m^2)	0.151	0.134
GA (Weeks)	0.128	0.203
Parity	0.09	0.371
Previous abortion	0.07	0.486

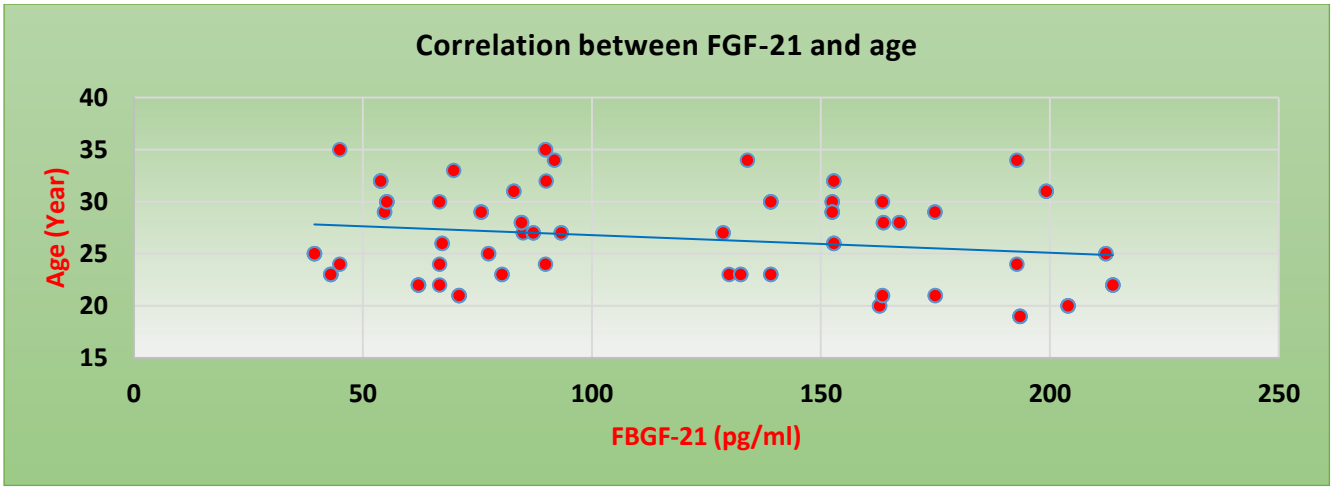

Figure (4): Correlation between FGF-21 and age

Discussion:

Missed miscarriage constitutes approximately 15% of clinically diagnosed pregnancies. Women presenting with a missed miscarriage may have no self-awareness due to the lack of obvious symptoms[12]. Despite the growing incidence of missed miscarriage, the current clinical implement remains imprecise and stagnant due to the absence of sensitive tools of biomarkers as risk factors[13]. Since missed miscarriage is usually unexpected without reliable clinical phenotypes and often presents pre-clinically, the onset of missed miscarriage could only be diagnosed on the basis of ultrasonography. The lag of diagnosis for missed miscarriage hindered the timely clinical manifestations[14]. So, it is imperative to screen accurately and promote tools for early identifying missed miscarriage with the potential for clinical practice. A cluster of metabolic hormones, as fatty acid binding protein-4 (FABP4) and fibroblast growth factor-21 (FGF21), are actively involved in the endocrine adaptations during pregnancy through regulating micronutrient flux and metabolic homeostasis[15]. Importantly, abnormal high level in circulating FABP4 and FGF21 during pregnancy are commonly observed in those with gestational diabetes mellitus, and gestational hypertension, which are all known risk factors for the onset of spontaneous abortion[8]. In the present work, 100 women enrolled, of which

50 women were diagnosed with missed miscarriage (Case group) and 50 women with normal births (control group).

In this study, cases of missed miscarriage had significantly higher mean of FGF-21 compared with controls (P= 0.001). Receiver operating characteristic (ROC) curve, revealed that cut point of the FGF-21 level was 93.32 pg/ml. Hence, FGF-21 level > 93.32 pg/ml is a predictor for missed miscarriage. FGF-21 level was 96% sensitive, 96% specific, and 96% accurate in diagnosis of missed miscarriage. Whereas application of this marker has been described widely in other literature as gestational DM or obesity, just a few papers describing its use in missed miscarriage, as found in the current literature.

In the same accordance, yang and colleagues in a study done at 2021, revealed that circulating levels of FGF-21 was significantly and independently elevated in patients with missed miscarriage relative to the levels in the healthy controls (P<0.05). Moreover, a single measurement of FGF-21 serum level effectively discriminated missed miscarriage with an area under the receiver operating characteristics curve of 0.80 (95% CI: 0.73-0.87) [(16)].

Actually, the placenta is the place of interaction between mother and baby, and it is, also, a metabolically highly active organ. It produces many hormones and growth factors with endocrine and paracrine effects, secreting into both maternal and fetal blood, in dependence on the properties of the hormone. It is unclear whether FGF-21 is expressed in the placenta, and if so, whether the expression changes in pregnancies affected by GDM. Placental FGF-21 expression could affect placental metabolism and nutrient transfer and so, the growth of the fetus. Alternatively, FGF-21 could be secreted into the fetal circulation and directly affect fetal metabolism[(17)]. FGF21 is one of the endocrine branch of the fibroblast growth factor family, that binds to general FGF receptors 1–4.

The receptor activity is influenced by the presence of the co-receptor β-klotho, which is mainly expressed in adipose tissue, liver, brain, and pancreas in the rodent[(18)]. In the liver, expression of FGF-21 is regulated by peroxisome proliferator-activated receptor (PPAR)-α, while in adipose tissue FGF-21 is regulated by PPARγ [(19)].

Vomstein et al study in 2021, studied FGF-21 expression in the placenta but did not recognized placenta as a major contributor to the levels of FGF-21. They observed a lower FGF receptor and other vascular growth factor levels in the placentas of missed miscarriages patients. Moreover, a histopathological examination showed altered syncytial sprouts in patients with missed miscarriages. This explained the role of FGF-21 pathway, not only in placental endocrine metabolism, but also in vascular development of the placenta. So, the

study not only provided insights in potential biomarkers for missed miscarriage, but also in the complex regulation of placentation in general. For example, an altered vascularization pattern was also shown in recurrent miscarriage patients with altered natural killer cell levels, indicating a possible link to immunomodulatory therapeutic strategies in patients with missed miscarriage[20].

Missed miscarriage is one of the common complications of pregnancy at the early stage. However, ultrasound checking as the most routinely used traditional diagnostic approach of missed miscarriage is a time consuming process and usually delayed[10], which represents a major barrier to both large scale diagnosis and therapies in advance. By contrast, blood-based biomarker assay would considerably raise the possibility to predicate the development of missed miscarriage in a prospective manner, whereby therapeutic intervention could be applied timely. Therefore, there is a huge unmet need to develop blood-based screening to identify the subset of individuals with MA[21].

Moreover, the functions of FGF-21 in gestation have not been fully explored. A growing body of evidence demonstrates the circulating levels of FGF-21 increase progressively throughout pregnancy and enjoys indispensable roles in coordinating the physiological adaption during pregnancy[22]. It is essential for the cardiac hypertrophy to compensate for the growing cardiac output especially at late stage, whereas deficiency of FGF-21 impaired pregnancy-induced cardiac remodeling during pregnancy[23].

Meanwhile, a dramatic increase of serum FGF21 is observed in mice at the end of pregnancy when they prepare to lactation[24]. In comparison to those positive influences, it was also reported that overexpressed FGF21 harms the physiological development process of the female reproductive system and affects their fertility capacity. FGF-21 overexpression transgenic mice exhibited hypogonadism compared to their wild-type littermates, which eventually leads to failure in mating and infertility[25].

This study revealed a significant weak negative correlation between FGF-21 and age ($r = -0.217$, $P = 0.03$), no correlation with BMI, GA, parity and previous abortion ($P \geq 0.05$).

In general, Circulating FGF-21 levels in humans increase as the age increase from five to 80 years in healthy individuals independently of body composition[26]. In contradiction, low levels of FGF-21 are related to healthy aging in centenarians[27]. In addition, endurance exercise in elderly individuals reduces FGF21 levels. Thus, it has been suggested that the increases in FGF21 that parallel aging are related to the appearance of an age-related FGF21-resistant state, as has been proposed in metabolic diseases[28].

In the current study, mean and SD of 26.48 ± 4.3 years (ranging from 19 – 35 years). The highest proportion of case group was aged < 25 years (40%), about 68% of case group were obese and 52% of controls were

overweighed; most patients in case and control groups were multiparous (88% and 86% respectively). Regarding previous abortions, 42% of case group had more than one previous abortions; while 44% of controls had no previous abortions.

In comparison to other studies, yang and other co-authors reported that mean age of patients with missed miscarriage was: 29.9 ± 0.5 years; and that of normal pregnancy was: 28.9 ± 0.4 years), and regarding BMI (mean BMI of patients with missed miscarriage was: 21.60 ± 0.46 kg/m^2, while that on normal pregnancy was: 22.15 ± 0.38 kg/m^2). Both groups were homogeneous in all BMI catalog[16].

A total of 72 women included in Ahmed et al study in 2020, in which the age range of participants was 17-45 years with mean and SD of age was 31.29 ± 6.11 years. The highest percentages of participants were in their second and third decades of life (40.5% and 37.5% respectively) and about 86.1% of them were un-employed (housewives) and majority about 57% of them attending primary school, and more than half percentage (63.8%) of them have intermediate economic state[29]. In a retrospective study conducted by Fang and colleagues in 2018, 694 cases of healthy pregnant women and 795 cases of women with missed miscarriage were enrolled. Mean age of control was 30.0±6.4 years and that of missed miscarriage was 30.6±5.7 years. Twenty-nine cases (4.2%) in the control group and 34 cases (4.3%) in the missed miscarriage group had lower abdominal pain. Five cases (0.7%) in the control group and 6 cases (0.8%) in the missed miscarriage group had at least one occurrence of vaginal bleeding[30].

In the current study, mean of BMI was significantly higher in case group (P= 0.012), while no significant differences with age, parity, gestational age, previous abortion ($P \geq 0.05$).

Yang et al study in 2021 reported a different finding, in which no significant relation observed between patients with missed miscarriage and those with normal pregnancy in regard of age and BMI (P>0.05), but found that mean of gestational age at their first prenatal care visits of the control group was significantly greater than subjects with missed miscarriage (P<0.001) [16]. In the same accordance, Fang et al study in 2018 observed a non-significance difference between study groups in concern to age, parity, lower abdominal pain (P>0.05) [31]. Differences in results of the above studies can related to different sample size and to the differences in socioeconomic status of the participants.

Missed miscarriage may cause maternal morbidity, as endometrial injury, coagulative dysfunction, depression, and anxiety. Presently, many etiologic factors as parental chromosomal abnormalities, immunological factors, endocrine disorders, uterine abnormalities, hereditary thrombophilia, infections, and environmental factors have been identified for missed miscarriage[1].

Conclusion: Fibroblast growth factor-21 biomarker represents a non-invasive, early, fast and excellent predictor of missed miscarriage which can be used if confirmed by further studies.

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