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Correlation of Erythrocyte Trans Fatty Acids with Ovulatory Disorder Infertility in Polycystic Ovarian Syndrome

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Abstract

Trans fatty acids are considered to be the important modifiable factor of the ovulatory infertility disorder. The purpose of this study was to test the hypothesis that higher trans fatty acids of erythrocytes (RBC) are associated with the risk of ovulatory infertility disorder in polycystic ovarian syndrome (PCOS).

Thirty five infertile women with polycystic ovarian syndrome, defined by AES criteria and 29 age-matched healthy women as a control group were recruited for the study. After physical measurements and nutritional assessment, blood samples were collected. Fasting serum glucose and insulin were measured, and then insulin resistance was calculated by homeostasis model assessment (HOMA-IR). Erythrocyte fatty acids were measured by gas chromatography.

The patients group had higher waist circumference (WC), insulin levels, HOMA-IR than controls ($p< 0.05$). Also, case group had lower percentage of normal BMI (BMI<25), physical activity and education levels than healthy women ($p< 0.05$). Among RBC trans fatty acids only trans linoleate (18:2t) were significantly higher in case group than control women ($p= 0.019$). PCOS group tended to consume more food rich in TFAs than the control group. Logistic regression analysis also showed that only 18:2t is positively associated with risk of ovulatory disorder infertility in PCOS (OR= 1.225, 95% CI. 1.024-1.465; $P= 0.026$), which was not affected after adjustment for BMI, physical activity and education levels.

The results suggested that RBC trans fatty acids might be a predictor of increased risk for ovulatory infertility disorder in women with PCOS.

Key words: Trans fatty acid; Infertility; PCOS; Erythrocyte

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Introduction

Infertility is a reproductive system disease that describes as a failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse (1). Approximately a third of infertile women referring infertility clinics have ovulatory disorder, and 90% of infertile cases are diagnosed as polycystic ovarian syndrome (PCOS) (2). In fact, it is estimated that PCOS is cause of around 20–30% of all infertilities (3). PCOS is a heterogeneous disorder with uncertain etiology, and the main causes of elevated ovulatory infertility disorder in such patients are hyperandrogenism, ovulatory disorder, and polycystic ovaries (4). In various countries, according to the criteria used in different studies for diagnosis and sampling, prevalence of PCOS was reported between 2.2% to 26%. (5-7). In Iran according to National Institutes of Health (NIH) definition, AES (Androgen Excess Society) criteria, and Rotterdam criteria (ESHRE/ASRM) prevalence of PCOS is estimated 7.1%, 11.7%, and 14.6% respectively (8). Despite considerable progress in assisted reproduction technologies, preventing infertility by identifying modifiable risk factors is more ideal option at the population level (9).

Composition of dietary fats, including fatty acids, is one of the modifiable risk factors influencing various aspect of metabolic health. However, limited studies have investigated possible role of these nutrients in infertility and specifically on PCOS subjects (10). Trans fatty acids (TFAs) are defined as unsaturated fatty acids with at least one double bond in the trans position, instead of the physiologic cis configuration. The main sources of TFAs, formed during the industrial manufacturing, are hydrogenated vegetable oils. Also, there are smaller amounts of trans fatty acid in meat and dairy products which are produced by the action of bacteria in the ruminant stomach (11). In Iranian homes, hydrogenated vegetable oils are generally used for cooking and average daily intake of each person is estimated to be 14g per 1,000 kcal (12). Saturated fatty acids (SFAs) and TFAs (33% of total fatty acids) are the main combination of these products. In other word, the average intake of all calories derived from TFAs are 4.2%, which is about twice the amount consumed in many developed countries (13, 14). Little is known about reproductive health effects of trans fats. Data obtained through a Food-Frequency Questionnaire (FFQs) as part of the Nurses' Health Study have shown that substituting energy intake, from trans fats with unsaturated fats or carbohydrates, was associated with considerably increased risk of ovulatory infertility (15).

The method that has been used for assessing TFA consumption in previous studies is FFQs (15). Despite being commonly used, the main disadvantage of FFQs is being memory dependent which causes recall bias. Also, in countries without accurate food composition data for some nutrients such as TFAs, using appropriate biomarker of dietary intake is preferred (16). Because the production of trans double bonds fatty acids chain in human body metabolism is impossible, erythrocyte TFAs can be used as a more stable biomarker for measuring the medium-term consumption (i.e., months) of dietary TFAs (17, 18).

TFAs are a modifiable risk factor if their role is identified in ovulatory disorder infertility. The purpose of this study was to investigate erythrocyte TFAs content as a biomarker of diet in infertile women with ovulatory disorder compared with healthy aged-matched control.

Materials and Methods

Study population

Females aged 19 to 35 years visiting one of the private Reproductive Medical Centre during the period of February till April of 2013 for infertility and suspected to have PCOS underwent standardized initial evaluation. A total of 35 patients were identified as PCOS cases according to the AES (Androgen Excess Society 2006) criteria (4) and 29 age-matched healthy women (without any infertility and PCOS disorders) were recruited in the study as the control group. Inclusion criteria for case group were: being married, clinical and/or biochemical hyperandrogenism, ovarian dysfunction, oligoanovulation and/or polycystic ovaries by ultrasound scans of the ovaries. Exclusion criteria were: having congenital adrenal hyperplasia, androgen-secreting tumors, taking androgenic/anabolic medications, Cushing syndrome, severe insulin resistance syndrome, thyroid dysfunction, hyperprolactinemia, pregnancy, diabetes, hypertension, CVD, taking vitamins and supplements during the 3 months prior to the study, evidence of recent or recurrent infection, smoking or drinking alcohol.

Physical measurements

Weight and height were measured without shoes and with minimal clothing. Digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg for weight and a non-stretchable tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm were used for measuring, respectively. For calculating body mass index (BMI), weight in kilograms divided by the square of height in meters, was used. Waist circumference was (WC) measuring was performed by the tape around the waist just above the uppermost lateral border of the iliac crest (19).

Nutritional assessment

Dietary intakes of selected foods which are rich in trans fatty acid (hydrogenated vegetable oils, liquid oils, frying oil, butter, fast foods, milk, yogurt, cream, cheese, ice cream, biscuit and cake) were estimated using self-administered, semi-quantitative FFQ (20). Mean servings of foods rich in trans fatty acids consumed each day were calculated according to the exchange lists for diabetes (21).

Serum analysis

After 12-h overnight fasting, blood samples were collected. Serum and plasma samples were separated using centrifuge at 1500 rpm for 15 minutes by Beckman Avanti^{TM} 30 centrifuge (Beckman, Palo Alto, USA). Fasting insulin levels were measured using ELISA kits (Monobind Inc., AcuuBindTM, Norwalk , USA). Fasting blood sugar (FBS) was measured using enzymatic procedures by an automatic analyzer (Abbott, Alcyon 300, USA). For calculating insulin resistance (IR) homeostasis model assessment (HOMA) was used. (HOMA−IR= fasting insulin (μU/mL)×fasting glucose (mmol/L)/22.5) (22).

Erythrocyte fatty acid measurement

Blood samples were taken into the EDTA after 12-hour night fasting from the antecubital vein. Erythrocytes were used for fatty acid analysis. Whole Bloods samples were centrifuged at 2500 rpm for 10 minutes by Beckman Avanti^{TM} 30 centrifuge for erythrocyte fatty acids analysis. Plasma and buffy coat were discarded. Packed erythrocytes were washed with normal saline three times. Washed erythrocytes were then stored at –70^{o}C until analysis. Fatty acids in erythrocytes were analyzed by gas-liquid chromatography (Buck Scientific 610, Norwalk, USA) (23, 24). The relative amount of each fatty acid was presented as the percentage of total area on chromatograms. The analyses for the fatty acids were also carried out according to the major fatty acid classes: saturated fatty acids (SFAs: 14:0, 16:0, 18:0 and 20:0), trans fatty acids (16:1t, 18:1t and 18:2t), unsaturated fatty acids (UFAs: 16:1, 18:1, 18: 2, 18:3, 20:4, 20:5

Table 1: Descriptive and metabolic characteristics of control and infertile PCOS groups*

Variable	Case (n=35)	Control (n=29)	*P* value†
Age	26.36 ± 4.2	27.96 ± 2.47	0.074
BMI (kg/m^2)	26.05 ± 3.49	25.08 ± 3.51	0.282
BMI (%)			
BMI < 25	27.8	51.7	0.049
BMI ≥ 25	72.2	48.3	
Waist circumference (cm)	94.77 ± 10.36	85.07 ± 8.48	<0.001
Physical activity (%)			
Low	667	37.9	0.021
Medium	33.33	62.1	
Education (%)			
Diploma or less	66.7	34.5	0.01
University education	33.3	65.5	
SBP (mmHg)	118.67 ± 8.99	116.89 ± 6.04	0.209
DBP (mmHg)	78.19 ± 6.98	76.38 ± 5.16	0.274
Fasting blood sugar (mg/dL)	92.56 ± 8.78	89.86 ± 8.26	0.216
Insulin level (µU/mL)	17.05 ± 9.19	10.05 ± 3.26	<0.001
Insulin resistance (HOMA-IR)	3.77 ± 2.23	2.27 ± 0.85	0.001

*Plus-minus values are mean±SD.

†*P* value estimates are based on un-paired t-test for variables expressed as mean±SD and χ^2 test for variable expressed as percentages.

BMI, body mass index, SBP, systolic blood pressure SBP; DBP, diastolic blood pressure

Table 2: Erythrocytes fatty acid composition in subjects*

% of total fatty acid	Case (n=35)	Control (n=29)	*P* value†
14:00	1.22 ± 0.46	1.08 ± 0.40	0.186
16:00	38.91 ± 5.78	38.71 ± 7.12	0.901
16:1t	0.56 ± 0.25	0.53 ± 0.37	0.685
16:1c	0.92 ± 0.23	0.86 ± 0.43	0.54
18:00	13.42 ± 3.42	13.58 ± 3.49	0.859
18:1t	1.96 ± 0.71	1.87 ± 0.83	0.622
18:01	12.48 ± 1.19	12.99 ± 2.37	0.28
18:2t	0.98 ± 0.36	0.79 ± 0.29	0.019
18:2c	12.53 ± 2.87	13.94 ± 3.69	0.096
20:00	0.89 ± 0.38	0.88 ± 0.42	0.933
18:3c	0.88 ± 0.43	0.79 ± 0.44	0.375
CLA	0.51 ± 0.33	0.54 ± 0.29	0.738
20:04	13.05 ± 3.18	11.13 ± 3.15	0.019
EPA	0.59 ± 0.27	0.64 ± 0.33	0.481
DHA	0.93 ± 0.44	1.12 ± 0.68	0.177
TFAs	3.51 ± 0.88	3.18 ± 1.01	0.174
SFAs	54.45 ± 5.96	54.25 ± 5.23	0.887
MUFAs	15.91 ± 1.49	16.21 ± 2.41	0.563
Omega-3	2.39 ± 0.8	2.55 ± 1.05	0.528
Omega-6	27.15 ± 4.61	26.47 ± 4.62	0.568

* Fatty acid contents are expressed as a percentage of total fatty acids in erythrocytes. Plus-minus values are mean±SD. CLA: with 3 related papers, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, TFA: trans fatty acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids

† Un-paired t-test

and 22:6), mono unsaturated fatty acids (MUFAs: 16:1 and 18:1), omega-3 fatty acids (18:3, 20:5 and 22:6), omega-6 fatty acids (18:2 and 20:4).

Statistical analysis

Data were presented as mean ± SD. Statistical significance was tested using unpaired t-test (or Mann-Whitney U test for non-normally distributed data). Data regarding categorized characteristics were analyzed by Fisher-exact test and Chi-square. The studied groups were also compared after adjusting for confounding variables using logistic regression. For all tests, p-value <0.05 was considered statistically significant. Data were analyzed using SPSS software version 20.

Result

Basic characteristics of 35 cases and 29 age-matched controls women are shown in Table 1.

Women in case group significantly had higher WC, insulin levels and insulin resistance (HOMA-IR) than controls (P<0.05). Also, case group had lower percentage of normal BMI (BMI<25), physical activity and education levels than healthy women (P<0.05). Age, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP) and fasting serum glucose were not statistically different between the two groups. Erythrocytes fatty acid compositions are displayed in Table 2.

Table 3: Mean serving of food rich in *trans* fatty acids contents consumed by the control and PCOS groups according to the FFQs*

Variable	Case (n=35)	Control (n=29)	*P* value†
	Median (Q1-Q3)	Median (Q1-Q3)	
Hydrogenated vegetable oil (1tsp)	6.42 (0-12)	0 (0-0.51)	< 0.001
Frying oil (1tsp)	3.42 (0-6)	3.21 (1.28-4.5)	0.8
Butter (1tsp)	0.57 (0-0.96)	0.5 (0.21-0.57)	0.947
Fast Food (1exchage)	0.2 (0.07-0.33)	0.11 (0.08-0.33)	0.504
Cheese (1oz)	1 (0.31-1)	0.5 (0.24-0.5)	0.006
Ice-cream (1/2cup)	0.21 (0.03-0.38)	0.07 (0.01-0.17)	0.055

* The values are shown as medians and interquartiles (Q1-Q3)
† Mann withney test

Among RBC trans fatty acids only trans linoleate (18:2t) was significantly higher in case group than control women (p= 0.019). Also, arachidonic acid (20:04) was significantly higher in patients than controls (p= 0.019). No other significant differences were observed in other fatty acids or the major classes of fatty acids between the PCOS and control group. The PCOS group consumed more total serving of food rich in trans fatty acids contents than the control group. According to the FFQs, the patients group consumed significantly more

hydrogenated vegetable oil (p<0.001), cheese (p=0.006) and ice-cream (p=0.055) than healthy women. There was no significant difference in the reported consumption of other specific food rich in trans fatty acids (Table 3).

Table 4 shows the trans fatty acids content in erythrocytes and the risk of ovulatory disorder infertility in PCOS. Logistic regression analysis showed that only was 18:2t positively associated with the risk of ovulatory disorder infertility in PCOS; each 0.1% increase in 18.2t content of erythrocyte had 22% (95% CI. 1.024-1.465; p= 0.026) higher risk of ovulatory disorder infertility in PCOS, even after being adjusted for BMI, physical activity and education levels (Model 1: OR=1.218, 95% CI. 1.016-1.46; p=0.033), (Model 2: OR: 1.216, 95% CI. 0.994-1.487; p=0.057). There was not significant association between total trans fatty acids, 16:1t and 18:1t and PCOS before and after adjustment for confounding variables.

Table 4: Odds Ratio of ovulatory disorder infertility in PCOS associated with *Trans* Fatty acid content in erythrocytes

Erythrocyte TFA	% Median (Rang)	Odds Ratio	95% Confidence Interval	P value
Trans Palmitate (16:1t)	0.4.95 (0.1-1.23)	1.035	0.88-1.216	0.68
Model 1*		1.014	0.859-1.198	0.867
Model 2†		1.018	0.938-1.397	0.183
Trans Oleate (18:1t)	1.94 (0.35-3.54)	1.017	0.952-1.086	0.616
Model 1*		1.021	0.955-1.091	0.545
Model 2†		1.029	0.957-1.107	0.435
Trans Linoleate (18:2t)	0.94 (0.14-2.02)	1.225	1.024-1.465	0.026
Model 1*		1.218	1.016-1.46	0.033
Model 2†		1.216	0.994-1.487	0.057
Total *trans* fatty acids	3.39 (1.1-5.32)	1.038	0.984-1.096	0.173
Model 1*		1.037	0.982-1.095	0.187
Model 2†		1.055	0.992-1.121	0.086

*OR was adjusted for BMI

† OR was adjusted for BMI, physical activity and education

Discussion

PCOS is the common cause of anovulatory infertility among young women. Hyperinsulinemia and insulin resistance induced by overweight and obesity, particularly abdominal adiposity, have substantial role in the pathogenesis of PCOS (10). In our study, similar to the previous reports, subjects with PCOS had higher fasting insulin concentration insulin resistance (HOMA-IR), WC and rates of obesity than age-matched control women.

In this case-control study, we observed a statistically significant increase in the arachidonic acid (20:4) levels in PCOS relative to the mean value in the control group. Furthermore, there was significantly different erythrocyte trans linoleate (18:2t) contents between case and control groups, and positive independent association between 18:2t and risk of ovulatory disorder infertility in PCOS women. Consistent with these finding, also, patients consumed more food rich in TFA compared to healthy women.

Because PCOS is a chronic disease with hormonal and metabolic symptoms, lifelong strategies that prevent long-term health consequences are essential. Data on the relation between fatty acids and the ovulatory disorder infertility in PCOS are still scarce. The available studies have assessed fatty acids in the diet only with food records (15, 25, 26), not as a biomarker. Chavarro et al., in a prospective cohort study, estimated that total daily energy intake above 2% in the form of unsaturated trans fatty acids was associated with 94% risk of the occurrence of ovulatory infertility (15). A potential mechanism through which trans fatty acids may increase the ovulatory infertility disorder in PCOS is the insulin resistance (27).

TFAs have been found to down-regulate peroxisome proliferator-activated receptor γ (PPAR-γ) expression (28). PPAR-γ is a nuclear transcription factor whose activation improves ovulatory function in women with PCOS (15). Other health effects of higher consumption of TFAs include dyslipidemia, inflammation, endothelial dysfunction, weight gain, diabetes and coronary heart disease (CHD) (27). All of these complications may also adversely affect ovulatory function in the PCOS women.

TFAs isomers have not yet been investigated in infertile PCOS women. However, studies using biomarker of TFA consumption have shown that both 18:1t and 18:2t isomers contributed to the risk of CHD. Among the TFAs, the available data suggest that 18:2t isomers may be more strongly associated with CHD risk than 18:1t isomers (27). Although, most studies did not detect any effect for 16:1t, in our studied population, the levels of total TFA were relatively high (≈ 3.3% of total erythrocytes fatty acids); however, no significant association was found between total TFA and PCOS. These findings confirm the hypothesis that the biological effects of different TFA on ovarian function are not the same.

For synthesis of many active fatty acid metabolites such as prostaglandins arachidonic acid is considered a precursor (29). In consistent with our findings, the amount of arachidonic acid has been correlated with reduced fertilization rate (30). Increased levels of linoleic acid and reduced levels of arachidonic acid in dominant follicles have also been reported previously (30). Since this fatty acid is used in the production of inflammatory mediators, it seems that its increase in the phospholipids content has a negative effect on fertility. These findings are in agreement with the fact that fatty acids play a key role in the oocyte maturation process.

Due to the lack of necessary data about food composition of different fatty acids especially TFAs in Iran, using FFQ questionnaire to determine the level of trans fatty acids intake was impossible. On the other hand, recognizing

quantity of fat used in food preparation is sometimes very difficult. In fact, biomarkers reflecting the consumption of fatty acids are more accurate and preferable. As regards, humans cannot synthesize trans fatty acids, measuring trans isomers contents of human tissues would closely reflect trans fatty acids intake of an individual (18, 31). Erythrocyte membranes fatty acid contents, as a biomarker, reflect intake aggregated over the lifespan of erythrocyte, or ~120 day, the half-life of erythrocytes (32).

In our study, trans fatty acids were measured as a biomarker in the erythrocyte that are not subject to reporting errors; this could be considered one of the strengths of the current study include. For the first time, selected subjects were limited to PCOS with ovulatory disorder infertility and with no interfering health conditions. However, the number of study population was relatively low and generalization of the findings is somewhat limited. Assessment of different isomers of 16:1t, 18:1t and 18:2t trans fatty acids individually in a larger sample size would be warranted.

In conclusion, this study showed that trans linoleate (18:2t) in erythrocytes was associated with an increased risk of PCOS, while other TFA showed no significant change. Moreover limitation of partially hydrogenated oils consumption as a main source of TFAs may result in preventing effects of TFAs on infertility disorders and lead to substantial health benefits in women.

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