Taiwanese Journal of Obstetrics & Gynecology 57 (2018) 427–431

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Evaluation of thiol/disulphide homeostasis as a novel predictor testing tool of early pregnancy viability

Hatice Akkaya [a, *], Gülsüm Uysal [b], Barış Büke [c], Gamze Gök [d], Özcan Erel [d], Çiğdem Karakükçü [e]

[a] Department of Obstetrics and Gynecology, Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey
[b] Department of Obstetrics and Gynecology, Adana Numune Training and Research Hospital, Adana, Turkey
[c] Department of Perinatology, Kayseri Training and Research Hospital, Kayseri, Turkey
[d] Faculty of Medicine, Department of Clinical Biochemistry, Yildirim Beyazit University, Ankara, Turkey
[e] Department of Clinical Biochemistry, Kayseri Training and Research Hospital, Kayseri, Turkey

ARTICLE INFO

Article history:
Accepted 2 April 2018

Keywords:
Missed abortion
Thiol
Disulphide
Oxidative stress
Ultrasound

ABSTRACT

Objective: To evaluate serum dynamic thiol/disulphide concentrations in patients with suspected missed abortion (MA) and to determine whether this ratio has a predictive role in the viability in these pregnancies.

Materials and Methods: In this prospective cohort study, 48 out of 120 recruited pregnant patients were confirmed MA in the study group. Following the recommended waiting time (7–10 days), the remaining 72 viable pregnancies that met the inclusion criteria were categorized as the control group. A novel, automated, and spectrophotometric assay, which can measure both sides of the thiol/disulphide balance, was used. The cut-off values were used for the ROC curve.

Results: There were no statistically significant differences between the groups (MA and control) regarding maternal obstetric and demographic features. Significantly reduced levels of Total Thiol and Native Thiol were shown in patients with MA compared to the control group ($p = 0.016$ and $p = 0.001$, respectively). Serum levels of disulphide was significantly increased in the MA group (23.4 ± 7.8 μmol/l vs. 17.9 ± 4.99 μmol/l, $p < 0.0001$). Disulphide values of less than 17.68 predicted 80.8% of the viable pregnancies.

Conclusion: Significantly increased serum disulphide levels, one of the oxidative stress markers, and decreased antioxidant levels (total and native thiol) were found in patients with MA. Increased oxidative stress status is thought to play a role in the etiology of MA. Serum dynamic thiol/disulphide homeostasis may serve as a promising testing tool to rule out subsequent diagnosis of MA and may benefit as an early pre-treatment testing tool for viability.

Introduction

Non-viable products of conception that are retained for days, weeks, or even months in the uterus with a closed cervical os has been defined as missed abortion (MA) [1]. It is a silent arrest of embryonic or fetal development. Fetal pole without cardiac activity or an empty gestational sac confirm diagnosis during sonographic examination. When we consider the development of ultrasound imaging technologies, the diagnosis and incidence of MA is steadily increasing (3.89–14.1%) [2]. Treatment options are medical or surgical evacuation of the uterus [2,3]. Although parental chromosomal abnormalities (more than half of all lost pregnancies), hereditary thrombophilia, endocrinological disorders, immunological factors, infections, apoptosis, oxidative stress, and environmental factors have been attributed for the etiology, the exact pathophysiology has not yet been clarified [1–3]. However, it is thought to be multifactorial.

Paradisi et al. demonstrated higher IL-12 and impaired Th1/Th2 rates in patients with MA [4]. Tian et al. also found higher serum cortisol levels and increased IL-12 concentrations and higher stressor numbers in patients diagnosed with MA [5]. In another study, Sapmaz et al. found a highly positive correlation between IL-

* Corresponding author.
E-mail address: doktorhakkaya@gmail.com (H. Akkaya).

https://doi.org/10.1016/j.tjog.2018.04.034

6 (inconsistent with the classical view of T helper 1/T helper 2 activity change), c-reactive protein (CRP), leukocyte, and neutrophil values in patients with MA [6]. Therefore, both stress and cellular immunity imbalance were presented as risk factors of MA. In fact, pregnancy has been shown to be a process that goes with a cooperation of Th1 and Th2 activity instead of impaired Th1/Th2 rates. For this reason, pregnancy failure, such as MA, should be evaluated in human homeostasis.

Human cells are equipped with enzymatic and non-enzymatic systems for antioxidation in order to maintain redox homeostasis, which is vitally important for growth and survival [7]. Oxidative stress (OS) is the process of a shift to an impaired free radical production and removal,which results with oxidative damage [8].

In fact, there is a balance between the free radicals and antioxidant defense system. Homeostasis maintains internal stability, owing to the coordinated response of its parts to any situation or stimulus that would tend to disturb its normal condition or function.

Thiols are organic molecules comprising sulfhydryl groups and has an important role in redox hemostasis through oxidation and/or reduction reactions. Albumin and other plasma proteins, such as cysteine, glutathione, thioredoxin and homocysteine comprise plasma thiols. Under oxidative conditions, thiol groups are reversibly oxidized to form disulfide linkages, which are the bonds between two cysteine residues of proteins. There is an interconversion between thiol (the reduced state) and disulfide groups (the oxidized state); therefore, a balance between thiol and disulfide groups must be maintained [9].The dynamic disulphide is defined as half of the difference between the amount of the total and native thiols and is the result of the interconversion between thiol and disulfide groups. The dynamic disulphide is assigned in detoxification, apoptosis, many enzymatic processes, and cellular signaling pathways [10].

The aim of this study is to evaluate serum dynamic thiol/disulphide concentrations in patients with suspected MA and to determine whether serum dynamic thiol/disulphide homeostasis has a predictive role in the viability of these pregnancies. Thiol/disulphide homeostasis has been investigated via a novel technique, defined by Erel and Neselioglu in 2014, and enables the detection of both sides of the balance in a practical way [11].

Materials and methods

This was a prospective cohort study involving 120 consecutive women with a first-trimester pregnancy that underwent transvaginal ultrasonographic examination between April and December 2016. This study was conducted at a tertiary hospital in the Kayseri province of Turkey, and the study protocol was approved by the local ethics committee. After obtaining informed and written consent from all of the participants, obstetric and general health data were collected.

A total of 120 pregnant women suspected for MA were evaluated with regard to early pregnancy outcomes. Initially, all cases were assessed by ultrasonography. A single experienced clinician(H.A.) performed all ultrasound examinations via a Mindray DC-7 ultrasound machine (Shenzhen Mindray Bio-Medical Electronics Co., China) equipped with a 5–8 MHz multi-frequency endovaginal probe in the lithotomy position. Assessment of pregnancy viability and shape of gestational sac, yolk sac, and measurement of crown–rump length (CRL) were completed. Later, blood samples were taken from all cases.

The statistical program on the website of the statistical department of the University of British Colombia was utilized to calculate the sample size and the power of our study (http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html). According to this calculation, the inclusion of 38 patients in each study group with 80% confidence interval and $p < 0.05$ significance level was calculated as an enough for the sample size of this study.

Patient selection

Suspicious of MA regarding criteria was defined in the Society of Radiologists in 2012. The criteria were as followed: Crown–rump length of less than 7 mm and no heartbeat, mean sac diameter of 16–24 mm and no embryo, absence of embryo with heartbeat 7–13 days after a scan that showed a gestational sac without a yolk sac, absence of embryo with heartbeat 7–10 days after a scan that showed a gestational sac with a yolk sac, absence of embryo ≥6 wk after last menstrual period, empty amnion (amnion seen adjacent to yolk sac, with no visible embryo), enlarged yolk sac (>7 mm), and small gestational sac in relation to the size of the embryo (<5 mm difference between mean sac diameter and crown–rump length) [12].

It was also suggested to follow-up ultrasonography for 7–10 days to assess the pregnancy for viability when there were suspicious findings for pregnancy failure [12]. According to these mentioned criterias, we evaluated 120 pregnant patients suspected with MA. Blood samples were taken during the first ultrasonography evaluation. Then, ultrasonography was performed again after the waiting period. Forty eight ultrasound confirmed non-viable gestation cases were diagnosed with MA after the recommended waiting time (7–10 days) while, 72 viable cases comprised control group. Patients in the control group were followed up at least until the second trimester.

Spontaneous, singleton first trimester pregnancies were included in the study group. Multiple gestations, history of recurrent pregnancy loss, systemic disease, evidence of fetal or maternal infection, hypertensive disorders of pregnancy, gestational and pregestational diabetes mellitus, use of steroids, antibiotics, other anti-inflammatory drugs and any derivative of progesterone, illicit substances, or medications usage were stated as the exclusion criteria for the study group.

Sample collection

Blood samples were collected from the antecubital veins of all patients just after ultrasonographic diagnosis of MA before the recommended waiting time (7–10 days) in the study. After recommended waiting time viable cases were categorized as the control group. The blood samples were centrifuged at 3,000g for 10 min and were frozen and stored at −80 C until the day of analysis. Thiol/disulphide levels were analyzed with a newly developed method by Erel and Neselioglu [11]. They described this novel technique as an easy, inexpensive, practical, faster (turn around time of about 10 min), and fully automated in their study.This new method, which was modified by adding a formaldehyde solution to the classic Ellman's reagent, can measure both sides of the thiol/disulphide balance using an automated assay. The principle of the new method is sodium borohydride ($NaBH_4$), which is used to reduce the disulphide bonds to the thiol groups. The $NaBH_4$ residuals that are not used are totally removed using formaldehyde. Therefore, the extra reduction of 5,50-dithio-bis-(2-nitrobenzoic acid) (DTNB) is prevented. The difference between the total amount of thiol and the native thiol is divided by two to calculate the disulphide bond. Measurements were made using a Cobasc501 (Roche Diagnostics, Mannheim, Germany) and serum thiol/disulphide homeostasis values were stated as μmol/L.