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ISSN:2149-455X
Makale / Article

Serum Asymmetric Dimethyl Arginine Level is Associated with the Pain Intensity in Primary Dysmenorrhea

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Abstract

Background: Aim of this study is the investigation of the association between Serum Asymmetric Dimethyl Arginine (ADMA) levels and menstrual pain intensity as assessed by Visual Analogue Scale in primary dysmenorrhea patients.

Methods: Eighty-nine young women with primary dysmenorrhea (PD) were selected as study group. PD pain was graded using Visual Analogue Scale (VAS), as mild, moderate and severe. Serum ADMA levels was measured in all subjects.

Results: Mean age of study population was 21.08±2.21 and mean VAS score was 5.9101±2.18. Serum ADMA levels were 35.2(24) in mild, 54.0(28.7) in moderate and 56.5(32.8) in severe pain groups, (P= 0.029 and P=0.001, respectively). Mean ADMA levels difference were -18.8, ρ=0.046 in mild to moderate; -2.52, ρ=0. 945 in moderate to severe and 21.32 ρ=0.048 in severe to mild pain groups.

Conclusion: Menstrual pain intensity is correlated with the serum ADMA levels as assessed by VAS pain score in PD patients.

Keywords: Asymmetric Dimethyl Arginine, Pain Intensity, Primary Dysmenorrhea, Visual Analogue Scale.

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Introduction

Primary dysmenorrhea (PD) is chronic recurrent lower abdominal pain occurs during the menstrual periods healthy young women without any detectable etiology. Prevalence of PD varies between 40-60% among young age women[1]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the main first line treatment method and mentions briefly the use of hormonal intrauterine devices (IUDs), which have been shown to reduce menstrual bleeding and pain[2]. Definite pathogenesis of PD remains unclear but previous studies showed that prostaglandins and vasoactive mediators are increased in the endometrium and menstrual specimens[3]. Recent studies showed that some hormonal or vascular endothelial functional changes such as increased vasopressin and increased serum asymmetric dimethyl arginine (ADMA) levels cause vasoconstriction, uterine contractions and eventually uterine ischemia that is related to the menstrual pain in PD. PD also affects other systems and might lead to increase in the risk of cardiac arrhythmia or mental status change during menstrual period[1,3]. Women with dysmenorrhea have greater sensitivity to experimental pain both within and outside areas of referred menstrual pain compared to with women without dysmenorrhea[4].

ADMA is an important marker in a variety of clinical settings. Although it shows endothelial dysfunction as an underlying pathogenesis of atherosclerosis, hypertension and diabetic vascular complication, it is also associated with PD, PCOS and pregnancy related disorders such as preeclampsia and hyperemesis gravidarum[5]. Serum ADMA levels are significantly decreased by oral contraceptives in women with PCOS. Orally administered hormone replacement therapy (HRT) also has an effect on circulating ADMA levels[6].

Most important symptom of PD is chronic pelvic pain with varying degrees with or without associated symptoms. There are many studies about the pain intensity grading in the published literature for further understand the disease severity, guide the decision for treatment and motorize the therapy[7-10].

The Visual Analog Scale (VAS) is a pain intensity-grading tool and is being used widely in research and clinical practice. Reliability and validity of VAS score has been verified[11]. Pain intensity measured by the VAS is significantly correlated well with those measured by verbal and numeric scales. In this study, we aimed to evaluate the association of serum ADMA levels and menstrual pain intensity as assessed by VAS score in PD patients group.

Subjects and Methods

Patients

Study cohort has been selected among female university student population from January to October 2015. Eighty-nine young women with primary dysmenorrhea (PD) were selected as study group. The investigation conforms to the principles outlined in the Declaration of Helsinki and Ethical Committee approved the study. Informed consent was obtained, clinical and demographic data were collected in each subject. A detailed medical history was taken. This is a sub-study of our group that previously published[12].

Clinical Evaluation

Each subject had a complete gynecologic examination including pelvic ultrasound, systemic physical examination including cardiac and cardiovascular system and laboratory tests such as, hemogram, biochemistry tests, electrocardiography and echocardiography. Subjects with any gynecological disease, rheumatic disorder, inflammatory bowel disease, fibromyalgia, known malignancy, known premature coronary artery disease, congenital cardiac disease, such as family history of premature coronary artery disease, diabetes mellitus, hypertension, any endocrine disease such as diabetes mellitus or thyroid disorders, endometriosis, ovarian cysts or subject who were taking any oral contraceptive drugs were excluded.

Participant' main complaint was dysmenorrhea where pain was mostly located in lower abdominal and pelvic area.

VAS score10, which is a 10-cm line, with the end point 0 for "no pain" and 10 for "worst pain" was explained clearly to all participants. They were asked to make a mark on the line that represented their pain intensity, and measuring the distance from the "no pain" end to the patient's mark scored pain intensity level.

Grading from 1-4 was accepted as mild, 5-7 was accepted as moderate and 8-10 was accepted as severe pain groups.

Laboratory analyses

Blood samples were taken in the morning after 12 hours fasting on the 3rd day of menses from each subject. After clotting and centri-

fugation for 5 min at 3.000 g serum for ADMA measurement were separated and stored at -80 °C until the time of analysis. Blood glucose, serum creatinine, blood urea nitrogen, lactate dehydrogenase, alkaline phosphatase, aspartate and alanine aminotransferases, gamma glutamyl trans peptidase and bilirubin were measured by using autoanalyzer ARCHITECT c16000(Abbott Laboratories. Abbott Park, Illinois, U.S.A.).

ADMA levels were measured by using commercial enzyme-linked immunosorbent assay kits (Shanghai Yehua Biological Technology, Shanghai, China) according to the manufacturer's instructions. Intra-assay variability coefficient of ADMA ELISA assays was 9.2% 10.6%, respectively. ADMA levels were expressed as micrograms per liter (µg\L) and nanogram per mililiter (ng\mL) in serum samples, respectively.

Statistics

Data was analyzed using SPSS software, version 10.0 (SPSS Inc, Chicago, IL). Distribution characteristics of continuous data were determined using histogram examination and one-sample Kolmogorov-Smirnov test. Normally distributed data were presented as mean (standard deviation) and compared with 1-way analysis of variance. Further analyses was performed using Tukey's HSD, as needed. Pearson correlation and linear regression analysis were used to evaluate associations between continuous data. Categorical associations were evaluated using 2 test. Data binning was applied in the case of embryo number. This process is a data preprocessing technique used to reduce the effects of minor observation errors. The statistical program offered resulting into harmonic groups optimal binning thresholds. Statistical significance was defined by $P \leq 0.05$.

Results

89 subjects, mean age 21.08±2.21 and mean body mass index 22.4±1.9 were included in the study. Menstrual pain was categorized using VAS pain scale as mild[1-4], moderate[5-7] and severe[8-10]. There were 24 subjects in mild, 39 subjects in moderate and 26 subjects in severe pain groups. Other demographic, laboratory and clinical characteristics of the study group were shown in Table 1 Serum ADMA levels were 35.2(24) in mild, 54.0(28.7) in moderate and 56.5(32.8) in severe pain groups, (P= 0.029 and P<0.001, respectively) (Table 2, and Figure 1). Further analyses by Tukey's HSD of the groups revealed that ADMA was significantly higher in moderate and severe pain groups compared to mild pain group. ADMA mean level difference was -18.8, (p=0. 046) in mild to moderate; -2.52, (p=0. 945) in moderate to severe and 21.32 (p=0. 048) in severe to mild pain groups (Table 3).

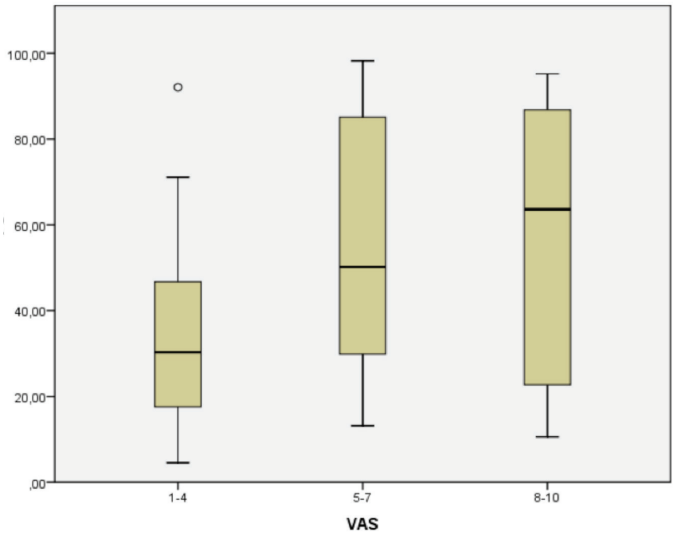

Figure 1: ADMA levels according to VAS groups

Discussion

This study showed that, serum ADMA levels are associated with the pain intensity as assessed by VAS score. Dysmenorrhea pain was graded as mild, moderate, severe and evaluated the association of them with serum ADMA levels. In comparison of pain groups, serum ADMA levels were significantly higher between severe and mild pain intensity groups. But, there was not any significant difference in the serum ADMA levels between moderate to severe pain groups. We have found that, more severe pain is associated with higher serum ADMA levels.

Dysmenorrhea is a chronic pain mostly affects young women's phycology, personal and social life[1,2,3]. Dysmenorrhea pain varies by the severity and associated symptoms. Pain intensity is thought to be one of the primary factors that determine the impact of pain on a person's overall function and sense of well-being. There are several available scores for quantifying the pain intensity and guide treatment decisions and monitoring the therapy. Previous studies showed that the visual analog scale (VAS), the numeric rating scale, the verbal descriptor scale or verbal rating scale, and the faces scale was the most commonly used measures of pain inten-

sity. The VAS is a pain intensity-grading tool commonly used in research and clinical practice, and its reliability and validity in pain assessment has been clearly demonstrated[7-10]. Pain intensity measured by the VAS is significantly correlated with those measured by verbal and numeric scales[7-10].

Table 1:Clinical, Demographic, Laboratory Data of the Study Group(N: 89)

Data		Mean (Std. Deviation), %	
Age		21.08±2.21	
BMI(kg/m2)		22.4±1.9	
Smoking		8(%8.9)	
Alcohol		5(%5.6)	
Physical activity intensity		Low-moderate: 65(%73), Intense: 26(%27)	
Age at menarche		13.2±1.3	
Dysmenorrhea Present		11 (12.4%)	
Dysmenorrhea Absent		78(87.6%)	
Pain Duration		1±0.76	
Visual Analogue Scale (VAS) Pain Scores		5.9101±2.18	
Asymmetric Dimethylarginine (ADMA)		49.6594±29.63	
(VAS) Pain Category			
		Frequency	Percent(%)
VAS Cat 1-4		24	26.8
VAS Cat 5-7		39	43.8
VAS Cat 8-10		26	29.2
Pain Location in Severity			
Lower abdomen		59/89	66.3
Inguinal region		44/89	49.4
Most Severe Time of pain			
Before the cycle		12/89	13.5
First day		70/89	78.7
Second day		13/89	14.6
Third and fourth day		4/89	4.5
Dysmenorrhea duration (Year)			
	Last 1 year	6/89	6.5
	Last 2 year	9/89	10.1
	Last 3 year	11/89	12.4
	More than 3 years	63789	70.8

It is known that, increased serum ADMA levels are associated with the PD and increased serum ADMA is a marker of endothelial dysfunction and it is a novel risk factor for atherosclerosis[1,2,11]. Serum ADMA levels have been reported in patients with hypertension, insulin resistance, hypercholesterolemia, renal failure, ankylosing spondylitis, in polycystic ovary syndrome (PCOS)[15], hyperemesis gravidarum and complicated pregnancies[1,2,11-17].

Table 2. ADMA Levels According to VAS Categories

VAS category	n	Serum ADMA, µg\L mean(SD)
1-4 (Mild)	24	35.2(24)
5-7 (Moderate)	39	54.0(28.7)
8-10 (Severe)	26	56.5(32.8)

Table 3. Comparison of ADMA Levels Between VAS Categories

(I) VAScat	(J) VAScat	ADMA	Mean Difference (I-J	Std. Error	Sig.	95% Confidence Interval
1-4	5-7	ADMA	-18.8	7.76	0.046	-37.35 to -0.26
5-7	8-10	ADMA	-2.52	7.89	0.945	-21.37 to 16.32
1-4	8-10	ADMA	21.32	8.96	0.048	-0.085 to 42.73

Recent studies also evaluated the serum ADMA levels during normal menstrual period. The menstrual cycle can be evaluated by dividing as follicular and luteal phases that regulates by hormones[18]. Estrogen and progesterone levels are lower in early period of the follicular phase, but it starts to increase toward the end of the follicular phase, peaks during the surge of LH and FSH. In early period of luteal phase and during the follicular phase the progesterone levels are very low, but it starts to increase in advancing of luteal phase[18]. Serum ADMA levels underwent small amount of changes during menstrual cycle. ADMA level was higher in the follicular phase than in the luteal phase of menstrual cycle. This finding was correlated well with that FMD was highest in the follicular phase. Furthermore, oral contraceptive therapy reduces the changes in serum ADMA levels in normal menstrual cycle. Estrogen-containing pills decrease the serum ADMA concentration but progestin-only pills don't decrease as compared to controls[6,21].

Estrogen has positive effect on endothelial functions inducing arterial vasodilation by activating endothelial nitric oxide synthase[6,21].

Cevik et al.[22] showed that estradiol may facilitate NO synthesis by reducing circulating levels of ADMA, and this may exert protective effects on the vasculature. It was demonstrated that relatively small changes in the concentration of ADMA affect gene expression in endothelial cells[22]. Identification of pathways regulated by ADMA may aid our understanding of how ADMA contributes to a wide range of pathologies. Two pathways of specific interest have been identified-BMP signaling and enzymes involved in arginine methylation. The effects on BMP signaling may be particularly important in renal disease and in the link between raised ADMA and pulmonary hypertension[22].

Role of ADMA in pain and pain sensation

ADMA as an inhibitor of NOS has important roles in pain pathogenesis. ADMA is structurally similar to L-arginine and liberated after posttranslational methylation of arginine residues within proteins and subsequent proteolysis. There is a competition between ADMA and L-arginine for the active site of NOS and thus regulates NO formation. Physiological concentrations of ADMA in the brain modulate neuronal NOS function and suppress NO-mediated excitotoxicity[25-27]. Recent data showed that ADMA modulates some physiological events in the central nervous system (CNS), such as the response to nerve injury and in nociception[25-31]. Asymmetric dimethyl arginine is actively regulated by dimethyl arginine dimethyl amino hydrolase (DDAH), of which there are 2 isoforms, DDAH-1 and DDAH-2; this supports the concept that ADMA confers an important physiological function. DDAH metabolizes ADMA to L-citrulline and dimethylamine, regulating the inhibitory influence of ADMA on NOS[25-31]. Serum ADMA levels underwent small amount of changes during menstrual cycle. ADMA level was higher in the follicular phase than in the luteal phase of menstrual cycle. This finding was correlated well with that Flow Mediated Dilatation was highest in the follicular phase. And also, increased serum ADMA levels in PD may increase the pain in uterine tissue eventually caused by endothelial dysfunction; uterine contractions and also it can increase the uterine pain sensation in CNS in patients with PD.

We can conclude that pain intensity in PD patients is associated with the serum ADMA levels as assessed by VAS pain score. This is the first study showed the association of pain intensity and serum ADMA levels in published literature. Further studies will be needed to clarify serum ADMA and pain intensity association with genetic and biochemical researches

Limitations:
Absence of follow-up data, single blood sampling and small sample size are the limitations of the study.

Details of ethics approval;
Ethics approval number:21,
date: 02.12.2014

Funding:
Sakarya University Scientific Research Office.
Disclosure Statement

The authors report no conflicts of interest.

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