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The Relationship between Pain and Vascular Function Biomarkers in Dysmenorrheal University Students

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Our aim was to establish if the secretion of contactin 1 (CNTN-1), a widely researched pain biomarker correlates with the severity of dysmenorrhea and circulating levels of vascular cell adhesion molecule 1 (VCAM-1) and angiotensin II (ANG-II). This study was a longitudinal randomized clinical study that involved 95 female students between 17-25 years. The control participant group were students who, without medications, had not experienced dysmenorrhea, while the inclusion criteria were primary dysmenorrhea without medications. Data was collected using demographic questionnaires that also contained the Numeric Rating Scale (NRS-11), while blood samples were collected for analysis of CNTN-1, VCAM-1 and ANG-II by ELISA. The participants' mean BMI's across the four pain strata were between 16.60-38.43 kg/m^2 and in addition to age and menarche, showed no correlation to either the NRS-11 scale ($r=-0.01214$) or their CNTN-1 levels ($r=0.009622$). The severe dysmenorrhea group showed statistically higher ($p<0.0001$) and positive correlation to systolic ($r=0.7304$) and diastolic (0.6588) blood pressures. The contactin 1 levels (7.00-55.70 ng/mL) increased with higher menstrual pain and as the pain increased, so did the mean VCAM-1 and ANG-II levels ($p<0.0001$). A positive linear correlation ($r=0.9691$) was observed between the NRS-11 scale of the participants and their CNTN-1 activities while the CNTN-1 levels positively correlated with their VCAM-1 ($r=0.9334$) and ANG-II ($r=0.8746$) secretion. In summary, the severity of dysmenorrheal pain elevates the contactin 1 levels which affects their vascular health and blood pressure.

Key Words: *Dysmenorrhea; Contactin 1; Angiotensin II; Blood Pressure*

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INTRODUCTION

Dysmenorrhea is a common gynaecological condition that manifests as chronic pain of the uterine origin, before and during menstruation. This condition can occur without any pathological pelvic disease (primary dysmenorrhea) or borne of a pelvic disease (secondary dysmenorrhea) usually in women above 20 years of age.[1,2] Symptoms associated with dysmenorrhea include diarrhoea, headache, nausea, and vomiting.[3] Various studies have reported varying dysmenorrhea prevalence across varying ages and ethnicities. Up to 90% of women between 17-24 years have been found with dysmenorrhea while up to 93% teenage Australians have indicated dysmenorrhea.[4,5] Reports about the prevalence of dysmenorrhea in older women have equally lacked consistence ranging between 15-75%.[4] Generally, as a debilitating gynaecological condition for women of reproductive age, dysmenorrhea contributes to economic losses, poor productivity, and poor overall quality of life. Factors such as excessive prostaglandin secretion, pelvic inflammation, obesity, and premenstrual syndrome all predispose women to dysmenorrhea.[6] Other risk factors include high menorrhagia, excessive caffeine consumption, as well as poor social support.[7] Attempts have been made in previous studies to understand if dysmenorrhea predisposes women to vascular complications such as high blood pressure, hypertension, and stroke, but whether the severity

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of dysmenorrhea correlates to the susceptibility to these vascular complications remained unexplained.[8-10] Based on this, we conducted this study using female university students to first establish the reliability of a pain biomarker, contactin 1, for the assessment of the severity of dysmenorrhea, by correlating with the NRS-11 pain scale of each participant, and also correlated with the biomarkers of vascular health; the vascular cell adhesion molecule 1 and angiotensin II.

MATERIALS AND METHODS

1. Study design

This study was a longitudinal randomized clinical study, involving ninety five (95) female undergraduate students of high institutions in Southern Nigeria. The Biochemistry Research and Ethics Committee, Imo State University, Owerri issued an ethical permit for this study (IMSU/BCH/ETS/20191111). Informed consent was obtained from each participant prior to the commencement of the study. The exclusion criteria were any use of analgesics, irregular menstrual cycles, any metabolic disease such as hepatic, cardiovascular, pancreatic or hormonal imbalance, and diseases that predisposed the participants to pain were excluded. The inclusion criteria were primary dysmenorrhea without medications. The control participant group were female students who, without medications, had not experienced dysmenorrhea.

2. Data collection

Demographic data was collected using a questionnaire. The questionnaire included strata for assessment of severity of pain. One of the widely used pain scales for endometriosis-related pain the Numeric Rating Scale (NRS-11) was included in the questionnaire for indication of severity of dysmenorrhea.[11] The rating was 0 for no dysmenorrhea, 1-3 for mild dysmenorrhea, 4-6 for moderate dysmenorrhea, and 7-10 for severe pain. In addition, to aid accurate selection of the appropriate pain scale, the participants were encouraged to relate the severity of menstrual pain to interference with activities of daily life. Thus, the obtained data was presented according to this NRS-11 scale. The blood pressure and blood samples of the participants was obtained either on the first or second day of dysmenorrhea, whichever was indicated as the day with most intense pain by the participant. The blood pressure was determined using a UNESCO International Analog Sphygmomanometer while the serum levels of the biomarkers CNTN-1, VCAM-1 and ANG-II levels of the participants were obtained with ELISA kits and as described in their assay manuals.

3. Statistical analysis

The result obtained was stratified into four classes of the pain scale according to the indication of the participants on the NRS-11 scale attached to the questionnaire. Data on the participant's characteristics was expressed in ranges and means±standard deviations. The mean differences of the groups were compared with one way ANOVA using SPSS version 22 and by their least standard deviations. Pearson's correlation coefficients (r) were calculated to estimate the extent of relationship between the NRS-11 pain scale and CNTN-1, NRS-11 and blood pressure, BMI vs. NRS-11 and CNTN-1. Scatter plots were performed to examine the linearity of the CNTN-VCAM and CNTN-ANG relationships. All statistical data comparisons were at 95% confidence intervals and two-tailed.

RESULT

Our study involved ninety five (95) undergraduate students across Southern Universities of Nigeria whose characteristics are shown in Table 1. The participants stratified according to the NRS-11 pain scale were between 17-25 years of age (p=0.2591) and menarche occurred between 11 and 13 years (p=0.6311) while their age of first dysmenor-

TABLE 1. Subject characteristic and their CNTN-1, VCAM-1 and ANG-II levels

Parameters	Normal (n=15)	Mildly painful (n=24)	Moderately painful (n=24)	Severely painful (n=32)	p-value
Age (yrs)	17-22 (19.80±1.65)	17-25 (21.37±2.33)	17-24 (20.29±1.85)	17-25 (21.09±2.02)	0.2591
BMI (Kg/m^2)	18.11-38.43 (25.61±6.43)	17.78-39.43 (25.94±6.68)	16.60-38.33 (26.39±7.08)	18.21-36.75 (25.59±5.30)	0.9725
Systole (mmHg)	120-130 (123.33±4.87)	120-130 (125.41±5.08)	120-140 (127.91±5.88)	130-150 (139.68±6.94)	<0.0001
Diastole (mmHg)	70-80 (77.33±4.57)	70-90 (78.75±6.12)	70-90 (82.08±6.58)	80-100 (90.00±5.08)	<0.0001
Menarche (yrs)	11-13 (12.00±0.75)	11-13 (12.04±0.75)	11-13 (11.95±0.80)	11-13 (12.12±0.79)	0.6311
Age of first DMR (yrs)	-	11-15 (13.00±1.25)	11-14 (12.70±0.95)	11-15 (13.46±1.04)	0.0882
Duration of DMR (days)	-	2-4 (2.54±0.58)	2-3 (2.45±0.50)	2-4 (3.09±0.73)	0.0013
CNTN-1 (ng/mL)	7.00-9.50 (8.5±0.74)	10.30-19.40 (15.62±2.96)	17.50-36.20 (25.90±4.94)	31.20-55.70 (44.59±5.87)	<0.0001
VCAM-1 (ng/mL)	503-804 (672.26±94.68)	525-1361 (917.70±194.87)	1058-1876 (1448.41±226.23)	1721-3127 (2422.75±420.07)	<0.0001
ANG-II (pg/mL)	20-27 (22.86±2.03)	21-29 (24.75±2.55)	24-36 (30.70±3.16)	29-39 (35.21±2.39)	<0.0001

BMI: Body mass index, DMR: Dysmenorrhea, CNTN-1: Contactin 1, VCAM-1: Vascular cell adhesion molecule, ANG-II: Angiotensin-II.

rhea was from 11-15 years (p=0.0882). Their mean BMI (p=0.9725) across the four pain strata were respectively 25.61±6.43, 25.94±6.68, 26.39±7.08, 25.59±5.30 kg/m^2. The participants experiencing severe dysmenorrhea showed statistically higher (p<0.0001) systolic and diastolic blood pressures (139.68±6.94 and 90.00±5.08 mmHg) and duration of dysmenorrhea, 2-4 years (p=0.0013). Those that indicated no dysmenorrhea had the lowest mean levels of contactin 1 (8.5±0.74 ng/mL), followed by the mildly painful, moderately painful, and the severely painful groups (15.62±2.96, 25.90±4.94, and 44.59±5.87 respectively). Also, as the pain increased, so did the mean VCAM-1 and ANG-II levels (p<0.0001) in the order; severely painful>moderately painful>mildly painful>normal.

We further used scatter plots to correlate the relationship between the severity of dysmenorrhea and contactin 1, a pain biomarker, as well as blood pressure, BMI, and vascular function biomarkers. We found a strong positive linear correlation (r=0.9691) between the severity of dysmenorrhea and the contactin 1 activities of the participants (Fig. 1). Both the systolic and diastolic blood pressures of the dysmenorrheal students positively correlated (r=0.7304 and r=0.6588) with the severity of menstrual pain (Fig. 2). No statistically significant relationship (r=−0.01214 and 0.009662) was observed between the participants' BMI and the severity of dysmenorrhea as shown in the scatter plots of BMI Vs NRS-11 pain scale (Fig. 3) and BMI vs. CNTN-1 (Fig. 4). When the participants' CNTN-1 activities were correlated against their vascular cell adhesion molecules 1 (VCAM-1) and angiotensin II (ANG-II) levels, a strong positive linear correlation was observed in their scatter plots presented in Figs. 5 and 6.

DISCUSSION

Notwithstanding that the role of contactin 1 as an adhesion molecule and its reported contributions in vasculogenesis which have all been documented, its primary func-

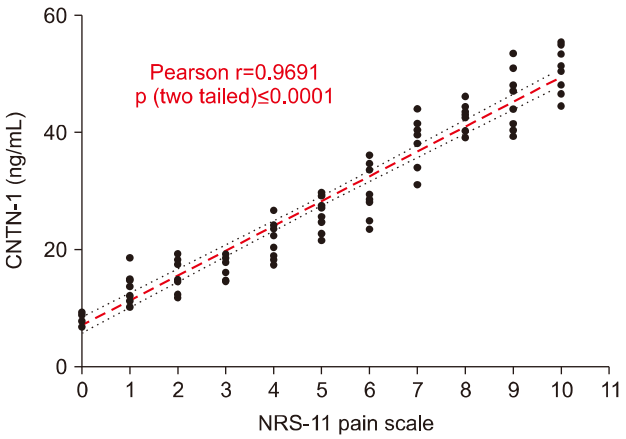

FIG. 1. Correlation between NRS-11 pain study scale and contactin 1 levels in n=95 undergraduate students, with n=80 positive response to dysmenorrhea.

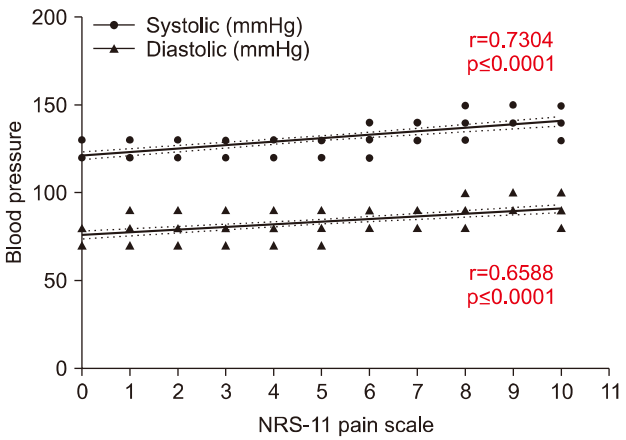

FIG. 2. Correlation between NRS-11 pain study scale blood pressure levels in n=95 undergraduate students, with n=80 positive response to dysmenorrhea.

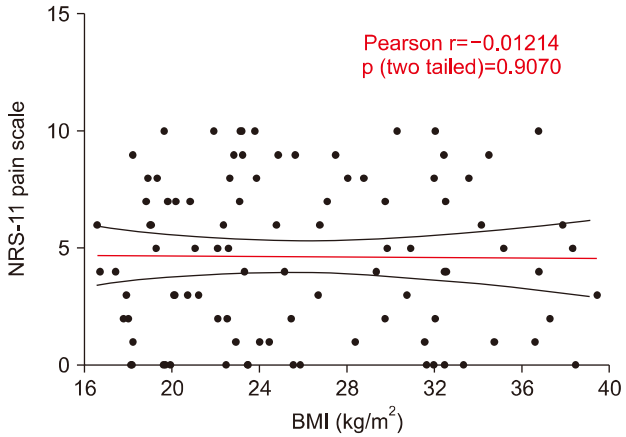

FIG. 3. Correlation between BMI and NRS-11 pain study scale response of n=95 undergraduate students, with n=80 positive response to dysmenorrhea.

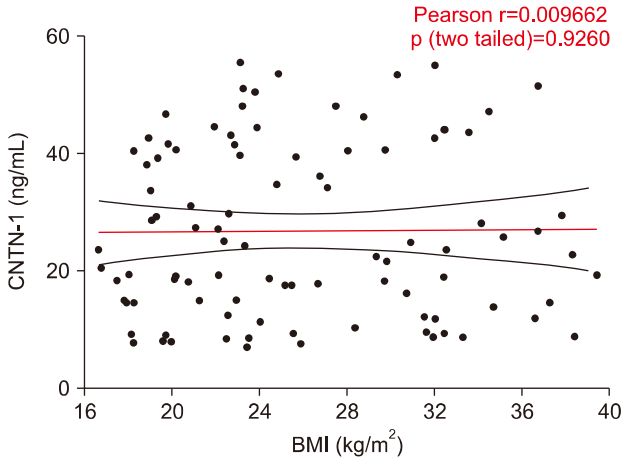

FIG. 4. Correlation between BMI and contactin 1 levels of n=95 undergraduate students, with n=80 positive response to dysmenorrhea.

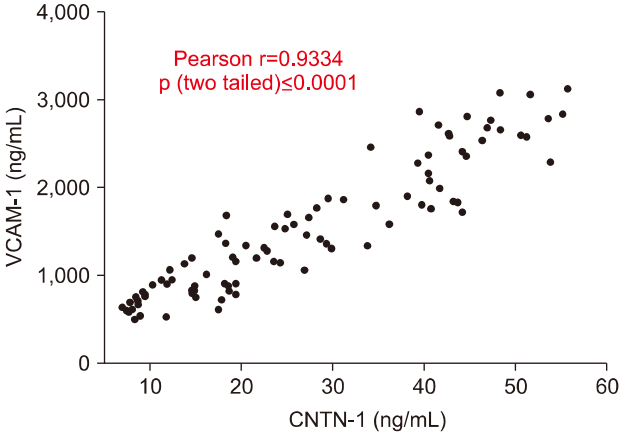

FIG. 5. Scatter plot of CNTN-1 Vs VCAM-1 of n=95 undergraduate students, with n=80 positive response to dysmenorrhea.

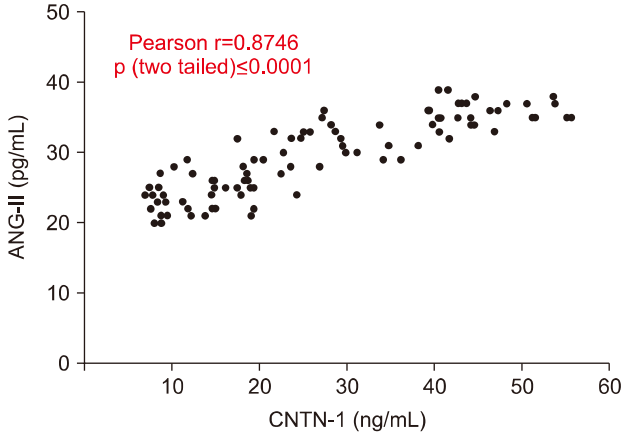

FIG. 6. Scatter plot of CNTN-1 Vs ANG-II of n=95 undergraduate students, with n=80 positive response to dysmenorrhea.

tion in our study was to serve a biomarker for menstrual pain.[12-14] We attempted to establish if the severity of dysmenorrhea using the pain biomarker (contactin 1), affects vascular health indicators. The participants who were selected from university students were adolescents because the highest prevalence of dysmenorrhea was found in adolescents.[15] Studies elsewhere suggest that with increasing age, dysmenorrhea subsides.[16] However, our study found no relationship between severity of the menstrual pains and the participants' ages, menarche, or the age of first dysmenorrhea. Further, some of the participants were obese (BMI up to 39.43 kg/m^2) or underweight (16.60 kg/m^2), which however did not have any effect on the severity of dysmenorrhea as shown in the scatter plots of BMI versus both the NRS-11 pain scale and CNTN-1 levels. To the contrary, BMI and underweight were positively correlated to dysmenorrhea, albeit in much older women.[3,17] Our findings further showed a strong relationship between the severity of menstrual pains and blood pressure. This is probably due to the influence of menstruation on cardiac autonomic regulation.[18] The blood pressure increases according to the severity of the menstrual pain. Some other studies have reported elevated blood pressures at onset of menstruation, but did not correlate with severity of dysmenorrhea.[8] It has been documented that more than 90% of women of reproductive age live with dysmenorrhea and mostly rely on analgesics for dysmenorrhea relief, which in most cases is poorly administered.[15] This difficulty is further compounded because the diagnosis of dysmenorrhea is made by physical examination making it very difficult to proffer reliable therapeutic solutions. As a result, we correlated the levels of a pain biomarker in dysmenorrheal adolescents and their response to the NRS-11 pain scale, and found a positive correlation. We chose CNTN-1 as the pain biomarker having being validated among other biomarkers in earlier studies, as a broad spectrum pain biomarker.[14] The CNTN-1 levels positively correlated with the NRS-11 pain scale in our study implying that CNTN-1 could provide an indication as to the severity of dysmenorrhea. Some other studies elsewhere have reported a significant association between severity of menstrual pain and the expression of progesterone and other some proteins in endometrial tissues.[19,20] We instead examined the activities of the vasoactive peptides, VCAM-1 and ANG-II, in the dysmenorrheal participants, as reliable diagnostic biomarkers for vascular health. They are certainly elevated during endothelial perturbations.[21] In agreement with these observations, our results showed elevated activities of VCAM-1 and ANG-II in response to the severity of dysmenorrhea. The strong positive correlation between the contactin 1 levels and the activities of these vascular health biomarkers in the dysmenorrheal participants suggests possible endothelial perturbation. Whether this is an indication of predisposition to vascular damage or future susceptibility to vascular diseases remains an interesting clarification to seek in our subsequent studies.

In summary, characteristics such as age, menarche, and BMI had no effect on the severity of dysmenorrhea in the students. A strong linear positive correlation was established between the severity of dysmenorrhea and secretion of contactin 1, a pain biomarker. Also, the dysmenorrheal students showed a positive correlation between the severity of menstrual pain and the activities of the vasoactive peptides, VCAM-1 and ANG-II.

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CONFLICT OF INTEREST STATEMENT

None declared.

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