Molecular profiles of hepatotoxicity and nephrotoxicity markers in dysmenorrheic (on treatment or not) students

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Abstract

Background: Dysmenorrhea is menstrual disorder that affects about 40% - 90% of women worldwide, it is associated with oxidative stress. The current treatment of this condition is administration of non-steroidal anti-inflammatory drugs, which when frequently used, may affect organs.

Objective: Assess the hepatotoxicity and nephrotoxicity side effects related to dysmenorrhea and its treatment

Materials and methods: A survey (questionnaire) was designed and implemented on 689 female students of the University of Dschang. After this, and following the inclusion criteria, 191 blood samples were collected for assay of hepatotoxicity markers (transaminases, albumin), nephrotoxicity indicators (creatinine, urea, total protein) and the inflammation associated indicators. The measurements were performed on fully automated Olympus AU 400 Analyzer, using standard reagent kits.

Results: Subjects with untreated dymenorrhea lasting more than five years had a significantly high level (p < 0.05) of ALT (39.47 ± 15.74 IU/L) and AST (44.37 ± 13.74 IU/L). Transaminases levels were significantly associate (p < 0.01) and positively correlate (0.251 for ALT and 0.223 for AST) with the disease duration. Dysmenorrheic individuals on medication for more than 9 years had significantly higher ALT (25.14 ± 7.85 IU/L) and AST (35.26 ± 0.70 IU/L) levels (p < 0.05) compared to those under treatment for less than 5 years (19.37 ± 8.27 UI/L and 27.68 ± 8.56 IU/L). The use of analgesics, regardless of the duration of treatment, had normal creatinine clearance (107.44 ± 30.86 ml/min), compared to those treated with either anti-inflammatory drugs (71.56 ± 26.44 ml/min), or a combination of analgesics and anti-inflammatory drugs (81.34 ± 31.97 ml/min), which was significantly reduced (p < 0.05).

Conclusion: Dysmenorrhea duration, type and duration of treatment potentially expose participants to liver and kidney disorders.

Introduction

Dysmenorrhea, a painful or cramping sensation in the lower abdominal and/or lower back area is often accompanied by other biological symptoms, including fatigue, dizziness, sweating, head-aches, backache, nausea, vomiting, diarrhea, all occurring just before and/or during menstruation [1,2]. Two categories of dysmenorrhea can be distinguished: primary (primitive) and secondary (organic), according to its pathogenesis. Primary dysmenorrhea is menstrual pain without pelvic disorder and secondary dysmenorrhea is menstrual pain associated with identifiable disease such as the endometriosis [3]. Primary dysmenorrhea is associated with a normal ovulatory cycle, with no pelvic pathology and has a clear physiological etiology [4,5]. After ovulation, there is a build-up of fatty acids in the phospholipids of the...
A Survey was conducted among 689 puberty-aged and non-pregnant students between 17 and 33 years old of the University of Dschang via a questionnaire. After taking into consideration the inclusion criteria (absence of pelvic pathology, problems of kidney and liver, none usage of intra-uterine deviceand pain-killers for other reasons, no pregnancy), 191 consenting students were selected and invited at the laboratory of the Dschang district hospital where their weights and heights were taking using an electronic balance and stadiometer respectively. 10 ml of urine were collected in a sterile container, and 9 ml venous blood were also collected in dry and citrate tubes. Blood samples was spun to obtain serum. Transaminases as well as albumin levels were measured to assess the liver functioning, while creatinine, urea and total protein were quantified to evaluate renal functioning. The measurements were performed on fully automated Olympus AU 400 Analyzer (Olympus Diagnostics GmbH, Germany) using standard reagent kits from laboratories: RECKON-India [ALAT(14FX17N)], MONLAB-Barcelone [ASAT(MO-165071) and creatinine (MO-1650)], SGM Italia-Roma [total protein LR (10031), albumin LR (10040) and urea UV LR (10239)]. The C-reactive protein and erythrocyte sedimentation rates were determined via agglutination using standard reagent kit from Human- Germany laboratory [c protein reactive (40034)] and on Westergren sedimentation tube respectively to detect any inflammation in the body. In urine, parameters such as protein and blood cells were evaluated by colorimetric assay of reactive strips while crystals, cylinders and urothelial cells were detected by microscopic observation. All these parameters were used for the evaluation of renal and hepatic functions.

**Statistical analysis**

Statistical analysis were carried out using SPSS 20.0 and Epi Info for window 13.0 and 6.0 software program respectively; continuous variables were expressed as mean ± Standard Error of Mean. The Waller Duncan test was used to separate means. Chi square and Fisher tests were used for the categorical variables. While the Rank correlation was applied to test the association between continuous variables. $p < 0.5$ was considered statistically significant.

**Results**

Of the 689 students surveyed, 191 agreed to give their biological samples (blood and urine) to the laboratory for analysis. Of the 191 participants, 42 were healthy, while 149 were dysmenorrheic. Of these, 83 were on medication; the results are in the table below

Table 1 summarizes the variation in haematological and biochemical parameters depending on the presence or absence of dysmenorrhea (healthy or sick). No significant difference was observed between the hepatotoxicity and nephrotoxicity independently of the status of the student.
The impact of the duration of dysmenorrhea on the various parameters of hepatotoxicity and nephrotoxicity is presented in Table 2. A significant increase (p < 0.05) was registered for transaminases, with dysmenorrhea over 9 years of the disease.

The impact of medications on the liver and kidneys is shown in Tables 3, 4. A significant increase in serum albumin (p < 0.01) with medication and depending on the duration of treatment was observed (Table 3). As presented in Table 4, a significant increase in transaminases was more pronounced over 9 years of treatment (p < 0.05).

The relationship between biochemical parameters and experimental parameters (presence of dysmenorrhea and its treatment) is represented in Table 5. In this table, we observe positive correlation/relationship between of transaminase and duration of dysmenorrhea and between the albumin and medication.

**Discussion**

The significant increase in the activities of transaminases (AST and ALT) in participants respectively whose medication and disease duration were over 9 years could be due to the oxidative stress induced by a combination of inflammation of the uterus and reactions of drugs or their metabolites. Dysmenorrhea is caused by tissue hypoxia/ischemia in consecutive uterine myometrium hypercontractility and arteriolar vasoconstriction of the uterine muscle; ischemia which releases noxious substances capable of exciting the nerve endings and triggering the alarm system (inflammation), which also causes an overproduction of reactive oxygen.

**Table 1:** Haematological and biochemical parameters depending on the presence or absence of dysmenorrhea (healthy and sick).

| Parameters       | Panel (n = 42) | Dysmenorrheic (n = 149) | Reference values | p value |
|------------------|---------------|-------------------------|------------------|---------|
| ALAT             | 17.42 ± 5.86a | 21.94 ± 8.31a          | 5-55 UI/L        | 0.25    |
| ASAT             | 24.61 ± 8.03a | 29.81 ± 7.18a          | ≤ 31 UI/L        | 0.15    |
| Albumin          | 3.51 ± 0.55a  | 3.49 ± 0.57a           | 2.5-5.4 g/dl     | 0.92    |
| Urea             | 21.37 ± 4.19a | 21.35 ± 3.33a          | 10-50 mg/dl      | 0.99    |
| Total protein    | 5.93 ± 1.33** | 5.66 ± 0.96**          | 6.6-8.3 g/dl     | 0.36    |
| Creatinine       | 1.16 ± 0.51a  | 1.22 ± 0.46            | 0.6-1.1 mg/dl    | 0.68    |
| Creatinine Clearance | 89.73 ± 21.41| 84.49 ± 20.27          | > 90 ml/min      | 0.66    |
| Erythrocytes sedimentation rate 1 | 16.50 ± 6.62** | 13.33 ± 7.82** | 4-7 min/h | 0.41 |
| Erythrocytes sedimentation rate 2 | 32.08 ± 8.19** | 29.49 ± 9.12** | 12-17 min/h | 0.65 |

The values in the table are presented as means ± standard errors of the means. Assigned values with different letters are significantly different by comparing the values of the groups with the reference probability level of 5% (Waller Duncian’s Test). n = number of participants per group. p = probability.

**Table 2:** Changes in haematological and biochemical parameters according to term dysmenorrhea.

| Parameters | < 5 years (n = 32) | 5-9 years (n = 23) | < 10 years (n = 11) | Reference value | p1 value | p2 value | p3 value |
|------------|-------------------|-------------------|-------------------|-----------------|----------|----------|----------|
| ALAT       | 19.99 ± 8.04a     | 21.19 ± 10.28a    | 39.46 ± 15.73a    | 5-55 UI/l       | 0.67     | 0.00     | 0.00     |
| ASAT       | 27.70 ± 9.99a     | 30.06 ± 9.263a    | 44.37 ± 13.74a    | ≤ 31 UI/L       | 0.57     | 0.00     | 0.00     |
| Albumin    | 3.26 ± 0.76a      | 3.56 ± 0.38a      | 3.23 ± 0.54a      | 2.5-5.4 g/dl    | 0.66     | 0.55     | 0.33     |
| Urea       | 22.01 ± 13.29a    | 23.48 ± 15.63a    | 21.94 ± 17.84a    | 10-50 mg/dl     | 0.58     | 0.73     | 0.92     |
| Total protein | 5.95 ± 1.11** | 5.56 ± 0.66**     | 5.48 ± 0.78**     | 6-6.3 g/dl      | 0.35     | 0.23     | 0.64     |
| Creatinine | 1.13 ± 0.46a      | 1.29 ± 0.53a      | 1.16 ± 0.52a      | 0.6-1.1 mg/dl   | 0.08     | 0.94     | 0.17     |
| Creatinine clearance | 98.00 ± 20.92a | 73.67 ± 16.60a | 93.54 ± 15.49a | > 90 ml/min | 0.12 | 0.91 | 0.16 |
| Erythrocytes sedimentation rate 1 | 12.62 ± 5.65** | 15.65 ± 3.94** | 16.63 ± 9.42** | 4-7 min/h | 0.33 | 0.24 | 0.67 |
| Erythrocytes sedimentation rate 2 | 29.25 ± 5.65** | 35.00 ± 9.35** | 38.27 ± 7.98** | 12-17 min/h | 0.17 | 0.12 | 0.62 |

The values in the table are presented as means ± standard errors of the means. Assigned values with different letters are significantly different by comparing the values of different groups, and those affected by the asterisk (*) are significantly different by comparing the values of the groups with the reference probability level of 5% (Waller Duncian’s Test). n = number of participants per group. p = probability.

**Table 3:** Changes in the parameters sought as a function of taking medication.

| Parameters       | Dysmenorrheic untreated (n = 66) | Dysmenorrheic treated (n = 83) | Reference values | p value |
|------------------|----------------------------------|---------------------------------|------------------|---------|
| ALAT             | 23.66 ± 7.72**                   | 20.55 ± 8.14**                  | 5-55 UI/l        | 0.16    |
| ASAT             | 31.30 ± 9.12**                   | 28.63 ± 5.53**                  | ≤ 31 UI/l        | 0.18    |
| Albumin          | 3.36 ± 0.63**                    | 3.60 ± 0.49**                   | 2.5-5.4 g/dl     | 0.01    |
| Urea             | 22.51 ± 9.71**                   | 20.42 ± 7.13**                  | 10-50 mg/dl      | 0.34    |
| Total protein    | 5.74 ± 0.93**                    | 5.60 ± 0.98**                   | 6-6.3 g/dl       | 0.39    |
| Creatinine       | 1.20 ± 0.49                      | 1.24 ± 0.43                     | 0.6-1.1 mg/dl    | 0.57    |
| Creatinine clearance | 88.85 ± 19.52** | 81.02 ± 10.92** | > 90 ml/min | 0.24 |
| Erythrocytes sedimentation rate 1 | 14.35 ± 7.58** | 12.53 ± 8.03** | 4-7 min/h | 0.39 |
| Erythrocytes sedimentation rate 2 | 32.76 ± 9.42** | 26.89 ± 8.59** | 12-17 min/h | 0.06 |

The values in the table are presented as means ± standard errors of the means. Assigned values with different letters are significantly different by comparing the values of different groups, and those affected by the asterisk (*) are significantly different by comparing the values of the groups with the reference probability level of 5% (Waller Duncian’s Test). n = number of participants per group. p = probability.
species (ROS) in the body, causing oxidative stress. This will result in oxidative damage in DNA, proteins, lipids, carbohydrates, which further leads to tissue destruction and eventually death of cells (hepatocytes) by necrosis or apoptosis [14]. Reactive metabolites formed during the processing of the drug in the hepatocytes can interact directly with the proteins, lipids or nucleic acids to initiate oxidative damage, or lipid peroxidation, leading to cell death by necrosis or apoptosis [15]. Dysmenorrhea (inflammation) leads to prolonged accumulation of ROS and could therefore lead to liver disease. Oxidative stress from ROS can lead to necrosis in various tissues such as renal tissue [16], causing changes in the activity of numerous control settings by them. The decline in the renal clearance of creatinine observed in this work could be dependent on the oxidative stress which affected the renal functioning. The significant decrease of creatinine clearance recorded in female students who dealt with NSAIDs showed renal disease, resulting in a significant increase and decrease of creatinine and its clearance respectively, as well as liver disease showed by the increase in transaminases enzymes activities.

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Table 4: Variation in biochemical parameters depending on the length of treatment of dysmenorrhea.

| Parameters       | < 5 years (n = 42) | 5-9 years (n = 31) | > 9 years (n = 10) | Reference values | p1 value | p2 value | p3 value |
|------------------|-------------------|--------------------|--------------------|------------------|-----------|-----------|-----------|
| ALAT             | 19.37 ± 8.27**    | 20.67 ± 7.79*      | 25.14 ± 7.85**    | 5-55 UI/l        | 0.49      | 0.04      | 0.13      |
| ASAT             | 27.68 ± 8.55**    | 27.77 ± 7.97*      | 35.26 ± 7.07*     | ≤ 31 UI/l        | 0.97      | 0.03      | 0.04      |
| Albumin          | 3.62 ± 0.55**     | 3.56 ± 0.46*       | 3.65 ± 0.37*      | 2-5.4 g/dl       | 0.62      | 0.85      | 0.62      |
| Urea             | 20.73 ± 12.09*    | 20.75 ± 13.87*     | 18.11 ± 5.14*     | 10-50 mg/dl      | 0.99      | 0.54      | 0.55      |
| Total protein    | 5.64 ± 1.08*      | 5.58 ± 0.98**      | 5.52 ± 0.52**     | 6-8.3 g/dl       | 0.79      | 0.73      | 0.87      |
| Creatinin        | 1.16 ± 0.37*      | 1.35 ± 0.48**      | 1.26 ± 0.47*      | 0.61-1.1 mg/dl   | 0.07      | 0.52      | 0.57      |
| Creatinin clearance | 81.97 ± 16.10** | 80.95 ± 25.39*    | 77.25 ± 27.59*    | > 90 ml/min      | 0.89      | 0.67      | 0.74      |
| Erythrocytes sedimentation rate 1 | 11.44 ± 9.65** | 13.45 ± 5.97 a*    | 14.30 ± 6.11 a*   | 4-7 min/h        | 0.52      | 0.53      | 0.86      |
| Erythrocytes sedimentation rate 2 | 24.5 ± 5.65*    | 29.35 ± 10.64**   | 29.3 ± 13.65 a*   | 12-17 min/h      | 0.27      | 0.47      | 0.99      |

The values assigned with a star (*) indicating a correlation at p < 0.05, the values assigned two stars (**) indicates a correlation p < 0.01. The r values in bold signify that there had significant correlation.

Table 5: correlation between biochemical parameters and dysmenorrhea (presence and duration) and medication (duration and type of drug).

| r values | Dysmenorrhea | Duration of dysmenorrhea | Medication | Duration of medication | Types of drugs |
|----------|--------------|--------------------------|------------|------------------------|----------------|
| Creatinin | 0.033        | 0.041                    | 0.047      | 0.145                  | 0.035          |
| AST      | 0.114        | 0.251(**)                | -0.109     | 0.180                  | -0.019         |
| ALT      | 0.092        | 0.223(**)                | -0.116     | 0.206                  | -0.123         |
| Albumin | -0.007       | 0.030                    | 0.210(*)   | -0.008                 | 0.099          |
| Total Protein | -0.072     | -0.122                   | -0.070     | -0.042                 | 0.016          |
| Urea     | 0.000        | 0.021                    | -0.078     | -0.052                 | -0.067         |
| creatinin Clearance | -0.034    | -0.047                   | -0.097     | -0.044                 | -0.098         |

The values assigned with a star (*) indicating a correlation at p < 0.05, the values assigned two stars (**) indicates a correlation p < 0.01. The r values in bold signify that there had significant correlation.

Conclusion

Participants treated over a long period (nine years) and those suffering from dysmenorrhea for the same period
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