

Research Article

Examination stress and its effect on ovulation of female undergraduate students

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ABSTRACT

Background: Students are susceptible to academic stress which is higher during examination period. Academic stress has been found to activate the Hypothalamic-Pituitary-Adrenal axis resulting in increased cortisol and progesterone levels in circulation. This study sets out to investigate the effects of examination stress on ovulation of 32 apparently healthy female students.

Methods: Serum levels of cortisol ($\mu\text{g/dl}$), glucose (mmol/L) and progesterone (ng/ml) was determined using blood samples collected on day 21 of the menstrual cycle before and after examination.

Results: The result showed significant elevation in pre-examination serum cortisol ($15.3 \pm 5.9\mu\text{g/dl}$) but significant reduction in Progesterone ($3.5 \pm 1.5\text{ng/ml}$) when compared with post-examination mean serum concentrations of cortisol ($10.5 \pm 5.1\mu\text{g/dl}$) and progesterone ($4.2 \pm 2.6\text{ng/ml}$) ($P < 0.001$ and $P < 0.001$) respectively. There was no significance difference in glucose level before examination ($5.4 \pm 0.8\text{mmol/L}$) and after examination ($5.3 \pm 0.7\text{mmol/L}$) $P = 0.282$.

Conclusions: These findings demonstrated that examination triggers stress, which altered progesterone level in female students.

Keywords: Cortisol, Progesterone, Glucose, Academic, Menstrual cycle, Ovulatory, Blood flow, Anovulation, Serum

INTRODUCTION

Stress is a nonspecific attempts by the body to adjust or adapt to the situation in order to re-establish normalcy.¹ This is why it is said that a wide variety of demands or stressors bring forth a common response.² Stress could be due to pressure at school, work or social status.

Sources of stress among Undergraduate students have been identified as poor academic performance, poor interpersonal relationships or skills, inadequate resources to learn, and inadequate time dedicated to assignments and review of what has been learnt, uncomfortable learning setups, overcrowded lecture halls and excessive

paperwork.³ Several studies have documented that academic activities cause high incidences of psychological stress among students.⁴⁻⁷

Examination stress is one of the most widely experienced stressor of students throughout the world.⁵ Persistent academic exercise under these conditions can induce a chronic stress which may adversely affect the performance of the students.⁸

Stressors like examination are more profound in female students resulting in behavioral, metabolic and physiological changes.⁹ Although some effects of some stressors in students have been worked on in country;

studies have not been done on ovulatory status on female undergraduate students.

This study was to examine the influence of examination stress on ovulatory status of female undergraduate students. This was achieved by analyzing serum cortisol level to establish stress and day 21 progesterone to assess ovulation status. Glucose was analyzed in this study to rule out hypoglycaemia as a cause of increase in cortisol.

METHODS

Subject recruitment

Thirty-two apparently healthy Female Undergraduate Students within the age range of 18 and 26 (21 ± 8) years were recruited into this study. Blood samples were collected from all the female students twice. Initial blood sample was collected on day 21 of the menstrual cycle just before second semester examination and subsequent blood samples were collected on day 21 of menstrual cycles after examination. Blood sample for cortisol and progesterone estimation was collected between 7:30am and 9am from the participants; the blood samples were dispensed into plain tubes and allowed to clot, centrifuged at 4000rpm and serum stored at -20°C until day of analysis. Questionnaires were also administered to the recruited female students. Information on menstrual cycle rate of flow, number of days of flow and length of menstrual cycle were provided by the participants on the questionnaire. Random blood glucose was analyzed on the spot.

Ethical approval and informed consent

Study design was approved by the Ethics Committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus. Informed consent was also sought from recruited students after the study design was thoroughly explained to them.

Inclusion/exclusion criteria

Female undergraduate students with regular menstrual cycle who were sitting for the semester examination were randomly recruited into the study. Students with irregular menstrual cycle and other known medical conditions such as fibroid, Poly Cystic Ovarian Syndrome and other ovarian diseases were excluded. Married students, pregnant students and students using contraceptive pills and students with hypoglycaemia were also excluded.

Estimation of cortisol

Cortisol was estimated from serum using reagent from Monobind Inc, 100 North Pointe Drive Lake Forest, California 92630 USA.

Principle: The Cortisol quantitative test is based on a solid phase enzyme-linked immunosorbent assay

(ELISA) which utilizes monoclonal anti- α - Cortisol antibody adsorbed to the microtitre plate that binds to its specific antigen present in the sample. The antigen-antibody reaction when coupled to a secondary antibody labelled with horseradish peroxidase in the presence of its substrate produces a reaction colour that is proportional to the concentration of the antigen in the sample.

Procedure as described by reagent manufacturer

Working solutions of the cortisol-HRP conjugate and wash buffer was prepared. 20 μl of each calibrator, control and specimen was dispensed into corresponding wells in duplicates. 100 μl of the conjugate working solution was dispensed into each well and incubated on a plate shaker (at 200rpm) for 45 minutes at room temperature. The microwell was washed 3 times with 300 μl of diluted wash buffer and tapped against absorbent paper to ensure dryness. 150 μl of TMB substrate was dispensed into each well and incubated on a plate shaker for 20minutes at room temperature. 50 μl of stopping solution was dispensed into each well. The optical density was read at 450nm with a micro-plate reader within 20 minutes.

Estimation of progesterone

Progesterone was estimated from serum using reagent from Pefemed Group, Inc, 358 Oyster Point Blvd Suite 5-6, South San Francisco CA94080.

Principle: The Progesterone quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA) which utilizes monoclonal anti- α - progesterone antibody adsorbed to the microtitre plate that binds to its specific antigen present in the sample. The antigen-antibody reaction when coupled to a secondary antibody labelled with horseradish peroxidase in the presence of its substrate produces a reaction colour that is proportional to the concentration of the antigen in the sample.

Procedure as described by reagent manufacturer

25 μl of standards, specimens and controls was dispensed into appropriately labelled wells. 100 μl of working progesterone-HRP conjugate reagent was dispensed into each well. 50 μl of rabbit anti- progesterone reagent was dispensed to each well, mixed thoroughly for 30 seconds and incubated at room temperature for 90 minutes. The microwell was rinsed and flicked 5 times with washing buffer. 100 μl of TMB substrate was dispensed into each well, mixed gently for 10 seconds and incubated at room temperature for 20 minutes. The reaction was stopped by adding 100 μl of stop solution to each well and gently mixed for 30 seconds, ensuring that all blue colour changed to yellow colour completely. The optical density was read at 450 nm with a micro-plate reader within 15 minutes.

Serum progesterone level between 2.0 ng/ml and 25.0 png/l at luteal phase was considered normal as stated by

the reagent manufacturer. Serum progesterone level less than 2.0ng/ml was considered anovulatory.

Estimation of glucose

Glucose was estimated from whole blood using Accu-check Active glucometer by Roche Diagnostics 9115 Hague Road Indianapolis, IN46256.

Principle: The glucose concentration in the sample is determined by amperometric means: glucose is oxidized in the reactive zone of the test strip by means of glucose dehydrogenase (glucose dioxidoreductase), during which hexacyanoferrate III is reduced to hexacyanoferrate II. The hexacyanoferrate II produced by the reaction is reoxidized by one of the electrodes containing palladium. The electron flow released is proportional to the glucose concentration of the sample and is measured by Accu-check meter.

Procedure as described by acucheck diagnostics

Choice finger was located and disinfected using spirit swab. The glucose meter was turned on and strip inserted into it, according to manufacturer's instruction. Following a gentle massage, the finger was pricked using a sterile blood lancet. The finger was gently massaged to

release a drop of blood. The drop of blood was placed on the test area of the inserted strip to cover the entire test area. The blood glucose result was read as displayed on the meter screen. Glucose level between 2.2 mmol/L and 11.1 mmol/L was considered normal.¹⁰

Statistical studies

The variables were expressed as mean \pm standard deviation (SD). The mean differences were assessed using paired Student's t-test, while levels of association were assessed using Pearson's correlation coefficient. Significant level was considered at $p < 0.05$. All statistical studies were done using Statistical Package for Social Sciences (SPSS).

RESULTS

The mean \pm SD Pre-examination cortisol ($\mu\text{g/dl}$) value was 15.3 ± 5.9 while the post-examination cortisol ($\mu\text{g/dl}$) value was 10.5 ± 5.1 ($P < 0.001$). The mean \pm SD pre-examination progesterone (ng/ml) value was 3.5 ± 1.5 , while the post examination progesterone (ng/ml) value was 4.2 ± 2.6 ($P < 0.001$). The mean \pm SD pre-examination glucose (mmol/L) value was 5.4 ± 0.8 , while the post examination glucose (mmol/L) value was 5.3 ± 0.7 ($P = 0.282$) (Table 1).

Table 1: Mean (\pm SD) values of cortisol ($\mu\text{g/dl}$) estradiol (pg. /ml) and progesterone (ng/ml) pre and post-examination.

Parameter	N	Pre-Examination	Post-Examination	T	p-value
Cortisol	32	15.3 ± 5.9	10.5 ± 5.1	3.667	< 0.001
Glucose	32	5.4 ± 0.8	5.3 ± 0.7	0.581	0.282
Progesterone	32	3.5 ± 1.5	4.2 ± 2.6	1.545	< 0.001

Table 2: Level of associations between pre-examination cortisol ($\mu\text{g/dl}$), estradiol (pg. /ml) and progesterone (ng/ml).

Parameter	n	r	P
Cortisol vs Progesterone	32	-0.090	0.624
Cortisol vs Glucose	32	0.225	0.216
Progesterone vs Glucose	32	0.417	0.017

Table 3: Proportions of students with normal and abnormal ovulatory status.

		n	Progesterone
Ovulatory (Progesterone $> 2.0\text{ng/ml}$)	Pre-examination	26(81.3%)	3.4 ± 1.5
	Post-examination	32(100%)	4.4 ± 2.2
Anovulatory (Progesterone $< 2.0\text{ng/ml}$)	Pre-examination	6(18.7%)	1.1 ± 0.8
	Post-examination	0(0%)	

There was significant positive correlation between pre-examination Progesterone and pre-glucose ($r = 0.417$, $p = 0.017$). There was no significant correlation between

pre-examination cortisol and pre-examination progesterone ($r = -0.090$, $p = 0.624$); there was no significant correlation between pre-examination cortisol

and pre-examination glucose ($r = 0.225$, $p=0.216$) (See Table 2). When progesterone values were considered for each students pre and post-examination, six students (18.7%) out of the 32 students had progesterone values less than 2.0ng/ml pre-examination while all the students had progesterone value greater than 2.0ng/ml post-examination (Table 3).

DISCUSSION

This study revealed that there was significant increase in serum cortisol levels pre-examination when compared with serum cortisol levels post-examination.

The finding in this study demonstrates that the increased cortisol level pre-examination may be due to stress of examination. This may be an indication that demand is higher on the student to meet up with their academic goal. This agrees with the studies carried out by several researchers among Undergraduate students pre- and post-examination.^{8,11,12} They independently reported increase in serum cortisol pre-examination and decrease in serum cortisol post-examination. This result buttress the fact that stress of any origin, whether physical or mental can greatly enhance secretion of Adrenocorticotrophic Hormone and consequently cortisol.

Blood glucose was measured also to ensure that the increased cortisol is not due to hypoglycaemic effect among the recruited subjects. The students had similar blood glucose levels pre and post-examination which indicates that elevated pre-examination serum cortisol is due to examination stress and not induced by hypoglycaemia.

There was increase in serum progesterone level post-examination, when compared with pre-examination level. The decrease in pre-examination serum progesterone levels and increase in cortisol level pre-examination is consistent with patterns indicative of stress since both cortisol and progesterone have the same precursor. Other researchers observed that there was significant decrease in serum progesterone pre-examination when compared with serum levels of progesterone post-examination during the luteal phase of the menstrual cycle of students. They also reported significant decrease in pre-examination progesterone levels, when compared with post examination progesterone.^{8,13}

This study tried to determine the effect of examination stress on ovulatory status of female undergraduate students. Day 21 progesterone levels less than 2.0 ng/ml was considered anovulatory. A further analysis of the progesterone results revealed that 6 (18.7%) of the students has day 21 progesterone less than 2.0ng/ml, hence considered to have failed to ovulate during the pre-examination period. However, it was observed from the results that ovulation had taken place post-examination as post-examination serum progesterone level returned normal (greater than 2.0 ng/ml). This shows that

examination stress temporarily altered the ovulatory function of the students.

Further study of the menstrual cycle characteristic of the students such a length of menstrual cycle, rate of blood flow and number of days of flow, indicates that 3 (9.4%) of the students had increased menstrual cycle length, 7 (21.9%) of the students had reduced menstrual blood flow rate, 4 (12.5%) of the students had reduced number of days of menstrual flow while 1 (3.1%) of the students had increased number of days of menstrual blood flow. This was with reference to their respective menstrual characteristic chartings for many months before the menstrual cycle into the pre-examination period. After the examinations, the menstrual characteristics reverted to pattern many months prior to pre-examination period. This observation therefore showed that examination stress has direct effect on the menstrual cycle characteristics of some the students.

The findings of alterations in the menstrual characteristics of some of the students may not be unconnected with the pressure and stress associated with examination. This could be achieved by the impact of stress on cortisol and progesterone levels because stress has overriding ability over the mechanism of regulation of hormonal synthesis and secretion in humans. For instance, the luteal phase of the menstrual cycle is constant for almost all females as it has been established that menstrual blood flow begins at about the 14th day after ovulation.¹⁴ This implies that the variation in the menstrual cycle length in females is as a result of the variations in the length of follicular phase of females.

CONCLUSION

Stress activates the Hypothalamic-Pituitary-Adrenal axis, which in turn affects ovulation and some menstrual characteristics on undergraduate students. Stress was observed in all the students during examination. Evidence of anovulation was seen in a fraction of the students while stress lasts. However, this anovulation was temporary as ovulatory function was restored after examination. This anovulation observed in a fraction of the students may be explained by the fact that individuals exposed to a particular stressor respond to that stressful situation differently. Stress also altered menstrual cycle characteristics such as menstrual cycle length, number of days of menstrual blood flow and rate to menstrual blood flow during examination.

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