BMJ Open

Cortisol dynamics in undergraduate nursing students during clinical practice: protocol for an exploratory cross-sectional study

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To cite: Loureiro F, Família C, Barroso MH, *et al*. Cortisol dynamics in undergraduate nursing students during clinical practice: protocol for an exploratory cross-sectional study. *BMJ Open* 2023;**13**:e071062. doi:10.1136/bmjopen-2022-071062

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-071062).

Received 14 December 2022
Accepted 22 June 2023

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ABSTRACT

Introduction This paper presents a protocol for the Investigation of Cortisol Dynamics in Undergraduate Nursing Students, a funded project aiming to understand the fluctuations in anxiety and salivary cortisol levels due to clinical setting changes and the anxiety associated with clinical practice.

Methods and analysis This study is an exploratory, cross-sectional, observational study that will be conducted at a health and science school in Portugal. Data collection will involve phycological assessment instruments for personality, anxiety, stress, depression and saliva cortisol levels. The target population consists of undergraduate nursing students enrolled in our institution for the academic year of 2022/2023 (N=272), of whom we aim to recruit 35% (N=96) to the study.

Ethics and dissemination The project obtained approval from the Institutional Review Board of the Egas Moniz—Cooperativa de Ensino Superior, CRL, on 5 July 2022 (ID: 116/21.22) and ethical approval from the Egas Moniz Ethics Committee on 28 July 2022 (ID:1110.22). Informed consent will be obtained from those who wish to participate, ensuring students' voluntary participation in the project. The results of this study will be disseminated through open-access peer-reviewed publications and presented at scientific events.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study's strengths include the collection of diverse data and the presence of professionals from various fields on the research team.
⇒ The limitations include the single-centre setti, the study's exploratory nature and the use of a convenience sample.
⇒ Retention of participants will be a challenge due to the collection of multiple saliva samples, requiring additional strategies to prevent drop-out.

INTRODUCTION

Within the nursing degree, students undergo various internships in a diverse healthcare setting. This period is highly anticipated by the students, yet it is also often accompanied by anxiety,[1] as they will be exposed to the realities of the nursing profession and will have direct contact with individuals who may be sick or healthy. Additionally, internships serve as evaluation experiences where nurse supervisors challenge students.

Nursing students are known to experience higher anxiety levels than other student populations.[2] This anxiety is particularly heightened in clinical settings as opposed to classroom environments.[3] Previous studies have explored nursing students' physiological and emotional responses to stress[4] and their coping strategies.[5] The stress experienced by nursing students has been shown to impact their academic outcomes. Furthermore, it can contribute to psychiatric disorders such as depression and other complications, including sleep disorders and substance abuse, all of which can have short-term and long-term effects on patient care.[6]

Clinical practice has previously been identified as a significant source of stress for nursing students in theoretical articles,[7] systematic[8 9] and integrative[10] literature reviews. The anxiety experienced by nursing students during clinical experiences has been linked to factors such as dropping out of nursing education programmes,[11] resilience and well-being,[12] burn-out,[13] academic performance,[14] and mental health indicators.[2] Furthermore, studies[15 16] have demonstrated a correlation between the personality characteristics of nursing students and their performance. These findings suggest that future professionals may display varying levels of vulnerability or resilience to stress based on their traits.

Salivary cortisol is widely recognised as the most extensively studied endocrinological biomarker for assessing stress exposure. It offers a non-invasive method for measuring

stress levels.[17] When the body experiences stress, cortisol levels in the serum increase and these changes are also reflected in the saliva cortisol levels, making it a reliable biomarker.[18] Cortisol exhibits a diurnal circadian pattern, typically peaking around 30 min after awakening and gradually decreasing throughout the day.[19 20] Studies have demonstrated that measuring cortisol in saliva, particularly in the early morning, provides an accurate alternative to measuring cortisol in the serum or plasma.[21]

Saliva cortisol levels in nursing students have been previously assessed in various contexts, including before assessments[17] in simulation-based education classes,[22] in laughter yoga intervention[23] and a three steps intervention yoga.[24] Saliva cortisol levels have also been used to evaluate stress and the effectiveness of interventions such as the aroma inhalation method,[25] Chinese five-element music therapy on students with depressed mood,[26] friendship network position,[27] effects of lavender and rosemary essential oils on test-taking anxiety[28] and music therapy in stress reduction.[29] Studies have also investigated cortisol levels and stress in nursing professionals, showing a direct association,[30] with high levels observed on working days compared with days off.[31]

By combining psychological assessment instruments with salivary cortisol level measurements, our research aims to elucidate the relationship between clinical practice and stress levels in nursing students. This study will be conducted by a multidisciplinary team from the Egas Moniz School of Health & Science, leveraging the diverse expertise of project participants to achieve our objective.

To the best of our knowledge, this comprehensive analysis has not been previously conducted on nursing students across all years of their curriculum. By comparing students from different years and different clinical practice settings, our original study will provide valuable insights for academic professionals, healthcare professionals and nursing students.

Aim

The main objective of our study, the Investigation of Cortisol Dynamics in Undergraduate Nursing Students (ICDUNurS), is to gain a comprehensive understanding of the fluctuations in anxiety and salivary cortisol levels that occur as a result of changes in the clinical setting and the inherent anxiety experienced during clinical practice. By examining these dynamics, we aim to shed light on the impact of clinical practice on nursing students' stress levels and how it may correlate with their physiological and psychological traits.

This research will contribute to the existing knowledge in the field and provide valuable insights for improving the support and well-being of nursing students during their clinical placements. By considering the individual characteristics of nursing students, such as their personality traits, coping mechanisms and psychological well-being, we can tailor the sequence of their internships to maximise their academic performance and overall well-being.

Adopting a personalised approach to internship sequencing can create a supportive and conducive learning environment for nursing students. This approach can potentially enhance their grades and optimise their overall experience during clinical practice. Ultimately, our study aims to provide evidence-based recommendations to improve the support systems and strategies in place for nursing students, ensuring their success and well-being throughout their educational journey.

METHODS AND ANALYSIS

Study design and setting

According to Burkett's classification,[32] this is a cross-sectional, observational and exploratory study, given the lack of previous studies on this topic specifically for the nursing student population. The study design is reported based on the STROBE statement for cross-sectional studies (https://www.strobe-statement.org/download/strobe-checklist-cross-sectional-studies-pdf), ensuring methodological rigour and transparency. The study adopts a quantitative approach, using psychological assessment instruments and saliva samples for data collection. Quantitative data analysis will be performed using specific statistical analysis software.

The study will be conducted at a health and science school in Portugal. In the nursing programme offered at our institution, which can be considered a traditional programme, students undergo seven clinical practices throughout their degree. These internships are organised to enhance their learning experiences and are structured as follows: in the first year, students practise in a single setting focusing on nursing home care; in the second year, they rotate through three different settings, including medical, surgical and palliative care; in the third year, they experience three different settings including paediatrics, obstetrics and primary care; and in the fourth year, they engage in clinical practices across four settings including community, psychiatric, critical care and an optional setting.

Students from all these clinical settings will be enrolled in this study, allowing for comparisons across different settings and years. Considering this organisational structure, the research project has been designed in four distinct phases, each serving a specific purpose and outlined in detail below.

Study phases

First phase

This first phase aims to determine the baseline levels of stress, anxiety and salivary cortisol among the participants in a controlled environment, free from additional sources of stress, despite the typical academic environment. The study protocol will be presented to all participant students, and the research goals will be clearly explained, as well as the importance of strict adherence to the study. This phase will be scheduled outside of the evaluation periods to ensure that the data collection is not influenced by the

potential stress caused by ongoing assessments. Before the clinical experience, all participants involved in the study will complete three psychological assessment instruments. The Revised NEO Personality Inventory (NEO-PI-R) is a known and accepted instrument across most cultures that allows personality assessment.[33] Additionally, students will complete the Depression, Anxiety and Stress Scale (DASS-21), which is a well-established instrument for measuring depression, anxiety and stress.[34] The third instrument is KEZKAK, a questionnaire measuring nursing students' stressors in clinical practice.[35] The first saliva sample for cortisol analysis will also be collected during this phase. This baseline assessment will be conducted at the beginning of the academic year across all years of the nursing programme, allowing us to capture the students' initial stress and cortisol levels.

Second phase

The same students enrolled in phase 1 will be requested to complete the DASS-21 and the KEZKAK Questionnaire immediately before commencing their internships. This assessment will enable us to evaluate their stress, anxiety and cortisol levels before they begin clinical practice. Additionally, a second saliva sample will be collected according to the following schedule, which aligns with the start of each academic year's clinical practice: 1 month after for fourth-year students (November), 4 months after for second-year and third-year students (February) and 7 months after for first-year students (May). This longitudinal approach will provide valuable data on the students' stress response and cortisol dynamics throughout their clinical practice experience.

Third phase

In this phase, we aim to investigate whether a correlation exists between changes in the clinical setting and fluctuations in students' stress and cortisol levels. To achieve this, the participating students will be asked to complete the anxiety, stress and depression scale (DASS-21) and KEZKAK survey during the transition periods between clinical practice settings. Additionally, saliva samples will be collected from the students according to the following schedule: March (second-year students and third-year students), May (second-year students and third-year students) and December, February and April (for the fourth-year students). These data collection points will allow us to examine the potential impact of changing clinical environments on nursing students' stress and cortisol levels.

Fourth phase

The final phase of the study will take place at the end of the academic year, and its purpose is to evaluate the anxiety, stress and cortisol levels of all nursing students on completing their clinical practice. During this phase, the participants will be asked to complete the anxiety, stress and depression scale (DASS-21) and KEZKAK questionnaires. In addition, the final saliva samples for cortisol analysis will be collected in June for all academic years. This comprehensive assessment will provide valuable insights into the students' stress levels and cortisol dynamics after their clinical practice experience.

Student recruitment

Detailed information about the research project will be provided to potential participants before enrolment to guarantee the recruitment of suitable candidates for the study. The research team will be responsible for the recruitment process, and to prevent any potential coercion, no teacher who evaluates students during clinical practice will be involved in the recruitment procedure.

The project will be announced in classes, and additional research details will be available through a link shared on popular social networks such as Facebook and Instagram. This link will contain general information about the research project, including its purpose, eligibility criteria, time and commitment required from the participants, research location, confidentiality and privacy insurance, ethical approval, and additional contact information for further inquiries. Students interested in volunteering for the study will register their intention to participate by accessing the provided link.

After 3 weeks, the interested students will be contacted via email to confirm their interest in participating in the study. They will receive further information about the study's details and will have the opportunity to ask any additional questions they may have. This process ensures that the potential participants have ample time to consider their decision and obtain all the necessary information before formally committing to the study.

Inclusion and exclusion criteria

The inclusion criteria for the participants in the study are nursing students from all academic years who are enrolled in the clinical nursing curriculum unit and provide their consent to participate. Exclusion criteria include registered students who are not participating in clinical practice. Additionally, a literature review was conducted, which highlighted the potential influence of corticosteroid therapy and autoimmune disorders on cortisol levels. Consequently, participants with these conditions will be excluded from the study to ensure the accuracy and reliability of the cortisol measurements.

Sample size

As an exploratory study, we aimed to obtain a representative sample of nursing students from our school. Therefore, all nursing degree students in their first, second, third and fourth years of study for the academic year of 2022/2023 (N=272) ill be eligible to participate. To facilitate registration, we created a dedicated webpage (https://nursecortisol.wixsite.com/icdunurs) where students can sign up. We aim to achieve a sample comprising 35% of the enrolled students from each year of study. Specifically, we aimed to include 25 students from the 1st, 22 from the 2nd year, 26 from the 3rd year and 23 from the 4th year,

resulting in a total sample size of 96 participants (n=96). In case more students express their interest in participating than the target sample size, a random selection process will be employed to select the students.

Because this study is exploratory and has a limited budget, the sample size was not determined using formal statistical methods. Although a larger sample size would have increased statistical power, we believe that the qualitative aspects resulting from the sociodemographic characteristics, anxiety and stress questionnaires, and psychological questionnaires used in this study can yield valuable insights even with smaller sample sizes. Although we are aware that a smaller sample size may limit the generalisability of the findings, our focus is on gathering preliminary data and gaining a deeper understanding of the topic. Despite the limitations, we are confident this study will provide valuable insights into the relationship between cortisol levels and stress in nursing students.

Statistical analysis

The sample will be characterised using descriptive statistics such as mean, SD, median, minimum, maximum and range. These statistics will be calculated for the salivary cortisol levels, anxiety and stress, for each year of study, internship, and selected sociodemographic and personality characteristics. Boxplots will also be created to visually represent the distribution of salivary cortisol levels, anxiety and stress, for each year of study, internship, and selected sociodemographic and personality characteristics.

Linear mixed-effects models will be used to examine the effect of the year of study, internship, sociodemographic characteristics and personality on the salivary cortisol levels, anxiety and stress scores. These models will include random intercepts for each individual to account for within-subject correlations. The significance of these effects will be assessed through factorial type II analysis of variance with Satterthwaite's approximation for the df. Effect sizes (partial $\eta 2$) will be calculated for significant effects.

Assumptions of normality and homoscedasticity of the standardised residuals will be assessed through residual plots and the Shapiro-Wilk and Breush-Pagan tests. If these assumptions are not met, transformations of the dependent variable (cortisol levels, anxiety or stress) will be applied, followed by the development of new models.

Data management

Data management and protection in this study will adhere to the standards outlined in the General Data Protection Regulation (GDPR) UE 2016/679. The GDPR provides specific requirements and principles for handling personal data and protecting privacy, and it applies to organisations that collect data from individuals in the European Union. The following measures have been implemented to ensure compliance with the GDPR:

1. Saliva sample collection: participants will collect their own saliva samples in the morning, 30 min after awakening, before eating or brushing their teeth. Detailed instructions will be provided to all students during the recruitment phase. Additionally, email communication will be sent to all enrolled students explaining the collection process. Each student will receive a sample tube and a flyer containing the collection instructions.
2. Sample storage: samples will be kept at 4°C if cortisol determination will be made in 24 hours or at −20°C for posterior analysis. Once the analysis is completed, the samples will be appropriately disposed of and destroyed.
3. Phycological assessments: phycological assessment instruments will be applied by a qualified team member from the field of psychology who is a registered member of the Portuguese Psychology Council.
4. Data processing and access: the principal researcher will process the obtained data, and access to the data will be limited to the central research team. A randomly generated alphanumeric code known only by the principal investigator (PI) will be assigned to each. This code will be used throughout all phases of the study, ensuring the anonymity and confidentiality of the participants. Participant names/codes will only be accessible to the principal researcher, who will retain this information for 5 years following national research regulations.

Patient and public involvement

Four students, one from each academic year, were consulted regarding the study and provided valuable feedback on the study protocol and materials, including the NEO-PI-R, anxiety, stress and depression scale DASS-21, KEZKAK questionnaire and consent form. Based on their input, we made the following modifications: (A) the consent form was modified to provide more straightforward and more explicit information and (B) the dates for the collection of salivary cortisol samples were adjusted to a more suitable time frame based on their suggestions. No further student involvement was considered for the project at this stage.

Ethics and dissemination

The project obtained approval from the Institutional Review Board of the Egas Moniz—Cooperativa de Ensino Superior, CRL, on 5 July 2022 (ID: 116/21.22) and ethical approval by the Egas Moniz Ethics Committee on 28 July 2022 (ID:1110.22). Informed consent will be obtained from those who wish to take part to ensure the voluntary participation of students in the project. Underaged students' consent will be signed by their parents.

The initiative includes a proactive approach to disseminating the findings within the scientific community. Dissemination will be accomplished through various means, such as preparing reports and delivering presentations to relevant stakeholders. Furthermore, the research results will be shared through open-access peer-reviewed publications in reputable scientific journals. Additionally,

the project team aims to present the findings at scientific conferences, where they can engage with other researchers and professionals in the field, exchange knowledge and contribute to advancing nursing research. By employing these dissemination strategies, the study outcomes will reach a broad audience, fostering dialogue and promoting further exploration of the subject matter.

Study status

The recruitment phase, which included the first salivary collection and instruments application, took place in October 2022, at the beginning of the academic year. The second phase occurred as planned in November for fourth-year students, February for second-year and third-year students, and May for first-year students. At time of submission of this protocol manuscript, the study is in its third phase. We expect study completion (phase 4) at the end of June 2023.

DISCUSSION

The ICDUNurS study aims to provide a comprehensive understanding of stress, anxiety and cortisol levels among nursing students in various clinical practice settings and academic years. By including a diverse sample of students from all academic years, we can obtain a more integrated knowledge of this subject and also allow us to analyse the results separately by academic year and clinical setting. This approach allows for a more nuanced understanding of the experiences and challenges faced by the students at different stages of their nursing education.

Including the NEO-PI-R personality assessment tool in this study will provide valuable information on the relationship between the levels of stress and the student's personality traits. This area is underexplored in previous research, and the findings from this study can contribute to understanding how personality factors may influence stress and anxiety among nursing students.

Salivary cortisol is widely recognised as the most extensively studied endocrinological stress response biomarker for assessing stress exposure.[17] It is a non-invasive and non-stressful technique compared with venipuncture for blood collection to determine stress levels. In this study, we have chosen to use the passive drool saliva method with absorbing devices, which has been found to be effective in previous research.[31 36] The use of this method ensures accurate and reliable cortisol measurements.

Our study has three primary limitations that need to be acknowledged: potential drop-out, the study's exploratory nature and the single-centre setting.

To ensure participant engagement and minimise drop-outs, the research team will implement measures to remind participants of collection dates and the completion of questionnaires. Specifically, the PI will maintain regular communication with participants by sending email messages before each salivary collection and questionnaire application to provide timely reminders and maintain participant involvement throughout the study. In addition, the drop-out rate will be tracked to analyse whether it affects disproportionately specific subgroups (academic year) of students.

The second limitation is related to the exploratory nature of the study. Because of this, we did not perform a formal sample size calculation to achieve a predetermined power from the inferential statistic tests. This decision was made deliberately due to the lack of precise information on key parameters, such as estimates of variance components, correlation structure and effect sizes, which are essential for an accurate sample size calculation. Consequently, the study's results will be interpreted considering its exploratory nature.

Regarding the study's single-centred setting, we must consider that it may not accurately represent a larger population or different contexts. Thus, results may not be generalisable to all nursing programmes or clinical settings as our institution's specific factors may impact the findings. However, by involving professionals from diverse fields such as nursing, psychology and pharmacy, this study benefits from interdisciplinary perspectives, strengthening the investigation. Nonetheless, future research should include multicentred studies involving diverse nursing student populations, geographical locations or academic settings in order to increase generalisability.

Overall, the ICDUNurS study aims to provide valuable insights into nursing students' experiences regarding stress, anxiety and cortisol levels. This study seeks to contribute to the existing knowledge base and inform interventions and support systems for nursing students in their clinical practice by including a diverse sample, using the NEO-PI-R tool, and employing the non-invasive salivary collection method.

Contributors AVA serves as the principal investigator for this study. AVA and FL conducted the preliminary literature review and wrote the manuscript. AVA, FL, CF, RVB and MHB participated in the study design and protocol drafting. RVB contributed to the questionnaire's selection and authorisation for its use. CF advised on statistical methods and edited and commented on different manuscript versions. All the authors participated in, read and approved the final manuscript.

Funding This work is financed by national funds through the FCT - Foundation for Science and Technology, I.P., under the project UIDB/04585/2020.

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained directly from patient(s).

Provenance and peer review Not commissioned; externally peer reviewed.

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