Proposal Information

Proposal Id	375 2022
Title of The Proposal	Youth Health and Stress Study in Chitwan Nepal
Created Date	July 25, 2022
Applied Date	August 7, 2022
Duration	July 1, 2022 to June 30, 2023

Screening

Does your research relate to health?	Yes
Is there any international researcher involved in your study team ?	Yes
Is this your thesis ?	No
Education Level	Other

Administrative Information

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Signature

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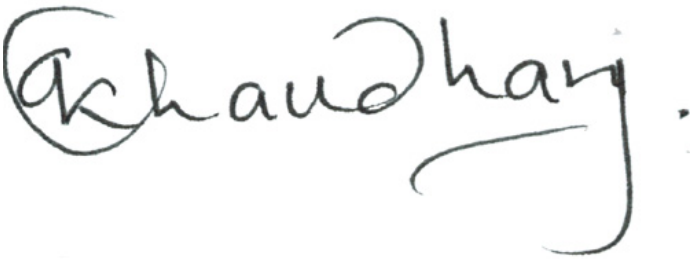

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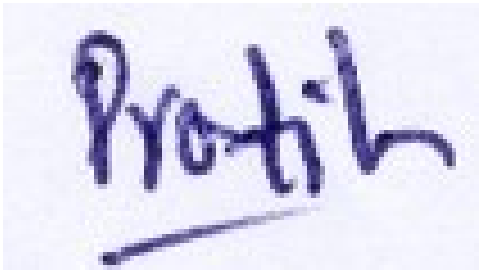
Signature



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List the name(s) and institutional affiliation to the researcher(s)(other than co-investigator) to assist your project in Nepal and abroad

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List the name(s) of nepali researcher(s)(other than co-investigator) or Nepalese Institution/hospital/NGO(s) etc. from whom you may seek co-operation

List the major equipment(s) in relation to your research project you plan to bring/import to Nepal

Financial Details

Human Resource Cost:	Rs 1157579
Field cost:	Rs 655629
Laboratory Cost:	Rs 2122227
Data management cost:	Rs 549814
Report writing and dissemination Cost:	Rs 336648
Logistic cost:	Rs 1520773
Monitoring and evaluation cost:	Rs 288432
Miscellaneous cost:	Rs 20210
Ethical Approval cost:	Rs 199539.36
Total budget of research:	Rs 6651312.00
Is this research funded?	Yes
What is the total amount funded?	Rs 6651311.00
Funding Organization Name	NIH
Postal Address	University of Michigan
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Technical Details

Title of Research :

Youth Health and Stress Study in Chitwan Nepal

Research Area :

Adolescent Health

Summary of the proposal (Structured):

Summary :

Population-scale measurements of psychological stress during the transition to adulthood are generally constrained to either self-reported measures or biomarkers that reflect only recent hours of brain response to stress. Recent breakthroughs in the use of hair to obtain stable measures of cortisol—a biomarker for the impact of stress on the human brain—has the potential to revolutionize this science. Hair-based cortisol provides a reliable measure of brain-experienced stress levels for the three months directly preceding collection of a hair sample. Laboratory processes for cortisol analysis of hair are now available

at a scale that can be used for general population research. Like some other biomarkers, hair could be collected by study participants themselves, greatly lowering the costs of adding such measurement to population-scale research. However, the field currently has no carefully studied protocol for large-scale hair collection, and no information about the selection biases likely to result from self-collection rather than professional-collection.
We will overcome this obstacle by using a large, randomized experiment to assess options for integration of hair-based cortisol measurement into population-scale studies. This experiment, comparing self-collection of hair for cortisol to professional-collection, will be integrated into a long-term family panel study with existing predictors of stress and the transition to adulthood from neighborhoods, households, parents, and individual young adults. These measures will support comprehensive evaluation of the selection bias in self-collection of hair samples. We will randomly assign 1,448 respondents aged 18-21 to two arms of an experiment comparing self-collection of hair to professional-collection. Analyses will examine success collecting hair, the quality of the hair sample, and the participant's self-evaluation of the process. We will link measures of these outcomes to thousands of existing measures of events over time in the individuals' own lives, their parents' lives, the lives of other household members, and the local community context. Analyses will feature exploration of all measures to identify any that are associated with refusal to participate, compliance with self-collection, quality of the hair samples, or self-reports of adverse responses to the hair-collection protocols.
Results from these analyses will provide the means to establish the limitations of large-scale hair-based cortisol self-collection. We will design an optimal protocol for integrating hair-based cortisol collection into population studies across settings and release that protocol to the public. We will also archive these innovative cortisol measures and collection procedures at ICPSR, allowing all researchers to launch innovative new analyses of psychological stress in the transition to adulthood.

Introduction:

Background :

The transition to adulthood is a demographically dense phase of the life course, with many intersecting, highly consequential transitions. These include exit from education, entry into work, entry into courtship and sexual relationships, marriage, childbearing, and divorce. The timing of these transitions and the occurrence of key events—especially sex, contraception, unintended pregnancy, and divorce—have strong long-term associations with subsequent health and wellbeing. As adolescents pass into the transition to adulthood, psychological stress can become a major influence on behavior. Adolescent comorbidity negatively affects cognitive, physical, psychological, and behavioral functioning, and perhaps not surprisingly, increases overall distress and leads to poorer treatment outcomes. Multiple studies have shown robust associations between adolescent psychological stress or mental disorders and subsequent negative outcomes in adulthood across a number of important life domains

Self-reported measures of psychological stress have long been the only option for researchers. Though many improvements have been made over time, self-reported measures of stress are imprecise and inconsistent across contexts and time, owing primarily to issues of recall bias, social desirability reporting, question order, context, interviewer characteristics, and location. This has been a key limitation to accurately and consistently understanding the role of stress in many complex relationships, such as the transition to adulthood.

The extraction of cortisol from human hair, an ideal, stable, reliable, valid, non-invasive marker of long-term stress in the form of hair cortisol concentration (HCC) has been an advancement. Researchers can now document and explore the body's response to stress, especially chronic stress, and its eventual role in recovery, adaptation, and eventually convalescence. No longer encumbered by the limitations of self-report measures of stress, HCC has liberated researchers to create retrospective physiological baselines, explore consequences of chronic stress, and target interventions based on these findings. Given the importance of HCC, large population-based studies have started to test its feasibility, and have found diverse populations generally accepting, with some potential selection bias issues. Self-collection of HCC measures has the potential to revolutionize the scientific study of population-scale psychological stress in

the transition to adulthood. However, creation of a protocol to accomplish this requires significant effort. Also, the self-collection of HCC measures creates the possibility of even broader selection bias (refusal, compliance, return of samples). Self-collection has been tried, but only at small scale and without any randomization or study of selection bias.

Rational/justification :

The transition to adulthood is a demographically dense phase of the life course, with many intersecting, highly consequential transitions. These include exit from education, entry into work, entry into courtship and sexual relationships, marriage, childbearing, and divorce. The timing of these transitions and occurrence of key events—especially sex, contraception, unintended pregnancy, and divorce—have strong long-term associations with subsequent health and wellbeing.55–64
As adolescents pass into the transition to adulthood, psychological stress can become a major influence on behavior. Approximately 50% of children and adolescents suffer from mental disorders.65-67 Population representative studies of adolescents demonstrate alarming lifetime prevalence rates across DSM-IV68 disorders: 31.9% Anxiety Disorders (i.e., phobias, generalized anxiety disorders, panic disorder, post-traumatic stress disorder), 14.3% Mood Disorders (i.e., depression, bipolar disorder), 19.6% Behavioral Disorders (i.e. attention deficit hyperactivity disorder, oppositional defiant disorder, conduct disorder), 11.4% Substance Use Disorders (i.e., alcohol, drug abuse/dependence), and 2.7% Eating Disorders.69 These psychiatric illnesses have profound consequences on psychosocial and emotional development, including poor academic outcomes and reduced educational attainment,70-72 greater interpersonal discord (e.g., peers, family),73, 74 greater risky behavior engagement (e.g., unsafe sexual behaviors, illegal activities),75, 76 and increased use of non-suicidal self-injurious behaviors (e.g., cutting, burning)77, 78 and suicidal behaviors (e.g., ideation, planning, attempts).79, 80 Adolescent comorbidity negatively affects cognitive, physical, psychological, and behavioral functioning, and perhaps not surprisingly, increases overall distress and leads to poorer treatment outcomes.81, 82
Multiple studies have shown robust associations between adolescent psychological stress or mental disorders and subsequent negative outcomes in adulthood across a number of important life domains. First, youth disorders are predictive of broad-based risky behavior engagement and illegal activities. Notably, whereas alcohol misuse increases the likelihood of risky behavior occurrence (e.g., sexual risk-taking) in early adulthood (ages 21-30 years),83 early conduct problems increase the odds of arrests and imprisonment.84 Second, youth-based mental disorders are associated with reduced educational attainment and greater welfare dependence. For example, both substance use and depressive disorders in youth are linked to poor educational attainment (i.e., greater rates of school dropout) both in secondary schools85, 86 and college,72 which often contribute to long-term welfare dependence and unemployment.86,87 Third, there is evidence that adolescent psychological stress or mental disorders can lead to higher rates of teen pregnancy, partner violence, and marriage dissatisfaction. In a 35-year longitudinal study in New Zealand, youth depression predicts increased rates of unplanned pregnancy and frequent intimate partner victimization (in females).88 In terms of behavioral disorders, conduct disorder in childhood increases the likelihood of teen pregnancy89 and is a known contributor of partnership discord and dysfunctional parenting (e.g., physical punishment, low parental warmth).90 Collectively, psychological stress and disorders in youth profoundly impact functioning across domains.

By collecting and archiving both the protocols and these new, reliable measures of stress during the transition to adulthood, this project will provide an important foundation for future research. This includes both current research to model the transition to adulthood using CVFS as well as proposals for new large scale research, either using the CVFS or entirely different populations.

Conceptual Framework

N/A

General Objective :

We will devise, test, refine, and share a new approach for population-scale collection of a superior biomarker of psychological stress (hair-based cortisol) during the transition to adulthood. Because psychological stress in the transition to adulthood has many potentially harmful health consequences—including increased substance use/ risk taking and decreased mental health/ cognitive performance—this improved approach has the potential to advance both understanding of the key risk factors and design of interventions to improve health outcomes.

To develop, test population-scale collection protocol of a biomarker of psychological stress (hair-based cortisol) during the transition to adulthood

Specific Objective :

Objective 1: To develop, test, refine and launch population-scale collection protocol (self-collection to vs professional-collection) of a biomarker of psychological stress (hair sample).

Objective 2: To analyze across both arms the success collecting hair, the quality of the hair samples, and subject self-evaluation of the process.

Objective 3: To extract cortisol from the hair sample at Center for Molecular Dynamics Nepal.

Objective 4: To conduct descriptive analyses comparing the two experimental conditions (self-collected vs. professional-collected).

Research Hypothesis

H:1 Compared to self-collection professional collection will have higher rate of sample collection; 2) better quality of sample; and 3 less biased sample. H:2 Individual's socioeconomic and demographic background is likely to be associated with HCC level. H:3 Individual's lifetime adverse experiences will be positively associated with higher level of HCC. H:4 Individual community context is likely to modify the observed relationship between individual's socioeconomic and demographic background and HCC level.

Study Variables

Dependent variables: Response rate of self- collection and professional collection, cortisol level, mental health, substance use and risk-taking behavior. Independent variables: Individual socioeconomic background: Age, sex, birth order, mothers' age at the time of birth, ethnicity/caste, education, work, travel, living arrangements, attitudes and beliefs, marital and childbearing history, and contraceptive use. Family background: Parents' age, ethnicity/caste, years of schooling, work histories, religiosity, age at first marriage, marital arrangement, childbearing, migration, travel, group participation, domestic violence, health service visits, attitudes and beliefs. Household background: Living arrangements; household size, structure, and sex and age composition; health of household members; annual income; livelihood; land holding; house quality, wealth, and assets; access to drinking water, toilets, electricity, transportation. Community characteristics: Distance from urban center, proximity to schools, health services, markets, transportation, community ethnic composition, education levels, and social service institutions.

Research Method :

quantitative

Research design :

Cohort

Description of research design :

The approach to this population-scale hair-based cortisol self-collection experiment has three parts: (1) preparation of the protocol, instruments, and collection kits; (2) hair sample collection; and (3) cortisol extraction. Part I - Preparation work (now complete) Instrument design. We designed tools for each arm: a recruitment script with eligibility screener, a video about how to collect the hair sample, a professional- and self-collection protocol with instructions for self-collection that will be randomized to eligible respondents; and an evaluation interview for subjects who did and did not provide a hair sample. The instruments, protocols, and hair collection kits replicate tools used for successful professional hair-collection protocols in U.S. national surveys conducted by SRC. SRC uses a rigorous protocol, with training materials, a certification process, and quality assurance plans. ISER-N translated each of these tools into Nepali, then back-translated and refined them to produce the final tools for this study. Respondent recruitment and eligibility screening will be done by phone. This mode is far less expensive than face-to-

face contact and widely used across all settings. Eligibility screener: The recruitment script screens respondents for eligibility for hair collection (residence, hair, age, steroid use) and factors that have been found to affect hair collection success (hair washing, use of hair products). CVFS monthly registry data already includes other key measures related to HCC (e.g. sex, pregnancy). Respondents will be eligible for hair collection if the respondent, a) has hair b) is between 18-22 years old, c) is still living in the study area, and d) has not used steroid medications in the past 3 months. The recruitment screener is identical for all respondents and administered during initial contact. Hair-collection protocol: We designed a hair-collection protocol and corresponding materials for each experimental group. The professional- and self-collection protocols are identical except for the instructions about who is collecting and how to return the hair sample. For the professional-collection protocol, we designed training materials and corresponding collection instructions. For the self-collection protocol, we designed self-collection instructions and instructional videos for long and short hair. Both the professional- and self-collection materials provide step-by-step instructions, including photographs to illustrate each step. All self-collection respondents will have access to the video demonstrating the process and centralized help by phone or text. Both the professional- and self-collection instructions assume low formal education in order to be inclusive of all respondents. Hair-collection kits: The self-collection kit includes scissors, comb, hair clips, strings, aluminum foil, a re-sealable plastic bag (specimen pouch), return envelope, procedure to collect hair sample and a copy of the consent form for the subject's records. The professional-collection kit includes gloves, permanent marker and an alcohol swab in addition to the items included in self-collection kit. (Only the self-collection kits will include envelopes to return the sample.) The aluminum foil, plastic bag and return envelope will be pre-labeled with a unique identification number and will not contain identifiable information. The professional-collection and self-collection instructions clearly explain how to use each item in the kit. Protocol evaluation interview: Similar survey interviews will be administered to subjects who provided a hair sample (Successful-Collection Survey) and subjects who did not provide a hair sample (Failed-Collection Survey). The evaluation interview includes questions designed to assess the collection protocol and other factors that may have influenced participation (e.g., a new hair cut/style, religious significance of hair). These measures will be used to investigate reasons for failure across study arms, including quantity of refusals, types of resistance, quantity and reasons for failed collection, and quality of the hair samples. Pilot testing: We will conduct pilot tests of the self-collection process. Using the recruitment script and eligibility screener, we will recruit subjects from outside of the CVFS sample and conduct professional and self-collection protocols. This pilot will include a protocol evaluation interview with each subject. Next, we will revise the protocols and instruments based on the results of the first pilot test then recruit new subjects to test the revised protocols and instruments. We will repeat this process until we are satisfied with the protocols and instruments (up to 300 pilot subjects). Part II - Hair collection field work Launch hair collection. Staff will call professional-collection respondents to administer the recruitment and eligibility script and schedule a collection visit. Staff will consent the subject, collect the hair, and administer the evaluation survey at this visit. Staff will call self-collection respondents to administer the recruitment and eligibility script, collect informed consent, explain the protocol and inform them of when the self-collection materials will be delivered and hair samples picked-up. ISER-N will transport the self-collection kits to the homes of subjects and pick-up the hair samples. The evaluation interview will be administered via phone after the protocol attempt. Part III - Cortisol extraction The cortisol extraction will be facilitated under the partnership of SRC/ISER-N with the Center for Molecular Dynamics Nepal (CMDN). ISER-N will transport CVFS hair samples to CMDN, CMDN will complete quality inspections, extract cortisol following the procedure described in Hollenbach et. al 2019, and provide these measures via electronic data file transfer. ISER-N will link the CMDN data back to respondents' CVFS IDs. CMDN will not have access to subject identifiers.

Total follow up duration :

0day

Follow up interval :

0day

How the follow up will be done :

This is one-time research. Once the hair sample will be collected, we will conduct a short successful survey

. Then we will not follow up the research participants.

What tests will be performed during follow up?

Same as above

Who will follow up the participants?

Same as above

Study Population

The study population includes young adults ages 18-22 years currently living in the study area (within Chitwan and surrounding area). The transition to adulthood that generally occurs between ages 18-22 is the most demographically dense phase of the life course, with many intersecting, highly consequential transitions. These include exit from education, entry into work, entry into courtship and sexual relationships, marriage, childbearing, and divorce. The timing of these transitions and occurrence of key events—especially sex, contraception, unintended pregnancy, and divorce—have strong long-term associations with subsequent health and wellbeing.18–27 As adolescents pass into the transition to adulthood, psychological stress can become a major influence on behavior. Approximately 50% of children and adolescents suffer from mental disorders.28–30 Population representative studies of adolescents demonstrate alarming lifetime prevalence rates across DSM-IV31 disorders: 31.9% Anxiety Disorders (i.e., phobias, generalized anxiety disorders, panic disorder, post-traumatic stress disorder), 14.3% Mood Disorders (i.e., depression, bipolar disorder), 19.6% Behavioral Disorders (i.e. attention deficit hyperactivity disorder, oppositional defiant disorder, conduct disorder), 11.4% Substance Use Disorders (i.e., alcohol, drug abuse/dependence), and 2.7% Eating Disorders.32 These psychiatric illnesses have profound consequences on psychosocial and emotional development, including poor academic outcomes and reduced educational attainment,33–35 greater interpersonal discord (e.g., peers, family),36,37 greater risky behavior engagement (e.g., unsafe sexual behaviors, illegal activities),38,39 and increased use of non-suicidal self-injurious behaviors (e.g., cutting, burning)40,41 and suicidal behaviors (e.g., ideation, planning, attempts).42,43 Adolescent comorbidity negatively affects cognitive, physical, psychological, and behavioral functioning, and perhaps not surprisingly, increases overall distress and leads to poorer treatment outcomes.44,45 Multiple studies have shown robust associations between adolescent psychological stress or mental disorders and subsequent negative outcomes in adulthood across a number of important life domains. First, youth disorders are predictive of broad-based risky behavior engagement and illegal activities. Notably, whereas alcohol misuse increases the likelihood of risky behavior occurrence (e.g., sexual risk-taking) in early adulthood (ages 21-30 years),46 early conduct problems increase the odds of arrests and imprisonment.47 Second, youth-based mental disorders are associated with reduced educational attainment and greater welfare dependence. For example, both substance use and depressive disorders in youth are linked to poor educational attainment (i.e., greater rates of school dropout) both in secondary schools48,49 and college,35 which often contribute to long-term welfare dependence and unemployment.49,50 Third, there is evidence that adolescent psychological stress or mental disorders can lead to higher rates of teen pregnancy, partner violence, and marriage dissatisfaction. In a 35-year longitudinal study in New Zealand, youth depression predicts increased rates of unplanned pregnancy and frequent intimate partner victimization (in females).51 In terms of behavioral disorders, conduct disorder in childhood increases the likelihood of teen pregnancy52 and is a known contributor of partnership discord and dysfunctional parenting (e.g., physical punishment, low parental warmth).53 Collectively, psychological stress and disorders in youth profoundly impact functioning across domains.

Sampling unit

Individuals

Sample size

1500 Individuals To achieve the goal of the study we plan to draw our sample from the Chitwan Valley Family Study (CVFS) sample. CVFS is a 26-year long community and whole family panel study and provides a representative sample of the study area. From CVFS panel sample. We will select approximately 1,500 respondents aged 18-22 in January 2023 who were interviewed in the 2016-18 CVFS

mental health survey and ongoing web survey of adolescents. First, we will administer the eligibility questionnaire. If the respondent is ineligible, then we will contact the respondent in three months to re-attempt enrollment. Among eligible respondents, we will randomize 500 to professional-collection and the remaining (approximately 1,000) respondents to self-collection. The unbalanced random selection will ensure sufficient self-collection cases to investigate both predictors of completing the self-collection and predictors of the quality of the sample collected (which is only possible for those who collect a sample). Except the data collected in previous survey no new data has been collected for this study. Statistical Power of the CVFS. The CVFS design provides high statistical power. in general for intergenerational estimates, and much higher power than related prior studies. The documented design effects in the CVFS are in a range from 1.1-1.3, yielding ample statistical power to detect odds ratios in the 1.10-1.20 range even on subsamples of the CVFS comprising as few as 1,448 individuals. The objective of our investigation into selection bias is to identify the largest drivers of successful hair collection. Thus, we expect the CVFS to provide the necessary statistical power.

Number of participants and Justification :

Imbedded within the larger, ongoing CVFS panel study, we propose to interview 1,500 CVFS subjects aged 18-21 in May 2022 (aged 18-22 at recruitment) and 300 additional subjects outside of the CVFS sample for pilot testing (1,800 subject total).

Statistical Power of the CVFS.
The CVFS design provides high statistical power. in general for intergenerational estimates, and much higher power than related prior studies. The documented design effects in the CVFS are in a range from 1.1-1.3, yielding ample statistical power to detect odds ratios in the 1.10-1.20 range even on subsamples of the CVFS comprising as few as 1,448 individuals. The objective of our investigation into selection bias is to identify the largest drivers of successful hair collection. Thus, we expect the CVFS to provide the necessary statistical power.

Sampling Technique

We plan to draw our sample from the Chitwan Valley Family Study (CVFS) sample. CVFS is a 26-year panel study of social, demographic, and ecological change in Western Chitwan Valley that includes the current Bharatpur Metropolitan City with an estimated population of 280528 in 2021.26,27 This panel is based on a stratified two-stage cluster area probability sample of the Chitwan Valley population in 1994.26 The study area was stratified into three strata based on distance from the town of Narayanghat, such that each stratum has approximately the same number of settlements (tols). The primary sampling unit (PSU) of selection was a settlement (range 5-1,000 households). Of the total of 198 settlements, 10 in each stratum were selected (30 total). Settlements were sampled with a probability proportional to the estimated size. In the second sampling stage, a sample of 4 SSUs were randomly selected from each selected PSU. Additional SSUs were selected to oversample specific ethnic groups (Newar and Tibeto-Burmese); 151 SSUs were selected, and all households within a SSU were included for a total of 1,581 households and 5,727 individuals aged > 15 years old. The original sample has been continually refreshed by adding newly age-eligible household members over time. We project that by January 2023, about 1,500 CVFS participants will be age-eligible and agree to participate in the proposed new study, after accounting for attrition due to death, lost/moved out, and non-response. From CVFS panel sample we will select all 1,500 respondents age 18-22 in January 2023 who were interviewed in the 2016-18 CVFS mental health survey and ongoing web survey of adolescents. First, we will administer the eligibility questionnaire. If the respondent is ineligible, then we will contact the respondent in three months to re-attempt enrollment. Among eligible respondents, we will randomize 500 to professional-collection and the remaining (approximately 1,000) respondents to self-collection. The unbalanced random selection will ensure sufficient self-collection cases to investigate both predictors of completing the self-collection and predictors of the quality of the sample collected (which is only possible for those who collect a sample). Except for the data collected in the previous survey no new data has been collected for this study.

Criteria for sample selection

The CVFS is a 25-year-long prospective panel study. In 1995 a systematic, equal probability selection of tols (similar to small villages) occurred. The result is a population-based probability sample of 151 tols, with stratification by distance from the urban center and oversampling to ensure high levels of variance in

key dimensions of social context and change. We will select all 1,500 current respondents age 18-21 in May 2022 who live in the 151 CVFS tols and who participated in a recent data collection (e.g., mental health survey, young adult web survey). We will randomize 500 to professional-collection and the remaining 1,000 respondents to self-collection. (The unbalanced random selection will ensure sufficient self-collection cases to investigate both predictors of completing the self-collection and predictors of the quality of the sample collected.) If the subject is screened ineligible, we may contact the subject at a later date to reassess eligibility. Exclusion Criteria: Since we have been going to collect the hair sample from the participants, we exclude the cases even though they meet the eligibility criteria mentioned above. Besides, the cases will be also excluded who have really short hair or no hair at all during data collection and who are taking inhaled steroids like Flovent or Pulmicort.

Data Collection Technique

Data collection will be conducted with two different techniques; professional-collection and self-collection. For professional-collection respondents, a trained interviewer will visit the subject's home on the scheduled collection date, collect the hair sample, and administer the evaluation survey. Self-collection respondents will collect their own hair sample using a hair sample collection kit and instructions delivered to their home. The professional-collection will involve the following steps: put on gloves, secure two small sections of hair at the back of the respondent's head using string, cut the hair as close to the scalp as possible, place the hair in the pre-labeled foil, fold the foil, place the sample containing foil in the specimen pouch, seal the specimen pouch, put it in the pre-labeled envelope, and return the sample to ISER-N. The self-collection will involve the following steps: wash the hands, secure two small sections of hair with string at the back of the head, cut the hair as close to the scalp as possible, place the hair on the pre-labeled foil, fold the foil, place the sample containing foil in the specimen pouch, seal the specimen pouch and put it in the pre-labeled return envelope for pick-up by study staff. A trained interviewer will later follow-up with respondent by phone to administer the evaluation survey.

Data collection tools

Data collection tools for this study are 1. Recruitment and eligibility script and Successful-collection survey questionnaire 2. Recruitment and eligibility script and fail-collection survey questionnaire 3. Consent forms 4. Hair self-collection instructions 5. Hair professional-collection instructions 6. Long hair video instructions 7. Short hair video instructions 8. Hair self-collection kits

Pretesting

Pilot testing: We will conduct pilot tests of the self-collection process. Using the recruitment script and eligibility screener, we will recruit subjects from outside of the CVFS sample and conduct professional and self-collection protocols. This pilot will include a protocol evaluation interview with each subject. Next, we will revise the protocols and instruments based on the results of the first pilot test then recruit new subjects to test the revised protocols and instruments. We will repeat this process until we are satisfied with the protocols and instruments (up to 300 pilot subjects).

Validity and reliability of tool

This study is an experiment, comparing the validity and relatability of hair self-collected to hair professionally-collected. The instruments, protocols, and hair collection kits will replicate tools used for successful professional hair-collection protocols in U.S. national surveys conducted by SRC.

Potential Biases

A goal of this study is to empirically assess selection bias in hair sample collection. Selection bias is a primary concern for large-scale population-based studies, threatening the validity of findings. Unfortunately, studies rarely have sufficient details on participants—and most importantly—non-participants, to do more than hypothesize about the impact of selection bias on their findings and potentially run sensitivity analyses. Given the newness of the HCC biomarker, it is not surprising that most studies involving hair collection have been in nonprobability samples, which is known to increase vulnerability to selection bias and may be accentuated in culturally sensitive collections like HCC. Self-collection protocols have been tested, but never with an experimental design that could be used to determine efficacy and bias. The CVFS, by contrast, has reliable and valid measures of key drivers of selection bias—such as individual respondent pre-conditions, parental life histories (including mental

health), household dynamics (including wealth, income, as-sets, migration), and neighborhood dynamics (including amenities, natural disasters, war, interventions). This study is uniquely positioned to empirically assess what most can only hypothesize; who does and does not participate in a study, and what are the differences between these people (individual, family, neighborhoods), and, in self-collection, who struggles to deliver an adequate sample for HCC.

Limitation of the study

This study will take place in the Western Chitwan Valley of Nepal, which places some limits on the generalizability of the findings to other locations.

Plan for supervision and monitoring

Monitoring and quality control activities are built into our management plans at several levels. Project monitoring is one of the PI's main responsibilities in fulfilling his scientific and management role. While final responsibility for quality control rests with the senior staff on the project, intermediate-level managers and supervisors are responsible for the critical tasks of implementing monitoring procedures, developing customized quality control activities to meet the monitoring needs of the project, and assessing the degree to which staff are meeting quality standards and regularly communicating the results to senior project staff. A checklist will be used for quality audit purposes which will cover the proper following of a) research study design and methodology; b) self and professional hair collection procedures; c) ethical considerations; d) discussion guidelines; and e) qualitative notes.

Plan for data management and analysis

Because biological sex is a key determinant of success with data collection protocols, predictors of the transition to adulthood, and HCC samples, we will conduct all analyses separately for males and females, and also test the statistical significance of interactions with sex in analyses combining males and females. First, we will conduct descriptive analyses comparing the two experimental conditions (self-collected vs. professional-collected). This comparison will document the differences with respect to hair collection success (refusal, resistance by type, non-contact, and collection outcome), hair sample (quality and quantity), HCC extracted (quality and quantity), and participant’s self-evaluation of the process (resistance and satisfaction level by type). Second, we will explore the potential influence of selection bias on the self-collected hair by comparing hypothesized drivers of selection bias (community-, household- and individual-level) on study participation, with a focus on experimental condition heterogeneity, and above primary study outcomes. Descriptive analyses comparing experimental conditions. To assess differences between self-collected and professional-collected conditions, we will start with descriptive analyses (either proportions and proportion z-tests or t-tests and chi-square tests, as appropriate) comparing across groups (self- and professional-collection) of primary outcomes (hair collection success, hair sample, HCC extracted, and summary measures of participant evaluation). To illustrate our approach, consider associations with hair collection success. We will fit a simple model: $Yij = \beta0 + \beta1 haircollectionarm + \sum\beta kXij + \mu0j$ (Eq. 1) Where Yij is a dichotomous measure of hair collection success for individual i in community j, β0 is the model intercept, β1 represents the estimate of the effect of intervention hair collection arm (self-vs-professional), and Xij represents a vector of k number of measures of other HCC-related factors that affect hair collection success (e.g. hair length, last time cut, frequency of cutting). As our design features individuals clustered within communities, we use a multilevel approach. We include μ0j, the estimate of the neighborhood-level random effect of the intercept (assumed to be normally distributed with a mean of 0 and variance of $\sigma\_\mu0^2$). Conditional on μ0j, the Yij are assumed to be independent. We will test each association with and without covariates to assess the robustness of our association estimates, but all models will contain the random effect of the intercept (μ0j). Our team has substantial experience using statistical tools for adjusting multivariate model estimates for multilevel clustering, including methodological contributions to multilevel model estimation. To explore the potential impact of differential response rates across study arms, we will also conduct sensitivity analyses with inverse probability weighted models. After the initial model with hair collection success, we will fit additional models for each primary outcome to compare the experimental conditions across multiple outcomes required to evaluate feasibility of self-hair collection in large population-based studies. Selection bias analyses. To explicitly explore selection bias and its potential impact on primary study outcomes, we will fit multivariable logistic regression models to investigate the differences in sociodemographic characteristics (e.g. age, sex, household size, SES, education, ethnicity) and exposure to adverse lifetime experiences (e.g. potentially traumatic events, adverse childhood experiences), between those who

completed the hair collection and those who did not. Using linked CVFS measures we will begin by assessing associations with sex, birth cohort, and ethnicity. Each of these is established before birth, and prior research indicates each of these may be a factor associated with population-based study participation. To illustrate our approach, building on the model in section, now consider associations with one of the primary potential drivers of selection bias, sex. We will use a simple model: $Yij = \beta 0 + \beta 1 haircollectionarm + \beta 2 sex + \sum \beta k Xij + \mu 0j$ (Eq. 2) Where Yij is a dichotomous measure of participation in the study for individual i in community j, β0 is the model intercept, β1 represents the estimate of the effect of intervention hair collection arm (self-vs-professional), β2 represents the estimate of the effect of sex, and Xij represents a vector of k number of measures of other HCC-related factors that affect hair collection success, including demographic factors. As in our primary experimental condition analyses, as our design features individuals clustered within communities we use a multilevel approach, including estimates of the neighborhood-level random effect of the intercept, and are conditional on μ0j, the Yij are assumed to be independent. We will test each association with and without these covariates to assess the robustness of our estimates of association, but all models will contain the random effect of the intercept (μ0j). Our study is designed to address the problems facing all population-based multilevel studies, including the potential for high correlations among contextual characteristics, selective migration into specific contexts, high levels of mobility that create mismatches between contextual and individual measures, and endogenous influences on the contextual characteristics themselves. The CVFS includes dozens of measures of factors known to shape the transition to adulthood and we will investigate all of them. This includes measures of community context (access to schools, jobs, health care, travel and social support groups), household resources (income, wealth, housing quality, household goods, food access), parental background (education, employment, religiosity, marriage/childbearing, travel/migration, attitudes/beliefs), and individuals' own experiences in the transition to adulthood (education, employment, courtship/marriage/childbearing, religiosity, travel/migration). We will estimate associations of each of these factors with selection bias, first one at a time, then together by grouping, and then all together. Particularly novel and important, for this specific cohort of young people making the transition to adulthood, CVFS includes pre-COVID-19 clinical interview measures of mental disorder histories from mothers, fathers, and the young people themselves. Using all of these measures, for the first time, we will empirically estimate the factors that may influence selection bias in study participation for large population-based studies involving self-hair collection for HCC testing. All the survey data and HCC data will be entered twice by different data entry staff (using a data entry program specifically designed to eliminate keying discrepancies). All phone survey will be done in CATI and will be automatically entered in the computer to allow for immediate follow-up on discrepancies. A set of quality control tables will be generated on a regular basis to identify potential problems in the data. Data processing and harmonization will begin as soon as data are entered in the computer. Harmonization refers to the process of creating comparability across data sets—for this study, harmonization across previous studies – mental health survey, and web survey data will be crucial. Once field data are collected, the coversheet will be detached from the instrument and stored in a locked cabinet. The instruments will be used for data entry and checking and will only contain a numeric code for identification. The data files will have no identifying information, except the numeric id. During field work, the data files and digitized files will be stored on ISER-N secure servers and will be backed up. The backup server keeps file versions, backed up nightly, back two years and deleted files are kept for one year. ISER-N has a long history of storing and securing data with high security (password protection/no identifying information). ISER-N will share the datasets with the investigators who will store the data on a secure server owned by the University of Michigan. All coded data and documentation files will be available to the research community includes new and existing data, individual interview and prospective panel data—and links across data components pose a significant risk of respondent identity disclosure. Therefore, these detailed, geographically linked measures will be archived by the University of Michigan's ICPSR (Interuniversity Consortium for Political and Social Research) and disseminated following strictly established procedures for restricted data of this type. ICPSR has pioneered safe methods for disseminating these types of data through their DSDR (Data Sharing for Demographic Research) archive. ICPSR is the largest data archive in the United States with an international consortium of about 700 academic institutions and research organizations. DSDR is designed to help accelerate the distribution of complex data collected by demographers to the research community. Specifically, ICPSR/DSDR maintains and distributes the panel study's restricted release data files. These data will be available to the researchers in SAS SPSS, STATA and R format for analysis.

Expected outcome of the research results

The study will compare results from the two experimental conditions (self-collected vs. professional-collected). This comparison will document the differences with respect to hair collection success (refusal, resistance by type, non-contact, and collection outcome), hair sample (quality and quantity), HCC extracted (quality and quantity), and participant's self-evaluation of the process (resistance and satisfaction level by type). It explores the potential influence of selection bias on the self-collected hair by comparing hypothesized drivers of selection bias (community-, household- and individual-level) on study participation, with a focus on experimental condition heterogeneity, and above primary study outcomes.

Plan for utilization of research findings

We will disseminate our findings locally in Nepal and globally through academic journal articles, dissemination events (webinar), and fliers. These findings may be used to develop new policies and programs on family, health and wellbeing. Depending on the results, we may use the findings to develop new research proposals to investigate young adult health and stress. The findings from this study will also allow researchers to launch innovative new analyses of psychological stress in the transition to adulthood. Finally, we will disseminate our findings from the descriptive and selection bias analyses at conferences and in peer-reviewed publications.

Ethical Consideration

Are human participants required in this research?

Yes

How many participants are required for the research?

1800

What is the frequency of the participant's involvement in the research?

The subjects will be contacted who will eligible for the study. First contact is only for recruitment. If participant will meet the eligibility criteria (age between 18-22, and living within Chitwan) will be selected for the study. Selected participant will mail the hair sample collection kit. After certain time (days), research staff will visit the participant and collect the hair sample and also will do the successful survey. The participant who don't provide their hair sample, will be asked fail survey question.

Responsibility of the research participants :

Research participants are expected to provide a hair sample collected by a professional interviewer, or self-collection following the instructions provided to them. Subjects are also expected to answer survey questions honestly, openly, and to the best of their ability.

Are vulnerable participants involved?

NA

Are there any risks involved for the participants?

Yes

Expected risk for human participants :

The overall level of risk to participants in this study is extremely low. There are no physical risks associated with participation in the interviews or hair sample collection, and no deception or purposeful creation of anxiety will be involved. Moreover, respondents' identities are maintained with strict

confidentiality—all data are encoded to protect individuals' identities. The code key linking study identifiers to personal identifiers will be kept in an access-restricted, password protected electronic file at ISER-N. Informed consent will be obtained from each research participant for data collection activity including the survey interview and the hair sample collection. Respondents will be told about the scientific use of the hair samples, the voluntary nature of participation in the hair sample collection, and that they may decline to provide a hair sample with no penalty. The informed consent will explicitly state that the results of genetic analyses will not be revealed to subjects or their physicians or be placed in their medical records. Moreover, respondents' identities are maintained with strict confidentiality all data are encoded to protect individuals' identities. No more than minimal risk is expected. There is the risk of respondent identity disclosure, which we believe the likelihood to be rare with the safeguards and data security measures that we have in place and describe above.

Expected benefits for human participants :

As with all CVFS data collection, respondents' benefits are primarily advances in the scientific study of health and potential for more effective public policies to promote health and wellbeing. Results from this specific study will provide the means to establish the limitations of large-scale hair-based cortisol collection and the biases that may be introduced by using self-collection. This information is crucial as self-collection may be more feasible in many settings, and could enable a significant scientific advance in the integration of psychological stress into studies of the transitions to adulthood. With the results from this study we will design an optimal protocol for integrating hair-based cortisol collection into population studies across settings and release that protocol to the public. This project will also create a transformative new resource for the scientific community to study biomarker measures of stress in a large cohort experiencing the transition to adulthood.

How informed consent is obtained from the research participants?

written

Please specify

The trained interviewer will obtain comprehensive written consent from the professional-collection subjects for their participation in the study. At the data collection visit, the interviewer will read the consent form in the Nepali language to the subject and will answer all questions the subject has during (or after) the consent process. The interviewer will proceed with the hair collection and evaluation interview after obtaining comprehensive written consent from the subject documented by a signature on the consent form (or thumb stamp if subject is illiterate). A copy of the consent form will be given to the subject for their records. The trained interviewer will follow a similar consent process for the self-collection subjects. During the recruitment phone call, the interviewer will read the consent form in the Nepali language to the subject and will answer all questions the subject has during (or after) the consent process. The interviewer will inform the subject about the process to deliver a hair collection kit after obtaining comprehensive written consent without signature from the subject. A copy of the consent form will be delivered to the subject for their records.

Who is responsible for obtaining informed consent?

Well-trained interviewers are responsible for obtaining comprehensive written informed consent from all study participants.

Is there anything being withheld from the research participants at the time the informed consent is being sought?

No

Is the research sensitive to the Nepali culture and the social values?

N

Is health insurance (if applicable) being made available to the research participants?

No.

Is Investigation going to be conducted in Nepal ?

Y

Name of Investigation lab

Intrepid Nepal Pvt Ltd

Type of Sample

Hair Sample

Detail Description of Investigation

INPL will conduct ELISA based assessment of cortisol on hair strands shared from the field collected by ISER-N. This will be a quantitative exercise and data will be shared as a definite value compared to controls.

Main person involved in Investigation

The main person involved in the Investigation: Saman Man Pradhan

Is there going to be a transfer of any biological materials from the country?

No

Does the study involve transfer of DNA sample?

No

Document

S.N	Name	Document
1.	Conceptual framework	Yes
2.	References	Yes
3.	Data collection tools	Yes
4.	Failed Survey Questions	Yes
5.	Flow diagram	Yes
6.	Work Plan	Yes
7.	Instruction for long hair sample collection.	Yes
8.	Instruction for short hair sample collection.	Yes
9.	Consent_Self_Hair Collection_English_Revised	Yes
10.	Consent_Professional_Hair Collection_Nepali_Revised	Yes
11.	Consent_Professional_Hair Collection_English_Revised	Yes
12.	Informed consent form	Yes
13.	NHRC Application Reference list	Yes

Study Timeline

Start date	End Date
2022-07-01	2023-06-30
2022-07-01	2023-06-30

Status History

S.N	Date	Status	Description	Document
1.	November 30, -0001	Draft	Proposal is saved as a draft	N/A
2.	August 7, 2022	Under_administrative_consideration	Proposal from researcher is accepted by NHRC.	N/A