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Nash-wo-Numa (childhood growth & development) study protocol: factors that impact linear growth in children 9 to 15 years of age in Matiari, Pakistan

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ABSTRACT

Introduction Adolescence is a time of significant physical and emotional change, and there is emerging concern that adolescents living in low- and middle-income countries (LMIC) may face substantial challenges in relation to linear growth and mental health. Data on the global burden of stunting after 5 years of age are limited, but estimates suggest up to 50 per cent of all adolescents in some LMIC are stunted. Additionally, many LMIC lack robust mental health care delivery systems. Pakistan has one of the world's largest populations of adolescents (10 to 19 years) at approximately 40 million. The Nash-wo-Numa study's primary objective is to assess the prevalence and risk factors for stunting among early adolescents in rural Pakistan. The study also aims to determine the prevalence of poor mental health and identify factors associated with common mental health concerns during the childhood to adulthood transition.

Methods This cross-sectional study will include girls (n= 738) 9.0 to 14.9 years of age and boys (n=687) 10.0 to 15.9 years of age who live in the rural district of Matiari, Pakistan. Participants will be assessed for anthropometrical measures, puberty development, nutritional biomarkers as well as symptoms of depression, anxiety and trauma using validated scales.

Ethics and dissemination The proposed study aims to complete the picture of child and adolescent health concerning linear growth and mental health by including puberty indicators. Ethics approval has been granted by the Ethics Review Committee at the Aga Khan University, Karachi, Pakistan, #5251-WCH-ERC-18 and Research Ethics Board at SickKids Hospital, Toronto, Canada, #:1000060684. Study results will be presented at relevant conferences and published in peer-reviewed journals.

Trial registration number NCT03647553; Pre-results.

Strengths and limitations of this study

- This study is the first assessment of puberty, stunting and mental health in young adolescents living in rural Pakistan.
- It includes boys who have largely been ignored from puberty literature in low and middle income countries.
- The study is not school-based which allows for inclusion of those not enrolled in school.
- Since most births occur in the home setting and are not registered, accuracy of age determination may impact outcomes.

INTRODUCTION

The United Nations (UN) *Sustainable Development Goals* and the UN Secretary General's *Global Strategy for Women's, Children's and Adolescent Health* place adolescent health in a position of prominence.[1 2] The recent focus on the second decade of life is due to the large international population of adolescents and the realisation of the importance of health during a second *window of opportunity* to improve nutrition and other health outcomes.[3] To sustain the investments and achievements made through child well-being initiatives, a focus on the second decade is now necessary. About a quarter of the world's population is between the ages of 10 to 24 years and 90 per cent of these 1.8 billion young people currently live in low- and middle-income countries (LMIC).[4] Pakistan, the sixth largest country in the world, has an estimated 40 million adolescents representing just over one-fifth of the total global adolescent population.[5]

Knowledge about adolescent health is evolving quickly but many research gaps persist.[6 7] Current abilities to respond to this *call for action* are limited by a lack of evidence and monitoring outside the essential focus of HIV, sexual reproductive health, infectious diseases, injury and violence. Indicators related to the health of early adolescents aged 10 to 14 years lack evidence and many monitoring and intervention mechanisms relating to only female reproductive health beginning

at age 15.[8] As many early adolescents attend school, there exists an opportunity to direct monitoring and interventions through existing educations systems. Early adolescence usually coincides with puberty presenting an occasion to impact future health outcomes; yet, little data on nutrition and growth or mental health of early adolescents exists in LMIC.

Poor nutritional status during early adolescence is an important determinant of future health outcomes. Chronic undernutrition is characterised by long-term exposure to an inadequate diet, vulnerability to infection and an inability to 'catch-up' in linear growth. This results in impaired linear growth, which is frequently quantified in height-for-age z-scores (HAZ). Short stature or stunting in childhood resulting from chronic undernutrition, is associated with reduced lean body mass and deficiencies in muscular strength and working capacity.[9] With about 15% to 20% of total stature and 45% of adult bone mass achieved during adolescence, it may represent the final opportunity to impact adult height.[10–14]

Stunting is multi-causal, with identified risk factors in children under 5 years of age including community, family and child factors.[15–20] As an indicator for undernutrition, stunting is defined as having HAZ-score less than −2 SD below the median, when compared with a reference population like the WHO - Growth Standards.[21] Data on the global burden of stunting after 5 years of age are limited, but estimates from some countries suggest that up to 50 per cent of all adolescents are stunted.[22] Stunting after 5 years of age is not monitored in Pakistan, however, data from government-led studies provide a glimpse into an equally unacceptably high prevalence of stunting in children over 5 years in Pakistan. The National Health Survey of Pakistan (1990) reported a stunting prevalence of 19% in boys and 25% in girls at 13 to 14 years of age[23] and about 20 years later the stunting prevalence of children aged 10 years from the National Nutrition Survey (2011) was 26.6% for Pakistan, 34.0% for the Province of Sindh and 49.3% for the District of Matiari.[24]

A conceptual framework to better understand determinants of early adolescent health relevant to this study was developed based on a *Conceptual Framework for Early Adolescent Health* and the *WHO framework on Childhood Stunting* (figure 1).[25 26] Indicators were categorised as follows: (a) *distal factors* that do not impact health and undernutrition directly and (b) *proximal factors* are those more likely to have a parallel or inter-related relationship with health and undernutrition. This study explicitly addresses most of the distal and proximal factors in the conceptual framework.

Given the estimated large number of early adolescents with persistent stunting in Pakistan, the potential impact

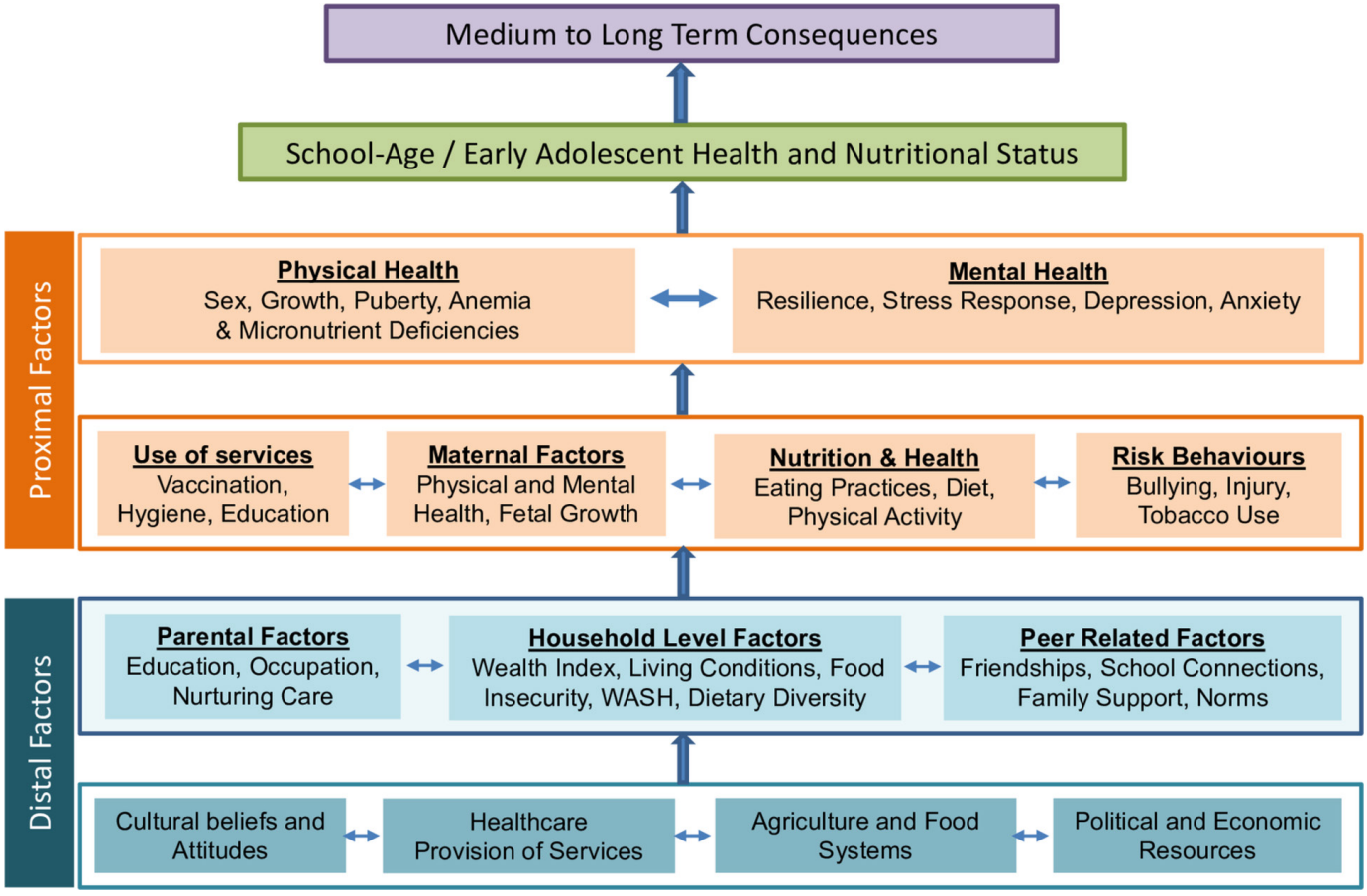

Figure 1 Framework for school-aged/early adolescent health and nutritional status anthropometry. WASH, water, sanitation and hygiene.

on the nation's future health and economical development may be devastating. Since it may take generations for secular trends to have an impact on stunting, it is clear that in countries like Pakistan, interventions to reduce stunting need to target more than one time-point in the life cycle. Key health outcomes during early adolescence relevant to this study are reviewed below and include linear growth, puberty, depression, anxiety, anaemia and micronutrient status.

Linear growth

Accelerated linear growth or growth spurts occur twice before one reaches adult height; the first from conception until 2 years of age and the second during puberty. During growth spurts, 'windows of opportunity' or plasticity in linear growth may enable an improved growth trajectory thereby allowing a previously stunted individual to achieve their genetic height potential.[10] The timing, sensitivity and mechanism of a second 'window of opportunity' to impact linear growth during adolescence remain uncertain. In typically developing children, the timing of puberty onset and childhood height impacts adult height.

The literature supporting the potential for increased linear growth during adolescence is derived from data indicating that chronic undernutrition delays the signal of senescence at the bone plate by decreasing oestrogen production, allowing for a longer period of linear growth that can result height gains.[27 28] Another hypothesis is the reduction of disease burden during pubertal growth as the mechanism for improved linear growth.[29] Nevertheless, one's ability to augment linear growth during adolescence involves complex mechanisms including pubertal timing, undernutrition severity and duration of the underlying causes of impaired linear growth which must disappear or, at the very least, diminish with age.[30]

Puberty

Sexual maturation of the body during adolescence is called puberty. While puberty usually takes place between the ages defined during early adolescence, it can begin as early as 8 years of age and can extend beyond 19 years of age. Hormonal changes during puberty spur dramatical changes in the composition of the body. During normal puberty, height and body weight increase, bone mass and muscle mass increase, blood volume expands and the heart, brain, lungs, liver and kidney all increase in size.[31] Although growth and sexual maturation are genetically determined, they are susceptible to nutritional, environmental and hormonal factors and, subsequently, possible modifications. The age at puberty onset and growth rate during the pubertal growth spurt are two important parameters in determining adult height. Both are sensitive to undernutrition and contribute to sexually dimorphic adult height, but the adolescent growth spurt can vary in intensity and duration from one child to another. Because of common endocrine pathways, it is likely that linear growth and puberty are regulated in parallel in response to chronic food insufficiency rather than one impacting the other.[28 32]

Depression and anxiety

There seems to be an important relationship between depression and linear growth and undernutrition. Several studies have examined the role of overall diet as well as specific food components like *n-3* fatty acids, vitamin B12, as well as zinc, selenium and iron on depression.[33–36] Single nutrient pathways are unlikely to play a significant role since many nutrients are involved in the production of individual neurotransmitters and numerous neurochemical pathways are likely to be responsible for complex feelings and mood.[37]

Although depression and related co-morbidities are common among adolescents in LMIC, there are currently few services specifically for adolescents suffering from depression.[38–40] Moreover, mental health services in LMIC face many challenges such as cultural issues, financial and human resource limitations and lack of robust healthcare delivery systems.[41]

Anxiety disorders are among the most common psychiatric conditions with prevalence rates of between 10% to 20% reported in children and adolescents of all ages.[42 43] In a recently completed large face-to-face survey, one in three adolescents met criteria for any anxiety disorder while 8.3% met the criteria for a severe anxiety disorder.[44] Co-morbidity with depression is also high with anxious children having an 8 to 29 times the risk of additional depression.[45] Anxiety disorders are debilitating, affecting all aspects of a child's life including social adjustment, academical achievement and home functioning.[46] Anxiety disorders rarely remit without treatment and even with remittance, high recurrence rates are evident.[47–49] Low remittance rates are associated with early age of onset, older age at intake and more severe baseline symptoms.[46 49] Evidence suggests that childhood anxiety disorders progress on as anxiety disorders in adulthood, with some evidence that anxiety disorders may progress onwards on to both depression as well as bipolar disorders and substance abuse.

Anaemia and micronutrient status

Iron and vitamin A deficiencies rank among the leading causes of global morbidity and mortality.[50–53] Little evidence exists in the literature on the prevalence of micronutrient deficiencies among school-aged children despite their increased risk. Nutrient deficiency data among adolescents in Pakistan are limited. The Pakistan National Nutrition Survey (2011) reported a prevalence of anaemia of 53.6% among girls 15 to 19 years of age,[24] but did not collect data on boys. A study in northern Pakistan reported a 58.8% rate of anaemia among boys and 70% among girls aged 6 to 11 years.[54] Despite high levels of sunshine and food fortification, Pakistan has a 53% prevalence of vitamin D deficiency and among the population under 20 years, vitamin D deficiency prevalence is 67.5% for males and 72.1% for females.[55 56] There

are no national Pakistani data reporting anaemia or other micronutrient deficiencies prevalence among school-aged children.

Dietary diversity and food insecurity

Diets in resource-poor settings commonly lack variety and quantity, thereby limiting intake of micronutrients and macronutrients paramount to one's health and well-being. The ability of the household to access food is one key aspect of food security. According to the World Food Programme Food Security Bulletin (2017), Pakistan is ranked as having a *moderately high* Global Hunger threshold.[57] Food Insecurity Experience Scale (FIES) and Household Dietary Diversity Score (HDDS) are two other important tools used to identify the food environment of households.[58–60] FIES was developed in 2013 by the Food and Agriculture Organization (FAO) of the United Nations for global and country monitoring of the severity of food insecurity in the previous 12 months. Since 2014, the Gallup World Poll has collected data using the FIES. The FIES consists of eight dichotomous questions and results range on a scale from mild-to-severe food insecurity. Results are classified based on the total number of affirmative responses ranging from 0 to 8. While the FIES does not measure food insecurity directly in children or adolescents, estimates of the percentage of children and/or adolescents living in food-insecure households are generally used. There exists a lack of data regarding food insecurity among households with adolescents living in Pakistan; however, in South Asia the prevalence of food insecurity (FIES) for households with children under 15 years of age is 13% for those living in *severe food insecurity*, 30% in *moderate-or-severe food insecurity* and 38% of households with *not enough money to buy food*.[61] The HDDS reflects household access to food variation and is a count of the food groups consumed over a given reference period. The value of this variable will range from 0 to 12 and represents the total number of food groups consumed.

Common hormonal, nutrient and energy pathways support the epidemiological evidence showing an association between undernutrition, impaired linear growth and delayed puberty. However, limitations related to the current understanding of the biological mechanisms of growth during puberty persist. Poor nutritional status has been shown to delay puberty onset and impair linear growth but its association with depression during early adolescence remains unclear. Growth during early adolescence is not assessed or reported in any systematic manner and therefore, both worldwide historical rates and current prevalence of stunting among early adolescents remain, frustratingly, unknown. Additionally, the underlying nutrient pathways of these effects remain elusive. Moreover, limited research exists on factors that impact the timing of puberty in boys. The role of nutritional status and puberty on mental health outcomes like depression among adolescents in Pakistan has not been fully explored. In fact, very little research has considered these associations in LMIC where undernutrition and micronutrient deficiencies are highly prevalent. In order to implement appropriate programmes to ameliorate the health of adolescents in the rural district of Matiari, these relationships must be better understood.

AIMS OF THE NASH-WO-NUMA STUDY

The primary objective of the Nash-wo-Numa study is to determine the prevalence and severity of stunting among girls aged 9.0 to 14.9 years and boys aged 10.0 to 15.9 years living in Matiari.

Secondary objectives are:

- To determine the prevalence and severity of low body mass index, anaemia, micronutrient deficiencies, depression, trauma and anxiety.
- To determine the burden of aggregate health conditions within participants.
- To identify factors associated with depression, trauma, anxiety, stunting, low body mass index, anaemia and micronutrient deficiencies.
- To assess the impact of dietary diversity and food insecurity on participant nutritional status and mental health.
- To describe variations in pubertal development in relation to nutritional status and mental health.

METHODS AND ANALYSIS

The proposed study will be cross-sectional in design (beginning on 2 November 2018 and expected to be completed by December 2019), conducted in the District of Matiari, Pakistan. Participants will be 9.0 to 15.9 years of age. Anthropometrical measures, puberty stage, nutrition biomarkers and mental health will be assessed. A summary of the study activities is outlined in table 1. This study has been funded by the Cundill Centre for Child and Youth Depression, Centre for Addiction and Mental Health, Canada.

Sample size

The prevalence of stunting is the primary outcome used for the analysis of precision sample size calculation. A prevalence of stunting of 36% for girls and 31.8% for boys aged 10 to 10.9 in the Province of Sindh was previously reported.[24] A two-sided 95% CI for the one proportion CI formula (simple asymptotic) was employed to obtain a sample size. A sample size of 738 (246 × 3 age groups) for girls is large enough to detect a stunting prevalence of 36%±6% and a sample size of 687 (229 × 3 age groups) for boys is large enough to detect a stunting prevalence of 31.8%±6%. The sample size for the randomisation lists for enrolment will be inflated by 30% to account for refusal for study participation as a high number of study refusals are anticipated due to the sensitive nature of the study. The sample size was calculated using Power Analysis and Sample Size Software.[62]

Study setting

The study will be conducted in Matiari District in the Province of Sindh, Pakistan. The population of Matiari (2017)

Table 1 Study activity schedule

Study activity	Staff member	Approximate time to complete	Source
Enrolment:			
Eligibility screen	Study field staff	15 min	Parent
Informed consent	Study field staff	10 min	Mother
Informed assent	Study field staff	10 min	Child
Assessments:			
Anthropometrical measures	Study clinic staff	15 min	Mother Child
Questionnaire	Study psychologist	45 min	Mother Child
Anaemia testing by HemoCue	Phlebotomist	10 min	Child
Blood draw for biomarker assessment	Phlebotomist	10–15 min	Child
Puberty assessment	Physician	10 min	Child

was estimated to be 769 349 with approximately 85% being rural.[63] There are approximately 48 000 school-aged children between 9.0 and 15.9 years of age living in the district, which is representative of rural conditions in Pakistan.[64] The existing district health system infrastructure for community healthcare encompasses Basic Health Units and Rural Health Centres offering primary healthcare services and Taluka Head Quarter and District Head Quarter Hospitals catering to secondary healthcare services. The Aga Khan University in Karachi has conducted a number of research studies on maternal and child health in the Matiari District for over 15 years. Necessary rapport has been established between the community representatives, health system personnel and the Centre of Excellence in Women and Child Health, Aga Khan University.

Eligibility criteria

Due to later puberty in boys, female permanent residents of Matiari who are between 9.0 to 14.9 years of age whereas male permanent residents of Matiari between 10.0 to 15.9 years of age will be eligible to participate in the Nash-wo-Numa study. One participant (girl or boy) per household will be sampled. The participant's birth mother must also be available to participate in the study.

Female participants who are pregnant or have been pregnant will be excluded from the study. Participants must not be participating in any other nutrition trials, but other household members may participate in other studies. Participants with known chronic or genetic diseases that impact growth will be excluded. An illustrative list of conditions include: congenital heart disease, metabolic disorders (eg, diabetes), cancer, genetic disorders (Down's syndrome, Turner's syndrome), blood disorders (symptomatic thalassaemia, sickle cell anaemia) and chronic disorders (kidney ie, nephrotic syndrome, gastrointestinal ie, Crohn's, bone dysplasia, immunodeficiency disorders).

Sampling strategy

All 53 000 households covered by Female Health Workers in the catchment population of 26 health facilities in Matiari District were surveyed as part of a companion study between December 2016 and May 2017.[64] Information regarding the number of occupants, sex and age were collected for each household. This data will be used for selecting participants using computer-assisted random sampling. Randomisation and catchment weighting will be done with statistical assistance from the Aga Khan University. This will ensure the sample is representative of all parts of the district.

Procedures

Study staff will visit eligible households during off-school hours to maximise the likelihood of finding participants and their mothers at home. At this time, eligible participants meeting the inclusion criteria will be invited to participate in the study. Study staff will follow protocols established by the Centre of Excellence in Women and Child Health, Aga Khan University, in Pakistan to obtain informed written consent from a legal guardian and assent from participants who agree to participate in the study. The participants' birth mothers (hereafter referred to as mothers) must also agree to participate in the study. All participants will be informed about the right to withdraw from the study without prejudice.

Study staff will then make arrangements for transportation to field-based clinics in Matiari for the participant, their mother and a chaperone if required. Study vehicles will be available to transport the participant, mother and chaperone to the clinic. The study team will provide family refreshments during the field-based visit.

Anthropometry

Anthropometrical assessments of mothers and children will be conducted by study staff at the field-based clinic. Study staff will be trained in stadiometry using standard methods.[65 66] Weight and height will be recorded to the nearest 0.1 kilograms and centimetre, respectively, for each participant in light clothing and without shoes using a Seca digital floor scale (model 813) and Seca stadiometer (model 213); Mid-Upper Arm Circumference (MUAC) will be determined using standardised procedures and a MUAC measuring tape (Seca 201).[67] All measurements will be conducted in duplicate and independently by two study personnel. If the two measurements differ by more than 1 cm for height, more than 0.5 kg for weight, more than 0.5 cm for MUAC, a third measure will be taken and recorded using standardised procedures.[65 66] The average (mean) of acceptable paired measures will be used in the

analysis. Maternal anthropometry is important in determining maternal health, as well as relationships between linear growth and maternal height.[68] The technical error of measurement (TEM) of height, weight and MUAC will be assessed at specified intervals and study personnel with low TEM will undergo anthropometrical methodology training.[69]

Questionnaires – verbal data collection

The maternal questionnaire has six modules. Module one seeks to collect data related to household composition and household characteristics and socio-economical status. The second module relates to parity, birth history and childhood characteristics that impact subsequent growth. The third module pertains to parental characteristics including age, education and occupation. Questions for the first three modules were adopted from Pakistan Demographic and Health Surveys.[70] The fourth module collects information on household food consumption and nutrition through the administration of the household version of the FIES and the HDDS.[58 60] Module five probes the maternal impression of the adolescent participant's mental health and well-being. It includes two scales and aims to determine the mental health of the participant according to the mother. The first is the parent-report of the Screen for Child Anxiety Related Emotional Disorders (SCARED), which has been validated in similar settings.[71 72] This instrument contains 41 items and measures anxiety across five subscales: panic/somatic, separation anxiety, generalised anxiety, social anxiety and school phobia. The second is the parental version of the Strengths and Difficulty Questionnaire (SDQ).[73] This is a more general assessment of mental health composed of 25 questions, which have been validated for use in 3–16 year-old participants. This scale screens for emotional and behavioural problems in children and adolescents. The questionnaire incorporates five scales: pro-social, hyperactivity, emotional problems, conduct (behavioural) problems and peer problems as well as questions that assess the extent to which identified problems impair functioning. It has been translated into many languages and is widely used and validated in Pakistan.[74] Module six relates to the mother's own mental health and involves administering three scales to determine the household psychological environment. The first scale, the Warwick-Edinburgh Mental Well-being Scale (WEMWBS) is a 14 item scale of more general mental well-being covering subjective well-being and psychological functioning, in which all items are worded positively and address aspects of positive mental health.[75] Higher scores are associated with higher levels of mental well-being. The WEMWBS has been validated in many countries with those aged 16 and above.[76 77] The second instrument is the WHO's Self-Reporting Questionnaire (SRQ).[78] The SRQ is a screening instrument, which consists of 20 questions designed for screening for the existence of mental disorders in developing countries (141, 152). The SRQ has been used in similar settings in Pakistan and is also a validated tool.[74 79–81] The third instrument is the Conflict Tactics Scale–2 (CTS2); it is validated, and the most widely used instrument for measuring intimate partner violence in Pakistan.[82 83] The CTS2 contains 20 questions to measure the extent of specific tactics aimed at measuring attitudes on partner conflict comprising five domains including negotiation, psychological aggression, physical assault, sexual coercion and injury. Higher scores indicate more use of the tactic.

Much of the participant questionnaire has been drawn from two WHO tools; The Health Behaviour in School-aged Children (HBSC) survey and the Pakistan Global School-Based Student Health Survey (GSHS).[84 85] The HBSC is a cross-national survey conducted in collaboration with the WHO and has been used for over 30 years to gain insight into young people's well-being, health behaviours and social context. The GSHS was developed by the WHO, in collaboration with Unicef, Unesco and UNAIDS and with the technical assistance from the Centre for Disease Control. Ninety-four countries including Pakistan (2008) have administered the GSHS to young people aged 11 to 16 years in LMIC school settings to assess behavioural risk factors and protective factors related to 10 key health areas. The participant questionnaire contains nine modules. Modules one and two aim to collect information related to health and nutrition. Modules three and four relate to school and physical activity, while the use of electronic media and social support are the focus of modules five and six. Modules seven and eight pertain to trauma, injury and risk behaviour. These final modules employ standardised mental health tools to gauge the mental health of the participant. The first mental health tool is a modified Childhood Trauma Questionnaire (CTQ), which has been adapted from its list of 28 questions to contain 23 questions appropriate for this setting. Questions include a maltreatment inventory to understand how participants have experienced and handle upsetting or traumatic experiences.[86 87] Lastly, module eight employs two standardised tools: the SCARED and the Mood and Feeling Questionnaire (MFQ); both of which have been validated in similar settings.[72 88–91] The SCARED instrument contains 41 items and measures anxiety across five subscales: panic/somatic, separation anxiety, generalised anxiety, social anxiety and school phobia.[92] The MFQ contains 33 items all of which consist of a series of descriptive phrases regarding how the subject has been feeling or acting recently. It has been extensively used as a depression screening instrument. Because of the sensitivity of some of the questions, study psychologists will conduct the questionnaires and participants can refuse to answer any questions without prejudice. A comprehensive study safety plan has been developed in collaboration with study child psychiatrists and the Sir Cowasji Jehangir Institute of Psychiatry in Hyderabad to address potential self-harm or other negative outcomes identified during the questionnaires. Both parent and child will be completing the SCARED, however, the correlation between parent

and child report on screening measures for internalising disorders (for example, depression and anxiety disorders) is generally low.[93] Therefore, one informant will be chosen as the primary informant and the other will be examined secondarily. In this population, the child/youth report will be the primary informant.

Anaemia and nutrition biomarkers

Following the completion of the participant questionnaires, those who agree to haemoglobin testing will be tested for anaemia. Study phlebotomists will conduct the haemoglobin (Hb) testing by drawing blood through a finger prick. A drop of blood will be used to examine the concentration of haemoglobin using portable HemoCue Hb 301 analysers that provide results in less than a minute. Haemoglobin concentration will be recorded and levels of anaemia will be classified as mild, moderate or severe. Individuals whose haemoglobin levels fall below designated cut-off points will be referred for assessment and treatment to a public health facility.[94]

If the participant agrees to a blood draw, and with parental consent, the phlebotomist will obtain a blood specimen according to standard procedures.[95] Blood specimens will be taken by venipuncture in zinc free tubes and the time of day recorded. A total of 5 mL whole blood will be collected which is below the maximum allowable total blood draw for research.[96] Specimens will be evaluated for serum ferritin, C-reactive protein and alpha-1-acid glycoprotein, transferrin receptor, vitamin A (retinol), calcium, vitamin D (25(OH)D), zinc and folate. All blood specimens will be coded to link with an individual subject's records using a specimen identification system and transported, analysed and stored at Aga Khan University.

Measuring puberty

The wide-reaching impact of puberty on physical and psychosocial outcomes highlights the need for puberty measurements. Because the age of puberty onset, sequence and tempo may vary and, in some cases, may indicate a pathological process, it is important to have a consistent, standardised method of assessing a child's progression through puberty.[97] Clinically, Tanner Stages are the gold-standard in assessing pubertal milestones. Evidence of gonadarche (breast development and testicular size) and adrenarche (pubic hair) are evaluated on a standard five-point scale.

Improper or omitted puberty staging can lead to erroneous conclusions when assessing research outcomes related to growth and development during puberty. Puberty stage assessment for this study will be composed of two components: (a) self-assessed puberty staging based on *Puberty Development Scale* and facilitated by study physicians and (b) physical Tanner Staging assessed by study physicians.[98 99] Puberty stage will be then classified into one of three phases of puberty: *Pre-puberty*, *In Puberty* and *Completing Puberty* using the Puberty Phase classification developed by the Royal College of Paediatrics and Child Health (UK).[98] In addition to field training for puberty assessments conducted by a pediatric endocrinologist, study physicians will complete an online Tanner Stage training module including an evaluation component which has been proven to improve accuracy, confidence and comfort in pubertal examinations.[100 101]

Considering cultural sensitivities, the physical puberty assessment will consist of a visual assessment while each participant is lightly clothed in the presence of a chaperone/parent and by a physician of the same sex. In cases, where the physician is unable to determine puberty staging while the participant is lightly clothed, they will ask to move clothing to complete the assessment. The physical puberty staging is an optional component of the study.

Data analysis

Summary statistics for continuous data will be reported as a mean and SD for normally distributed variables and median and IQR for non-normally distributed variables. Categorical data will be reported and sample size and the proportion identified. Participant Body Mass Index (BMI) will be calculated and converted to BMI-for-age z-scores along with height-for-age z-scores according to chronological age using the WHO Growth Reference for Children and Adolescents[102] and WHO-package for R. A list of study variables and standard cut-off values are presented in online supplementary appendix tables 1-8.

The participant's overall mental health will be reported using scores from tools which were collected from the participant and maternal questionnaires calculated individually. General mental health will be determined by having a score of 17 or greater on the SDQ and depression by a score of 27 or greater on the MFQ.[103 104] All tools and scales will be scored using the standard cut-offs outlined in online supplementary appendix table 9. To determine the burden of aggregate health conditions within participants, the prevalence of multiple micronutrient deficiencies and multiple mental health outcomes will also be reported.

For secondary analyses, a hierarchical/nested approach for multivariate data analysis will be based on the Conceptual Framework (figure 1) as outlined by Victora *et al*.[105 106] Significance will be assessed at p values less than 0.05. All analysis will be executed using R V.3.5.1 statistical software.

Patient and public involvement

Patients and public were not involved in the study design and were not consulted to develop patient relevant outcomes. Participants and parents/guardians will be given anaemia tests results at the study visit. Participants were not invited to contribute to the writing or editing of this document for readability or accuracy.

DISCUSSION

Little is known about the prevalence or the effectiveness of interventions to help overcome stunting during adolescence in rural Pakistan. Linear growth during

puberty is difficult to characterise due to its dependence on maturation over chronological age. Because of the sensitive nature of collecting maturation data, much of the literature on stunting during adolescence examines only the effect of age on linear growth. Where research exists on puberty, it is usually limited to girls and with age at menarche being the marker of puberty reported most often. There is scant research on growth and puberty on children living in LMIC.

Strengths and limitations

A major strength of this study is the inclusion of boys in addition to girls as participants. The study is also not limited to a school setting thereby allowing a broader age range, which can include ages that are pre-pubescent throughout puberty and possibly puberty completion – at least in girls. An important strength of the study is the inclusion of two methods to determine puberty phase: self and physical assessments. Other strengths include the collection of biological samples for micronutrient assessment along with dietary diversity and household food insecurity data, which will provide ample data to explore correlations. Lastly, this study employs robust assessments of participant mental health regarding depression, anxiety and trauma using various validated scales/tools.

There are a few limitations to this study. First, accurate determination of age in rural Pakistan is difficult since most births happen outside a healthcare facility and are not registered. Rather, methods to obtain age employ recall of common events near the time of birth providing only birth date estimates. A second relates to incomplete puberty. Incomplete puberty is anticipated to be a factor in boys since many boys will continue to grow in height after the age of 16 years. The third limitation involves administering participant questionnaires while a parent or chaperone is present which can potentially limit the truthfulness of responses.

At an individual level, findings from this study will help identify and provide support for those at risk for anaemia and micronutrient deficiencies, as well as, those with depression or other mental health issues. Medical and mental health professionals will also be more informed about the prevalence of these conditions in this rural population. Most importantly, this study will draw attention to the burden of aggregate health conditions within participants. It will also serve as a foundation for future intervention studies, which aspire to improve the prevalence of stunting, anaemia and other micronutrient deficiencies, as well as, depression and other mental health issues during adolescence. Results from this study may assist policymakers as they set priorities for adolescent health.

Ethics and dissemination

Ethics approval: Informed written consent to participate will be obtained from the participant's parent or legal guardian. Written assent will also be obtained from participants. The study results will be disseminated via peer-reviewed publication and conference presentations.

Study status

The first participant was enrolled on 2 November 2018; enrolment is expected to be ongoing and planned data collection will continue until December 2019. This manuscript has been prepared following the Strengthening The Reporting of OBservational Studies in Epidemiology checklist.

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