Protocol

The Long-term impact of nutrition and education interventions on neurodevelopmental and nutritional outcomes: a cohort follow up

Version 1.2

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Principal Investigator:

Dr. Zulfiqar Bhutta Ph.D, MBBS, PRCPCH, FAAP [1]

Co-Principal Investigators:

Dr. Sajid B. Soofi MBBS, FCPS[1]

Dr. Jai K. Das MBBS, PhD[1]

Dr. Shabina Ariff MBBS, FCPS[1]

Co-Investigators:

Mr. Yaqub Wasan MPH, MSc [1]

Ms. Maira Niaz Ahmed, Study Psychologist[1]

Ms. Arjumand Rizvi, Data Analyst[1]

Institutions:

[1]Aga Khan University, Karachi, Pakistan

Funding:

Mind and Brain Institute, Aga Khan University Pakistan.

Contents

Study Summary

Title	The long-term impact of nutrition and education interventions on neurodevelopmental and nutritional outcomes: a cohort follow up
Short title	Cohort Follow-Up Study
Study design	Cross-sectional
Anticipated study duration	02 years
Study sites	District Matiari of Sindh, Pakistan.
Parent study short titles	Zinc Gates study
Number of subjects in parent studies	Zinc Gates study - 1596
Primary objectives	▪ To examine the long-term impact of early nutrition interventions on neurodevelopmental outcomes in a cohort of early adolescent girls and boys. ▪ To examine the long-term impact of early nutrition interventions on nutritional status in a cohort of early adolescent girls and boys.
Secondary objectives	▪ To assess the association of household and individual demographics with adolescents' nutritional and developmental outcomes. ▪ To assess the association of young adolescents' lifestyle/patterns with developmental and nutritional outcomes. ▪ To assess the association of household food insecurity, dietary diversity, and food recall data with developmental and nutritional outcomes in adolescents. ▪ To document the knowledge, attitude, and practices around climate change.
Primary outcome measures	▪ Neurodevelopmental: will be assessed using scale Weschler Intelligence Scale for Children (WISC-IV). ▪ Nutritional status: To assess growth (height, weight, and mid-upper arm circumference to the WHO standards for height-for-age (HAZ), weight-for-height (WHZ), weight-for-age (WAZ) for adolescents.
Secondary outcome measures	▪ Nutrition behavior: o Eating Habits using questions from Health Behavior in School-Aged Children (HBSC) study. o Minimum Dietary Diversity (MDD) scores using the Dietary Diversity questionnaire.

	▪ Household food insecurity will be assessed using Household Food Insecurity Experience Scale (HFIES). ▪ Individual and household-level demographic data will be used to disaggregate data based on several indicators – using the PDHS tool. ▪ Frequencies and proportions of knowledge, attitude, and practices of families about climate change – using UNDP modules. ▪ Meal patterns of young adolescent ▪ Lifestyle & physical activity ▪ Sleep patterns ▪ Health and wellbeing ▪ Personal hygiene practices ▪ Adolescents hitting discipline
Inclusion criteria	▪ Participants must belong to the previous study. ▪ Participants must provide written informed assent in addition to one of the parents providing written informed consent. ▪ Intend to comply with study procedures.

Background

Adolescence (10-19 years of age) is generally believed to be the healthiest period of life, boys and girls of this age group have been observed to experience faster physical, cognitive, and psychosocial growth. This rapid growth has a lot to do with the ways they understand, feel, make decisions, and adopt behaviors that have lifelong implications. (WHO, 2022) adolescents' health improvement will have multifold economic and health returns (Patton et al., 2016).

Lancet commission on adolescent health has emphasized the importance of investment in adolescents' largest ever population (1.8 billion) in human history. The majority living low and middle income countries (LIMCs) with limited opportunities to realize their optimal developmental potential. (Patton et al., 2016). In Pakistan, with its approximately 200 million population ranked 6th most populous country (UN Department of Economic and Social Affairs Population Division, 2019), the estimated young population ages 10-19 makes approximately 40 million , equates to > 20% of world's young population. (UNICEF, 2013). A substantial proportion (29.5%) living below the national poverty levels. (The World Bank, 2017). Towards the ending poverty, sustainable development goals pledge 2030 has been recognized and strategized to ensure the health and well-being of adolescent girls and boys. (Every Woman Every Child 2015).

Global Strategy for Women's, Children's, and Adolescent Health (2016-2030) highlighted the many-sided socio-economic and policy-related issues that negatively impact the health and wellbeing of adolescent girls and boys living in many settings. Nutrition and mental health interventions are among the key areas to promote adolescent health (E. W. E. C. WHO, 2015).

Maternal diet, nutritional status during pregnancy, and the early diet of the offspring play an important role in later health (Marshall et al., 2021). The short-term outcomes of nutrition interventions have been extensively studied in recent decades (Haider & Bhutta, 2017; Keats, Haider, Tam, & Bhutta, 2019) but the impact of these early nutrition interventions on long term health and development outcomes has been missing. Most of the key nutrition intervention trials including education, food supplementation, micronutrient supple, mentation and others have usually looked at the outcomes for a defined period and the evidence on the sustainability of these impacts is largely lacking. Therefore, this cross-sectional assessment aims to understand the impact of nutrition and education interventions introduced during preschool years on the nutritional and developmental outcomes in early adolescence may be of great importance to adolescent health policy makers and the researchers.

Nutrition in adolescence

A lancet review of 2416 population-based studies on around 129 million boys and girls of 5-19 years estimated that the prevalence of thinness (underweight) has not been reduced in adolescents from almost last three decades. Global estimates of median body mass index are 8.4% and 12.4% (-2SD) in girls and boys. Rates of both moderate (-2SD) and severe (-3SD) thinness are higher in South Asia, one third of male and roughly 20% females of 5-19 years age are underweight. While worldwide age-standardized prevalence of obesity has been observed far greater < 1% in 1975 to 5.6 and 7.8 in girls and boys in 2016. (Bentham, Di Cesare, BIlano, & Boddy, 2017; Murtagh & Collaboration, 2017)

Nutrition and growth in adolescence is particularly important to one's health and adult stature (Black et al 2013). A pooled analysis of demographic health surveys conducted in 58 countries on around 0.24 million adolescent mothers (15-19 years) showed significant left skewedness of height-for-age-z-scores (HAZ). See figure 1a. More adolescent mothers from Southeast Asia found to be stunted compared to other World Health Organization (WHO) demarcated regions, as shown in figure 1b. (Christian & Smith, 2018)

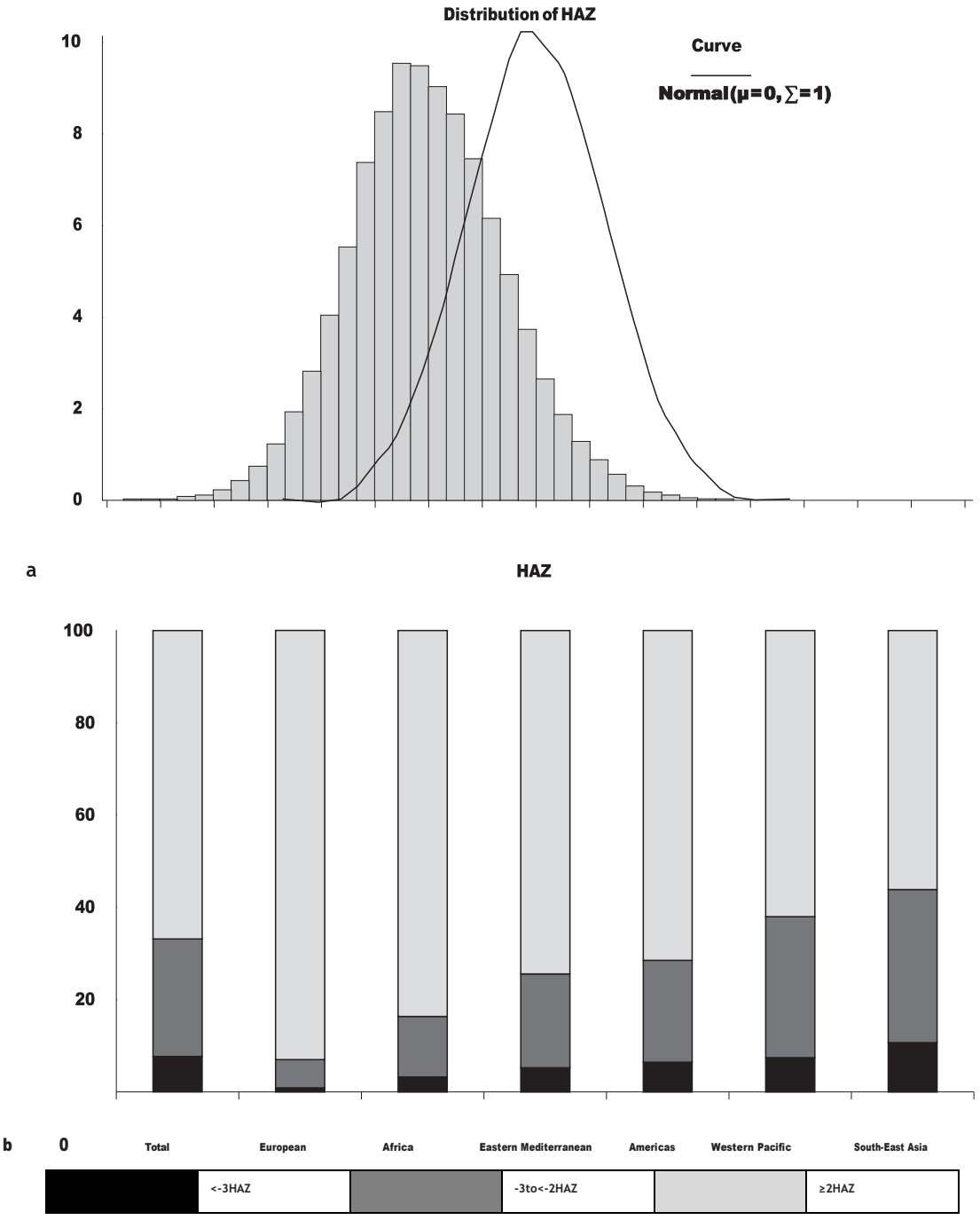

Fig 1. Stunting in older adolescents. a) Shows the distribution of height-for-age z- scores (HAZ) among 15–19 years old girls. b) Shows the proportion of these girls measuring <–3 HAZ , –3 to <–2 HAZ, and ≥–2 HAZ (data source: demographic and health surveys (DHS) – the most recent survey data from 58 countries, *n* =243,806 (Christian & Smith, 2018).

National Nutrition Survey of Pakistan (2018) produced first ever national estimates on nutritional indicators of girls and boys (aged 10–19 years). The survey findings revealed higher prevalence of malnutrition in boys compared to the girls of similar age band (10-19 years) i.e., underweight (boys: 21.1%; girls: 11.8%), overweight (boys: 17.8%; girls: 16.8%), obesity (7.6% and 5.5%) and short stature (boys: 31.7%; girls: 28.5%). Similarly, in a cross-sectional study conducted in district Matiari Sindh, Pakistan, involving 1385 girls and boys of 9-15 and 10-16 years respectively, showed that thirty seven percent of girls and 27 percent of boys were stunted – higher number of boys 21.8% versus 15.6% girls were underweight. Majority participants had micronutrient deficiencies, often coexist with other forms of malnutrition. Almost half of the girls (47%) and boys (48%) had two or more micronutrient deficiencies. More than half of the girls (60.5%) and boys (42.9%) were vitamin A deficient. Iron deficiency (ID) was prevalent in 31% girls and 22% boys (Campisi et al., 2021). Such widespread prevalence of multiple forms of malnutrition can have several adverse effects on one's health and productivity. For example, an analysis of the global burden of disease study (2013) estimated that iron deficiency only accounts for 15.6% and 18.8% of life years lived with disability (YLDs) in girls and boys respectively. (Mokdad et al., 2016)

The role of optimal nutrition for the continuum beginning at preconception, during pregnancy, at birth, and beyond extending through childhood and adolescence has received too little attention from researchers, clinicians, and policy experts in the past. (Marshall et al., 2021) Malnutrition in adolescence has lifelong and intergenerational impacts (Das, Lassi, Hoodbhoy, & Salam, 2018; Patton et al., 2016) Sufficiency of nutrition in children and adolescents is intrinsically connected to healthier next generations. More evidence is required to tailor the policy guidelines for the older children or young adolescents to improve their nutritional and mental health outcomes. (Das et al., 2018). Thus, we understand that this study would substantially add to generate evidence to understand the impact of multiple nutrition and education interventions introduced in early life on nutritional status and developmental outcomes in a cohort of early adolescents aged 10-15 years.

Neurodevelopment in adolescence

Adolescence marks a rapid phase of brain development after infancy. Significant neurological changes occur during early adolescence. The kind of these transformations in brain structure, functions and connectivity permits for greater developmental malleability, which is peculiar to this phase of age – allowing agencies to amend the behavior during adolescence. Such normative developments are needed for brain readiness to adopt with and address the varying challenges and demands of this phase of life and adulthood. Given greater developmental plasticity during adolescence – they are equally vulnerable to develop risky behaviors and mental disorders. (Paus, Keshavan, & Giedd, 2008; Rudolph et al., 2017) The structural changes, for example, maturation in the regions of prefrontal cortex (PFC) helps

function cognition and impulse control potential. (Crone & Steinbeis, 2017) In simple terms the region of brain that helps in decision-making and planning grows substantially during adolescence. (Backes, Bonnie, National Academies of Sciences, & Medicine, 2019).

Nutrition and health can have long-term effects on cognitive abilities through multiple pathways. First, this can have direct effects on the brain because of a reduction in the supply of nutrients or because infectious agents release neurotoxins or precipitate an immune response that affects the brain. Such incidents may damage the structure of the brain, for example by starving cell-growth processes of essential nutrients. Where such damage is irreversible, long-term effects on cognitive function may ensue. In addition to direct effects of illness, secondary behavioral consequences may emerge with time. A secondary consequence of this is that children do not explore and interact with their environment to the same extent. This, in turn, will reduce the level of stimulation the brain receives and stunt social and cognitive development. Nutritional and educational interventions (exposed at preschool age) from multiple sites have shown to be associated with improved nutritional and developmental outcomes in the cohorts of adolescents (Jukes, 2005) yet the evidence from Pakistan is missing in the available literature. Therefore, this long-term assessment will be instrumental to understand the impact of nutrition and education interventions introduced during preschool years on the nutritional, developmental status of early adolescents.

Study rationale

Within the growing body of literature pertaining to adolescent health, there exists a wide research gap (Nagata, Ferguson, & Ross, 2016; Salam, Das, Lassi, & Bhutta, 2016). Most of the data on younger adolescent lacks indicators on health beyond HIV, sexual reproductive health, infectious diseases, injury and violence. (Global Adolescent Working Group, 2017) Although, we see some literature on nutrition and growth in this age group in LMICs. Yet, in a recent UNICEF evidence gap map of effective interventions for adolescent well-being, only one of 71 studies targeted adolescent males (Bakrania, Ghimire, & Balvin, 2017). Moreover, much of the data available on adolescence health indicators is from national demographic surveys, the need for comprehensive research in adolescent nutrition is huge. Specifically, lack of data on systematic evaluation of nutritional interventions to define the amount of micro and macro nutrients, age at intervention and duration of intervention to observe improved physical and mental outcomes in adolescence (Christian & Smith, 2018) Pakistan's adolescent nutrition strategy also pledged continued evidence generation for guidance, learning and accountability (GAIN, 2020) Thus, we understand that this evaluation of long-term impact of nutrition interventions on nutritional and developmental outcomes in early adolescence would substantially add to fill this research gap. The results may help guide policy managers

to design appropriate interventions in early infancy and childhood to yield improved health outcomes in second decade of human life.

Context of study

Current study is a cross-sectional assessment of a cohort of early adolescent boys and girls who received nutrition and education interventions in their early life. Brief details on the parent study are summarized in table-1.

Table 1: Details on parent studies are summarized in table below, followed a brief description of these studies.

Cohort short title	Design and description of original studies	Major outcomes measured in study	Sample size at recruitment	Sample size for follow up evaluation	Current ages of cohort
Zinc Gates	RCT 3 arms Group A- Control Group B- MNP Group C-MNP+Zinc Age at recruitment: 6 months Intervention duration: 1 years	Height gain Morbidities (Diarrhea & ARI)	Matiari n= 1596	1005	11-14 years

Brief description of the parent study

Prospective, cluster randomized, controlled evaluation of the impact of zinc and / or micronutrient supplementation on intestinal flora, diarrheal disease burden, intestinal mucosal integrity, and growth among cohorts of children in Pakistan.

This trial was a community-based randomized controlled effectiveness trial in Bilal colony, a slum area of Karachi, and Matiari, a peri-urban settlement in Sindh. The estimated population in these 2 locations was 383,000. The purpose of the trial was to evaluate the effects of provision of two micronutrient powder formulations, with or without zinc, to children in Pakistan. The target population was infants from 3-6 months of age. A total of 2746 infants were recruited and followed till 2years of age. (Soofi et al., 2013)

The enrolled infants were placed in three groups given as under,

Group A (control) received no specific nutrition intervention other than the standard of care for preventive health education by the Lady Health Workers (LHWs), which didn't include any nutrition commodities. In addition, there was a health worker training in integrated management of childhood illness (IMCI) and facility strengthening for child survival interventions in all clusters. Specifically, these children had however received oral rehydration and 10 days course of treatment with 20 mg zinc daily for treatment of acute diarrhea episodes. These refresher LHW trainings included a specific focus on family education and

mobilization for exclusive breastfeeding (for about 6 months) and appropriate complementary feeding using home available foods.

Group B (additional daily multiple micronutrients, excluding zinc, as Sprinkles). In addition to the health systems interventions listed above, this group of children received a daily sachet of these micronutrients (Sprinkles) for 12 months from recruitment (i.e., between 6-18 months age). These Sprinkles added to commonly used and home available weaning foods. The Sprinkles were prepared by Genera Pakistan Pvt. Ltd (Rawalpindi) to required specifications as single serving sachets each containing microencapsulated iron (12.5 mg), vitamin C (50 mg), vitamin A (300 µg), vitamin D (5 µg) and folic acid (150 µg).

Group C (additional daily multiple micronutrients, including zinc, as Sprinkles). In addition to the health-systems and nutrition interventions listed above, this group of children received a daily sachet of micronutrients for 12 months from recruitment (i.e., between 6-18 months age) added to commonly used and home available weaning foods. In addition to the micronutrients specified above, these Sprinkles also contained 10 mg elemental zinc.

Overall goal

The overall goal of this study is to assess the long-term impact of nutrition interventions given in early childhood on the neurodevelopmental outcomes and nutritional status of early adolescent boys and girls (10-15 years).

Primary objectives

- To examine the long-term impact of early childhood nutrition interventions on neurodevelopmental outcomes in a cohort of early adolescent girls and boys (10-15 years).

- To examine the long-term impact of early nutrition interventions on nutrition status in a cohort of early adolescent girls and boys (10-15 years).

Secondary objectives

- To assess the association of household and individual demographics with adolescents' nutrition and developmental outcomes.
- To assess the association of household food insecurity, dietary diversity, and food recall data with developmental and nutritional outcomes in adolescents.
- To assess the association of young adolescents' lifestyle/patterns with developmental and nutritional outcomes.
- To assess the association of household food insecurity, dietary diversity, and food recall data with developmental and nutritional outcomes in adolescents.
- To document the knowledge, attitude, and practices around climate change.

Outcome indicators

Primary outcome measures

- Neurodevelopmental: assessed using scale Weschler Intelligence Scale for Children (WISC-IV).
- Nutritional status: To assess growth (height, weight, and mid-upper arm circumference) about the WHO standards for height for age (HAZ), weight-for-height (WHZ), –weight-for-age (WAZ) for adolescents.

Secondary outcome measures

- Nutrition behavior:
    - Eating Habits using questions from Health Behavior in School-Aged Children (HBSC)
    - Minimum Dietary Diversity (MDD) scores using the Dietary Diversity questionnaire

- Household food insecurity will be assessed using Household Food Insecurity Experience (HFIES)
- Referring PDHS tool, individual and household-level demographic data will be used to disaggregate data based on several indicators.
- Frequencies and proportions of knowledge, attitude, and practices about climate change – using the UNDP module.
- Frequencies and proportions of knowledge, attitude, and practices of families about climate change – using UNDP modules.
- Meal patterns of young adolescent
- Lifestyle & physical activity
- Sleep patterns
- Health and wellbeing
- Personal hygiene practices
- Adolescents hitting discipline

Methods

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Study design

This will be a cross-sectional study.

Setting

The study will be conducted in Matiari District in the Province of Sindh in Pakistan. The population of district estimated to be 769,349 and the majority living rurally (Pakistan Bureau of Statistics, 2017). The existing District Health System infrastructure for community health

care encompasses Basic Health Units (BHU) and Rural Health Centers 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 several research studies on maternal and child health in this district. Necessary rapport has been established between the community representatives, health system personnel, and Aga Khan University.

Inclusion criteria

- Participants must be part of one of our previous study.
- Participants must provide written informed assent in addition to one of the parents providing written informed consent.
- Intend to comply with study procedures.

Exclusion Criteria

- Child is or has been part of any nutritional trial other than parent study.

Sample size

The sample size calculation is briefly described here.

Zinc Gates (RCT)

To detect an effect size of 0.2 and above (2-point difference in mean with SD of 10) sample size of 335 children would be needed per group (total 1005) at 80% -90% power and 5% level of significance. This sample allows multiple comparisons using Tukey-Kramer (Pairwise) multiple comparison test.

Data Collection

Data collection will be started with the retrieval of cohorts followed by initial screening and written informed consent and assent for the detailed assessments.

Cohort retrieval and screening

Each field team will be provided with a list containing the name of the child, his/her father's name, and address along with the previous identification number. During this visit the field team will screen the participants for eligibility, included participants will be requested for written informed assent, and one of their parents will also give written informed consent.

Household questionnaire

This questionnaire contains multiple modules. These include household and individual demographics, knowledge, attitude, and practices about climate change, flood damages, child morbidities, food insecurity, dietary diversity, 24 hours food recall, and anthropometrics. This questionnaire will be administrated to mother and child at their home. These modules are briefly described as under,

Household demographics

Once informed consent and assent will be obtained, the study team will collect household demographic information, socio-economic status (SES), and educational and occupational information of the participant. These statistics will be collected using standard Pakistan Demographics and Health Survey (PDHS) questions. Information on eating habits will be collected using questions from Health Behavior in School-Aged Children (HBSC) study questionnaire.

Flood damages and climate change

In the post-flood scenario – widely believed to be evidence of climate change; this survey provides us with an opportunity to assess the communities' perceptions about climate change and estimate the damages and losses of recent rains and flooding in the target population.

Data on knowledge, attitude, and practices around climate change will be collected from the parents of participants. We adopted the standard set of questions commissioned by the United Nations Development Program (UNDP). This tool aims to assess the indigenous understanding of climate shift including their take on what climate change is, how it happens (causes) and what are the ways it is affecting them, the weightage people give to the issues, their perceptions of the importance of various contextual actions that can be taken, etc.

Child health

Relevant medical history for any hospitalization, chronic infections like (TB, HIV, etc.) or acute infections (like ARI, Diarrhea, Pneumonia, Malaria, etc.) will be collected from the primary caregiver.

Household food insecurity assessment and dietary diversity

Diets in resource-poor settings commonly lack variety and quantity, thereby limiting the 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 (*Pakistan Food Security Bulletin*, 2017). Food Insecurity Experience Scale (FIES) and Household Dietary Diversity Score (HDDS) are two other important tools used to identify the food environment of households) (Cafiero, Viviani, & Nord, 2018; Calfiero, Nord, & Viviani, 2016; Kennedy, Ballard, & Dop, 2011). FIES was developed in 2013 by the FAO for global and country monitoring of the severity of food insecurity in the previous 12 months. Since 2014, the Gallup World Poll (GWP) has collected data using the FIES. Thus, both scales will be used in this study to understand the food environment of households and its association with adolescents' mental, emotional, neurodevelopmental, and nutritional health.

Physical growth

Anthropometric techniques will be used to assess growth (height, weight, and mid-upper arm circumference) about the WHO standards for height for age (HAZ), weight-for-height (WHZ), –weight-for-age (WAZ) for children and adolescents.

Assessment tools for developmental outcomes

The developmental period of adolescent age can be difficult. It includes complex biological (physical growth), psychological, cognitive (such as intellectual functioning, problem-solving, reasoning, ability to think hypothetically, to develop abstract concepts, and decision making), and moral development (i.e., social, emotional, and behavioral paradigms). It is important to note that such domains are in constant flux, interact with each other, and do not occur in isolation. We can measure this stage by "adaptive functioning" Psychiatric screening" and "intelligence quotient". Wechsler Intelligence Scale for Children-4th (WISC-IV) edition will be used to assess the neurodevelopmental outcomes.

Table 3: Summary of WISC-IV

Tool Name	Subtest	Age range	Administration time	Outcome measures
Wechsler Intelligence Scale for Children-4th Edition (WISC-IV)	• Verbal Comprehension Index • Perceptual Reasoning Index • Working Memory Index • Processing Speed Index	6:0 to 16:11 years	60 to 90 minutes	Assess the child's intellectual abilities

Wechsler Intelligence Scale for Children (WISC-4th edition)

Wechsler's intelligence scale for children is a standardized assessment tool. It is the most widely used assessment instrument for determining a child's intellectual abilities and particular strengths and weaknesses in cognitively understanding his or her world. This battery provides full-scale IQ, with five main indexes assessing, Verbal Comprehension (VC), Visual-Spatial Ability (VS), Fluid Reasoning (FR), Working Memory (WM), and Processing Speed (PS). Details are given below:

Table 4: Indexes of WISC-IV	
Index	Detail

Verbal Comprehension	This index measures a child's ability to access and apply acquired word knowledge. Specifically, this score reflects one's ability to verbalize meaningful concepts, think about verbal information, and express oneself using words.
Visual-Spatial ability	This index measures a child's ability to evaluate visual details and understand visual-spatial relationships to construct geometric designs from a model. This skill requires visual-spatial reasoning, integration and synthesis of part-whole relationships, attentiveness to visual detail, and sometimes using hand-eye coordination, and working quickly and efficiently with visual information.
Fluid Reasoning	This index measures a child's ability to detect the underlying conceptual relationship among visual objects and use reasoning to identify and apply rules. Identification and application of conceptual relationships require inductive and quantitative reasoning, broad visual intelligence, simultaneous processing, and abstract thinking.
Working Memory	This index measures a child's ability to register, maintain, and manipulate visual and auditory information in conscious awareness. These tasks measure one's skills in attention, concentration, and mental reasoning as well as visual and auditory discrimination. This skill is closely related to learning and achievement.
Processing Speed	This index measures a child's speed and accuracy of visual identification, decision-making, and decision implementation. Performance is related to visual scanning, visual discrimination, short-term visual memory, visuomotor coordination, and concentration. This skill may be important to a child's development in reading, and ability to think quickly in general.

TABLE 5: SUMMARY OF STUDY FORMS

S#	Form Name	Respondent / Participant	Primary Data/Purpose	Responsible personnel	Duration
1	Research Consent *Form-01* Research Assent *Form-02*	Mother & Participant	Obtain written informed consent and assent	Research staff	10-15 minutes

2	Household Questionnaire *Form-03*	Mother	Household and individual demographics, flood damages and climate change, child morbidities, child life style, food insecurity, dietary diversity, 24 hours food recall, and anthropometrics.	Research staff	50-60 minutes
3	Wechsler Intelligence Scale for Children-5th Edition (WISC-IV) *Form-04*	Child	Assess the child's intellectual abilities and strengths and weaknesses in cognitively understanding his or her world.	Research staff	60-75 minutes
				APPROX. TOTAL TIME	2-2/12 hours

Data management and analysis plan

Tablet-based questionnaires will be used to guide research personnel in conducting structured interviews with the study participants. The tablets run a custom-made data collection application, which includes built-in logic and range checks. Real-time data synchronization will be used to ensure data completeness. Given the qualitative and observational nature of psychological assessments, these questionnaires will be paper-based. The forms will be checked by desk editors for their completeness and consistency. These forms will be then entered in pre-designed data entry screens.

Household and participant characteristics

Summary statistics will be determined for all household and participant variables (i.e., demographic, socioeconomic, dietary diversity, household food insecurity, and household food consumption score). Demographic variables including household construction material, the source of drinking water, sanitation facilities, cooking fuel, household utilities, household density, assets, and livestock will be used to compute a Wealth Index using standardized methods (Rutstein, 2008). The final score will be divided into five equal parts to make a wealth quintile. STATA version 17 will be used for quantitative analysis.

Dietary diversity and food insecurity

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 0-8. While the FIES does not measure food insecurity directly in children, estimates of the percentage of children living in food-insecure households are generally used. There exists a lack of data regarding food insecurity among households where adolescents live in Pakistan but 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* (Pereira, Handa, & Holmqvist, 2017). 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.

Anthropometric measurements

Summary statistics for height (m), weight (kg), BMI, and MUAC (cm) will be determined. Height and BMI will be converted to height-for-age z-scores (HAZ) and BMI-for-age z-scores (BAZ) according to chronological age using the WHO Growth Reference for Children and Adolescents (de Onis et al., 2007) and WHO-package for R. Summary statistics for z-scores will be stratified by sex and calculated according to chronological age for mean, median and standard deviation. Participants with a HAZ <-2 SD will be classified as stunted; severe thinness BAZ ≤ -3 SD, thinness: BAZ > -3 SD to ≤-2 SD, normal weight: BAZ >-2 SD to <+1SD, overweight: BAZ >+1 SD to < +2 SD and, obese: BAZ ≥+2 SD. The prevalence of anthropometric indicator categories will be reported as the proportion of participants who did not achieve the respective cut-off. Participants with biologically implausible z-scores (BAZ < -5 or > 5, WAZ <-6 or>5, or HAZ < -6 or > 6) will be excluded using WHO cut-offs (2006). MUAC will be assessed using cut-offs outlined in the Integrated Management of Adolescent and Adult Illness (WHO) as the proportion of participants with MUAC < 160 mm (WHO).

Developmental outcomes

Wechsler Intelligence Scale for Children (WISC-IV)

This battery provides full-scale IQ, with five main indexes assessing, Verbal Comprehension (VC), Visual-Spatial Ability (VS), Fluid Reasoning (FR), Working Memory (WM), and Processing Speed (PS). Similarities and Vocabulary are the two primary subtests that comprise the Verbal Comprehension score. The two primary Visual Spatial subtests are Block Design and Visual Puzzles. Fluid Reasoning is measured with the two primary subtests, Matrix Reasoning, and Figure Weights. Digit Span and Picture Span are the two primary Working Memory subtests, and Coding and Symbol Search are the two primary Processing Speed subtests. The ten primary subtests, however, do not contribute equally to the Full-Scale Intelligence Quotient

or IQ score. The Full-Scale IQ is comprised of seven primary subtests: Similarities, Vocabulary, Block Design, Matrix Reasoning, Figure Weights, Digit Span, and Coding.

The descriptive statistics will be reported as mean, standard deviation, frequency, and proportions as appropriate. The outcomes will be compared by intervention or risk categories to identify their impact using an independent sample t-test and chi-square test for continuous and categorical outcomes, respectively. A multivariate regression analysis will be performed to assess the impact of various factors on a child's mental development. These factors include early intervention or exposure to childhood illnesses including diarrhea and ARI; household socioeconomic and food security status; age, sex, number of siblings, and parental education of the child.

Training

Extensive training sessions will be provided to study staff for all assessments based on standard manuals. The data collection team includes psychologists who will be trained on scales to be used for neurodevelopmental assessments, anthropometric assessments, and questionnaires.
Training will include lectures with PowerPoint presentations, handouts, hands-on practice, distribution of relevant SOP to study staff, and mock interviews/assessments.

Ethics

Research ethics approval

Research ethics committee (ERC) review board approval will be obtained from the ERC - Aga Khan University, Pakistan. All standard procedures related to assent and consent will be followed.

Consent and Assent

Parental consent will be obtained for each participant. Informed assent will be obtained from all study participants. Participants will be informed that they have the right to withdraw from the study at any time without penalty meaning that withdrawal from the study or refusal to participate in any section will not in any way affect their ability to receive any services normally offered to the community. All signed informed consent and assent forms will be retained in the study files and a copy supplied to the participant's guardian.

Confidentiality

Confidentiality of all the data collected from the population will be guaranteed through existing systems of data storage and anonymization of forms. Participant privacy and confidentiality in electronic and printed data, publications, and reports during and following

the completion of the study will be fully ensured. The field site offices and central storage facilities at AKU are fully secure with electronic access and monitoring around the clock.

Risks and Benefits

Risks

This study is observational in nature and therefore the methods of this study pose a minimal physical risk to participants. Questionnaires used in the study may, on rare occasions, cause distress for the respondent. If a participant or parent(s) or chaperone imparts that they are experiencing distress, or if they are visibly distressed, the research staff member will inquire more about how the participant is feeling about completing the questionnaires. If necessary, study staff will pause or delay the assessment.

Participants may also be concerned with the confidentiality of the information they provide; however, stringent survey procedures are in place to protect confidentiality.

Benefits

The study does not offer direct benefits to you. However, in case of the identification of any suspected or confirmed neurodevelopmental delay or disorder– the participant shall be referred to the nearest public sector mental health institution for further evaluation and treatment. For this, the study team shall offer transportation and appointment facilitation, if that support is required by families. In case of non-compliance with referral, the study psychologist will counsel parents on the importance of seeking care for their child. for During the screening assessment if a participant identified with suspected COVID-19 symptoms will be oriented on COVID-19 SOPs and referred to the COVID-19 district cell for further evaluation.

Societal benefits of the proposed study include the evaluation of the long-term impacts of the nutritional interventions given in early infancy on the adolescents' health outcomes. The study results are expected to inform future intervention studies in this population.

Study Timeline

The study activities are summarized in the table below.

Table 5: Study timeline

	2022							2023											2024					
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
ERC Approval																								
Staff Hiring																								
Training																								
Data collection																								
Data management																								
Data analysis																								
Manuscript preparation																								

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