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PREVALENCE OF ANEMIA AND FACTORS ASSOCIATED WITH IT AMONG THE FEMALE POPULATION GOING TO COLLEGES AND UNIVERSITIES IN KHYBER PAKHTUNKHWA (KPK), PAKISTAN.

Muhammad Sajid[1], Noor Ul Ain Nawaz[2], Sumaira Irum Khan[3], Junaid Ahmad[4], Shubana Hayat[4], Naseem Ullah[5], Zakia Subhan[6], Muhammad Usman Amin[6,7]*

[1]Department of Pharmacology, Gajju Khan Medical College, Swabi, KPK, Pakistan
[2]City University of Science and Information Technology, Peshawar, Pakistan.
[3]Department of Pharmacy, Mirpur University of Science & Technology (MUST) Mirpur AJ&K.
[4]Department of Microbiology, Hazara University Mansehra, KPK, Pakistan
[5]Isma Institute of Pharmaceutical Sciences, Gujrat, Punjab, Pakistan
[6]Department of Pharmacology, Khyber Medical University Institute of Medical Sciences, Kohat, KPK, Pakistan.
[7*]Department of Pharmacy, Abasyn University Peshawar, KPK, Pakistan.

***Correspondence Author;** Muhammad Usman Amin
*Email: musmanamin1999@gmail.com

Abstract
The study was conducted to investigate the prevalence of Anemia and its associated factors among females studying at colleges and Universities in KPK, Pakistan. This is the institutional based cross sectional study carried out in different academic institutes of KPK, Pakistan where 3000 female students aged between 18 to 25 years were included. The prevalence of anemia was assessed by taking the blood samples for blood test including hemoglobin (Hb), smear test, serum ferritin, vitamin B12 and folic acid. An interview oriented questionnaire was designed to obtain all the relevant data. Height, weight, mid arm circumference, body mass index (BMI) of each female was determined. Dietary habbits of the population was also included. The financial status such as monthly income of the participants was also assessed. The prevalence of anemia was 58% in the population under study. The BMI and anemia were found associated with each other. The imbalanced and inadequate diet along with monthly income was the key factors for anemia among females under study. About 46.8% population had mild anemia, 10% had moderate anemia and 1.2% had severe anemia on the basis of hemoglobin. The population with anemia was divided on mean corpuscular volume (MCV) basis into three groups, Normocytic normochromic anemia (73.2%), hypochromic anemia (24.4%) and macrocytic anemia (2.4%). The study revealed that anemia is very common among females, especially at the adult stage. It is the need of the hour to reform health care system so that the prevalence of anemia can be reduced.

Keywords: Anemia, Body mass index, Hemoglobin, normocytic anemia, microcytic anemia, Macrocytic anemia

Introduction

Health is defined by the world health organization as the presence of mental, physical and social comfort not as the absence of disease (Valderrábano, 2000). Anemia is a universal problem that is highly dominant in emerging areas of the world (Tatala *et al.*, 1998; Goodnough and Schrier, 2014). Anemia is a medical problem that reduces the quality of life and increases the risk of mortality and morbidity (Joosten, 2004).Anemia is associated with a decrease in red blood cells, reduced hemoglobin levels and malformed red blood cell morphology. It is pathophysiologically multifactorial and diverse disease (Kassebaum *et al.*, 2013). Anemia can either result from non-nutritional factors, such as infection, hemorrhage, drug toxicity, chronic disease states or from nutritional factors, including iron, vitamins, protein and copper deficiency. Iron deficiency remains the primary reason of anemia. It is estimated via many surveys that 75% of anemia is related to iron deficiency, followed by vitamin B12 and folate (folic acid) deficiencies (Al-Assaf, 2007; Haidar, 2010). The vitamin B12 and folic acid deficiency leads to macrocytic anemia. Anemia is associated to impaired mental growth and physical development (Chalco *et al.*, 2005). Anemia is defined by World Health Organization (WHO) as a hemoglobin (Hb) concentration below 13 g/dl in men over 15 years of age, below 12 g/dl in non-pregnant women over 15 years of age, and below 11g/dl in pregnant women. Anemia affects 1/3rd of the world's population; most of the time anemia reported was due to iron deficiency (Guralnik *et al.*, 2004; Tettamanti *et al.*, 2010; Goddard *et al.*, 2011) Iron deficiency anemia (IDA) is the most common nutritional insufficiency around the globe (Clark, 2009) This type of anemia causes reduced work capacity in adults and has an effect on mental growth in adolescents and children (Killip *et al.*, 2007; Alleyne *et al.*, 2008). Individuals with IDA have insufficient intake, impaired transport or absorption, physiologic losses of iron or chronic loss of blood secondary to disease (Agha *et al.*, 1992). In adults, IDA can result in a wide variety of adverse outcomes including reduced exercise or work capacity, GI disturbances, immune dysfunction, impaired thermoregulation and neurocognitive impairment. (Beghé *et al.*, 2004; Clark, 2008). Iron deficiency is most prevalent in infancy and in puberty (Leung and Chan, 2001). Blood loss during menstruation is the common reason of IDA in pre-menopausal women. Each milliliter of blood loss results in loss of around 0.5 mg of iron (Cohen and Gibor, 1980; Balducci, 2003; Pasricha *et al.*, 2010). Microcytosis [mean corpuscular volume (MCV) lower than the normal value] is significant feature of iron deficiency anemia. The treatment of iron deficiency should always be started with iron given orally. When this does not work because of large blood loss, intolerance to oral iron or iron malabsorption, parenteral iron can be given using iron gluconate, iron dextran or iron sucrose (Cook, 2005). Iron treatment can be given by mouth, intravenous or intramuscular route. Alternatively, recombinant erythropoietin and blood transfusions can also be used (Cuervo and Mahomed, 2001). Medicine supplementation is very effective in controlling and preventing anemia (de Souza Queiroz *et al.*, 2000). Anemia continues to be an endemic problem of large magnitude, and the increasing trends in several developing countries, point to the failures of current methods to lessen this burden. The WHO has started successful campaigns against a number of contagious diseases—but the battle against anemia is far from being won and more efforts should be focused on tackling this huge problem. Identification of the local causes of anemia and improvement and implementation of suitable approaches will be essential for progress of this significant worldwide health issue (Balarajan *et al.*, 2011).

Material and Methods

Study Design and population

Institution-based, cross-sectional study design was employed. Anemia was evaluated in the population by determination of hemoglobin and mean corpuscular volume which is one of the most suitable screening procedures (Tatala *et al.*, 1998). While factors associated with anemia were also assessed. This study was carried out at Abasyn University, University of Peshawar, Peshawar, KMU institute of medical sciences, Kohat, Government Degree College for women, Mardan, KPK, Pakistan during January 2018 to December 2018 .Total 3000 females were included in the study.

While the questionnaire was distributed among 3250 females .The objective and importance of study was explained to females and their consent was taken.

Inclusion Criteria

All females registered as regular students in the above mentioned colleges and universities were included. Females aged 18 to 28 years and who showed willingness to participate in the study were considered for the study

Exclusion Criteria

Females other than above mentioned colleges and universities, females ≤ 18 years and ≥ 26 years of age were excluded. Females going through their manusteral cycle were also excluded from the study.

Assessment of Anemia

According to World health organization the female students having hemoglobin level < 12gm/dl were considered as anemic. Anemia was assessed among the population on the basis of hemoglobin (Hb) concentration and mean corpuscular volume (MCV). Anemia was classified in to non-anemic, mild anemia, moderate anemia and severe anemia on the basis of hemoglobin concentration (Tatala *et al.*, 1998). Those found anemic were further classified in to Normocytic normochromic anemia, Microcytic hypochromic anemia and Macrocytic anemia on the basis of MCV in order to find the cause of anemia (Chulilla *et al.*, 2009).

Data Collection

A structured pretested interviewer-administered questionnaire was used to obtain sociodemographic information from female students. To obtain dietary habit, standard food frequency questionnaire adjusted for local food item was adapted and used to assess the usual intake of various types of food such as eating animal foods, green leafy vegetables, taking fruit after meal, and drinking tea/coffee for the past one month with their respective consumption frequency(Gibson, 2005).

The questionnaire was developed in English as all of the students could understand and speak English well. To ensure reliable data collection and attain standardization and maximize interviewer reliability, authors themselves interacted with students to do the interview. Authors supervised for proper data collection; all the questionnaires were checked for completeness and consistency in daily basis. Information regarding economical status of participants was also collected through the questionnaire. Economical status was evaluated on the basis of monthly income of the family. The monthly income of the family was categorized in to three classes such as < Rs.50,000 was considered as low income, > Rs.50,000 up to 200000 (medium) and > Rs.200000 was considered as high income (Bekele *et al.*, 2016).

Anthropometric measurements :

A questionnaire was designed which included height, mid arm circumferences and weight measurements. Trained person was appointed to take all anthropometric measurements. Body mass index (BMI) was calculated by using following formula:

BMI = weight in kilograms (kg) / height in centimeters (cm)

The females were categorized into underweight (BMI 16 to 18.5kg/m^2), normal weight (BMI 18.5 to 25 kg/m^2), overweight (BMI 25 to 30 kg/m^2), obese (BMI 30 to 35 kg/m2), (Leenstra *et al.*, 2004; Ausk and Ioannou, 2008; Eftekhari *et al.*, 2009) on the basis of BMI (WHO, 2001).

Specimen Collection

The specimen collection process in all mentioned colleges and universities was carried out by two trained laboratory technologists. Each step of specimen collection, processing, and analysis was supervised by experienced and trained laboratory technologist supervisors. A venous blood sample

was taken from the study participants; using disposable syringe, three-fourths of the syringe was filled and then blood was transferred to EDTA tube labelled with identification number

Biochemical analysis:

About 3ml blood taken from each female in EDTA tube was subjected for Hb analysis, hematocrit and total blood count. Iron or Ferrous (Fe) deficiency was defined as 12mg/dl serum ferritin concentration and 16% transferrin saturation while anemia was defined as 12g/dl (Hb level) in accordance with the WHO guidelines (Who, 2001).Various investigations such as hemoglobin concentration, red cell indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration were (MCH), Hematocrit (HCT), Red blood cell (RBC) count, White blood cell (WBC) count were carried out. The cyanomethaemoglobin technique was adopted for measuring Hb (Dallman *et al.*, 1984; Qurtom *et al.*, 1989; Eftekhari *et al.*, 2009; Qureshi *et al.*, 2012). Those females having Hb value 12g/dl or less than the specified value were subjected to special smear test. We classified the anemia on the basis of hemoglobin concentration and MCV. The hemoglobin concentration less than the specified value confirm the anemia occurrence. The MCV clearly indicated the cause of anemia weather it is because of iron deficiency, Vitamin B_{12} or folic acid deficiency (Sgnaolin *et al.*, 2013).

Statistical Analysis

The data in the study was tabulated and statistically analyzed through SPSS version 16. The statistical parameters like mean, standard deviation, frequency and percentage were measured.

Results

Total 3000 females were selected for study. Among these females, 16% were underweight, 54% had normal weight, 18% were overweight and 12% were obese. In Table 1, the mean value of the Hb, RBC count, WBC count, MCV, MCH and HCT for underweight, normal weight, overweight and obese are mentioned as shown in Table 1. It was evident that the average values of Hb among underweight, overweight and obese females were found less than 12g/dl in comparision to the normal weight females. Similarly other parameters such as MCV, MCH and HCT were also found less in case of underweight, overweight and obese females in contrast to normal weight participants.

Table 1 Comparison of hematological indices according to category of body mass indices (BMI)

Parameters	**Underweight** BMI 16-18.5 N=480 (16%)	**Normal weight** BMI 18.5-25 N= 1620 (54%)	**Overweight** BMI 25-30 N=540 (18%)	**Obese** BMI 30-35 N=360 (12%)	**Normal ranges**
Hb (g/dl)	11.01±0.93	13.50±1.19	11.16±0.87	11.24±0.75	12-15.5
RBC count (µl)	4.45±0.56	4.43±0.48	4.62±0.53	4.68±0.13	4.2-5.4
WBC count (billion/L)	7.63±1.92	7.94±2.12	9.17±1.85	8.48±2.10	3.50-10.5
MCV (fl)	79.33±8.22	89.51±6.58	80.21±1.14	80.51±2.90	81-96
MCH (pg)	25.03±1.36	27.9±3.27	25.58±2.72	25.03±1.36	27.0-31.2
HCT (%)	31.10±2.95	38.36±6.50	32.30±3.40	33.43±1.63	34.9-44.5

g/dl = gram/deciliter, **µl** = microliter, **billion/L** = billion cells per liter, **fl** = Femtoliters, **pg** = picograms

In order to determine the association between anemia and BMI, the Hb was checked of all the females. The female population under study was divided into four categories on the basis BMI in to underweight, normal weight, over weight and obese as shown in Table 2. It was evident from the Table 3 that Hb had decreased in a very significant way which was < 12g/dL among underweight

(98.1%), overweight (95.5%) and obese (92.5%). As it can be easily seen that about 58% of the female population under study were found anemic on the basis of Hb measurement. While only 25.9% cases were found less than the normal Hb value among the normal weight females with BMI 18.5-26.

Table 2 Association between BMI and Anemia

BMI (kg/m^2)		**Frequency**	**Hemoglobin(Hb) content (g/dL)**	
			< 12g/dL	**≥12g/dL**
Underweight	BMI 16-18.5	480(16%)	471(98.1%)	9(1.9%)
Normal weight	BMI 18.5-25	1620 (54%)	420 (25.9%)	1200 (74.1%)
Overweight	BMI 25-30	540 (18%)	516 (95.5%)	24 (4.4%)
Obese	BMI 30-35	360 (12%)	333 (92.5%)	27 (7.5%)
Total			**1740(58%)**	**1260(42%)**

From the Table 3, it is clearly evident that out of 3000 females, 42% (1260 females) were non-anemic having 12.57 ± 0.47 g/dl hemoglobin value, 46.8% (1404 females) have mild anemia with 10.99 ± 1.26 g/dl hemoglobin value, 10% (300) have moderate anemia having 8.78 ± 2.54 g/dl hemoglobin value and 1.2% (36 females) have severe anemia having 7.26 ± 0.25 g/dl hemoglobin value.

Table 3 Classification of anemia on the basis of hemoglobin (Hb) level

Classification of anemia	**Hemoglobin (g/dl)**	**Frequency (N)**	**%**	**Normal ranges**
Non-anemic	12.57 ±0.47	1260	42	12.0-15.5
Mild anemia	10.99 ±1.26	990	56.8	10.0-11.9
Moderate anemia	8.78± 2.54	600	34.5	8.0-9.9
Severe anemia	7.26± 0.25	150	8.6	Lower than 8.0

Females suffering from microcytic hypochromic and macrocytic anemia observed were 424 and 42. Therefore the serum ferritin was measured to confirm iron deficiency among microcytic hypochromic anemia. The Table 4 revealed that 24.4% females were found iron deficient having 9.16±1.52 µg/ml of serum ferritin in comparision to the normal value of serum ferritin (12-150 µg/ml).While vitamin B12 and folic acid were measured for the females suffering from macrocytic anemia. Out of 42 macrocytic cases 64.3% females were found to have vitamin B_{12} and 28.6% were found folic acid deficient.

Table 4 Serum ferritin, Vitamin B12 and Folic acid concentration

Variables	Serum levels	Frequency (N)	Percentage (%)	Normal value
Serum ferritin (µg/ml)	9.16±1.52	424	24.4	12 -150µg/ml
Serum Vitamin B12	176±1.05	27	64.3	200-900 pg/mL
Serum Folic Acid	1.5±1.25	12	28.6	2-20 ng/mL

The economical or financial status was assessed by inquiring about the monthly family income of the participants. It was found that average income of most of the families (64%) was < Rs.50000 which was considered a low income as mentioned earlier. The income > Rs.50,000 up to 200000 was observed among 22% participants, while only 14% families had income > Rs.200000.

The questions regarding the diet of the females involved in the study clearly indicated the type of food consumed by them. It was observed that tea/coffee intake was maximum i.e.83% (2490) while minimum intake was that of legumes i.e. 5% (150).Chicken was consumed on average was 50% (500). Red meat and green leafy vegetables were eaten up by 25% (750) females respectively.

Whole grains were ingested by 10% (300) females. The citrus and iron containing fruits were part of only 16% (480) female population's diet.

Discussion

Anemia is the most predominant nutritional deficiency complaint in the world. It affects all age groups. Anemia affects 1.62 billion people globally which parallels to 24.8% of the population. The highest occurrence of anemia is in the developing world where its reasons are multi-factorial (Stoltzfus, 2001; Johnson-Wimbley and Graham, 2011; Qureshi *et al.*, 2015). Anemia is a critical clinical problem in female population with an important public health impact. Data on the prevalence of anemia is wide-ranging and depends on the locality and inhabitants (Qureshi *et al.*, 2015).

The categorization of females (underweight, normal weight, overweight and obese) on the basis of BMI was carried to determine the association between the BMI and anemia. Our study has provided a very solid evidence for this association. As hemoglobin concentration and other parameters such as MCV, MCH and HCT were found less than normal value in most of the underweight, overweight and obese females. However the hemoglobin concentration was also found ≤ 12g/dl among 25.9% normal weight females. Our results are in compliance with another study where the hemoglobin and BMI were found associated with each other. This relationship clearly depicted the incidence of anemia among the population under study. In the developing countries the abnormal BMI and anemia are two major nutritional disorders. The young adult females who were underweight, overweight and obese were found to suffer from anemia (Keikhaei *et al.*, 2012).

Anemia in the population was assessed by hemoglobin determination, which is one of the most convenient screening methods (Tatala *et al.*, 1998). The hemoglobin levels results of our study revealed that, 42% were non-anemic, 46.8% had mild anemia, 10 % had moderate anemia and 1.2% had severe anemia. Our result was in compliance with another study, where mild degree anemia was incident in 46.34%, which was the most common, followed by moderate degree which was present in 43.44% of individuals under study. The severe anemia was present in 10.22%, which was least common degree of anemia (Toteja *et al.*, 2006; Qureshi *et al.*, 2015).

According to our results based on mean corpuscular volume (MCV) clearly indicated that, most of the cases (73.2%) observed were normocytic normochromic anemic. While 24.4% were microcytic hypochromic anemic and 2.4% were macrocytic anemia. These results were found in compliance with a study conducted in Pakistan in year 2012 in which 72% patients were having normocytic normochromic anemia (Qureshi *et al.*, 2012). The most common causes for normocytic normochromic anemia were renal failure, various hematological diseases and anemia of chronic diseases. Various body changes such as dysfunctioning of the body to use iron to make red blood cells caused by the chronic diseases were responsible anemia of chronic diseases. The most common cause of macrocytic anemia was the iron deficiency and microcytic anemia was recognized by vitamin B_{12} and folic acid deficiency(Chulilla *et al.*, 2009).

Nutritional factors are important for the production and prevention of iron deficiency anemia. The life style of females is often strongly related with their health status, which in turn affects their possibility of developing anemia. The occurrence of anemia reported for female individuals differ according to the location in which study is conducted (MOORE, 1955; Beghé *et al.*, 2004). From the questionnaire regarding diet, it was clearly evident that about 83% female population were having maximum intake of tea and coffee that may be the major cause of anemia among more than 50% females participated in our study. The association of anemia with coffee was also confirmed in another study (Kumera *et al.*, 2018)**.** Actually tea and coffee contain caffeine that was found to decreases the absorption of iron in by 35% after meal leading to iron deficiency (Morck *et al.*, 1983). Similarly, non-heme iron absorption which makes about 70-90% of iron in diet has been found to be inhibited by tea causing iron deficiency (Zijp *et al.*, 2000).

Similarly, the red meat and leafy vegetables was part of diet of only 25% females. Results revealed that healthy females having BMI more than 25 kg/m^2 were also suffering from anemia having

hemoglobin value lower than 12g/dl. Same findings were also evident from the literature indicating that main reason of it is the excessive use of junk food in every socio-economic status (Jain and Jain, 2012). Again the study confirmed that iron deficient diet was the most common cause of anemia. As green leafy vegetables and legumes were the best sources for non-haem iron (Hurrell and Egli, 2010).Another study has revealed clearly that those people who only eat vegetables and completely exclude meat from their diet mostly suffer from iron deficiency (Saunders *et al.*, 2013).
Financial factors such as family income was a very solid reason for the provision of proper and adequate diet. As our results revealed that the family income of most of the participants was found below Rs.50,000 which was considered a low income . The improper and insufficient food found among our participants is directly related to anemia which in turn associated with average income. Our study has revealed 58% prevalence of anemia among the females population, the family income of the anemic population was ≤ Rs.50, 000. This relationship of anemia with family income was in consistent with another study where the prevalence of anemia was found associated significantly with low family income (Bekele *et al.*, 2016).
Our study clearly revealed that anemia is the key health issue in fully grown females. At the present it is the need of the hour to prevent anemia at this stage. As the adult women with iron deficiency, are at a very great peril of long term mental and motor impairment. These females also face lack of concentration, easy distractibility and short attention span. Immune system is also affected by anemia leading to poor physical fitness and increasing sensitivity to infections. That's why iron should be taken on daily basis (Walter *et al.*, 1989; Jain and Jain, 2012).

Conclusions

This small, provincial population based study of college and University going females has indicated the occurrence of normocytic, microcytic and macrocytic anemia. The dietary and family incomes were observed to be related with anemia significantly among the college and university going females. In our study the major reason for anemia was due to iron deficiency. There is a need for an organized study to find out the causes and frequency of anemia at public level among females, males, children and elderly people. Future investigation is needed to define the optimal hemoglobin levels, to refine diagnostic testing to sort out the etiology of the unexplained anemia, and to evaluate rigorously therapies designed to augment erythropoiesis.

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Conflict of Interests

The authors have no conflict of interest

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