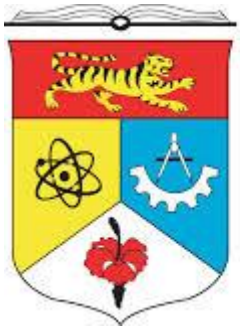

UNIVERSITI KEBANGSAAN MALAYSIA
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PROPOSAL OF

EFFECT OF IRON SUPPLEMENTATION AND NUTRITIONAL EDUCATION AMONG IRON DEFICIENT AND IRON DEFICIENT ANEMIC FEMALE ADOLESCENTS IN THE GAZA STRIP-PALESTINE

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ABSTRACT

Iron deficiency (ID) is the most common form of malnutrition worldwide, affecting more than 2000 million people globally. Iron deficiency anemia (IDA) is highly widespread in developing countries. Adolescent female constitute about fifth of total female population in the world. Adolescent is one of the most challenging period in human development. The sudden changes create nutritional needs. As a period of growth and development, is considered the best time to intervene, to assist in physical and mental development, and to prevent later maternal anemia. The prevalence estimates of IDA among adolescents is 30–55% worldwide. Adolescence is an opportune time for interventions to address anaemia. Not only is there a need (growth, preparation for pregnancy), but large numbers of both male and female adolescents can be reached easily if school attendance or participation in other group activities is high. Also, adolescents are open to new information and new practices since they are often striving for physical or academic excellence. The present study designed to determine the prevalence of anemia, ID and IDA and to study the efficacy of iron-supplementation and nutritional education on hemoglobin and ferritin levels among female adolescents aged 15-19 years living in the Gaza strip-Palestine. The study has two phases: the first phase will be a cross sectional descriptive study, which will enroll randomly 374 female students aged 15-19 years in the Gaza strip-Palestine. The study population in the study is female students enrolled in the secondary schools. Five female secondary schools will be selected randomly from five governorates in the Gaza strip. In each school, one to two classes of each grade will be selected randomly as well. According to the list of the student's names, the subjects will be selected upon odd number in the school records. The second phase is randomized controlled trial (intervention phase) that will be divided into three groups; the first group for iron supplementation, the second for nutritional education intervention and the last as control group. Data will be collected by questionnaires, anthropometric measurements, complete blood counts (CBC) and serum ferritin will be analysed. All ID and iron deficient anemic female adolescents will be monitored to evaluate the effectiveness of iron supplement and nutritional education. Statistical package for social science (IBM-SPSS) version 22 will be used for analysis of data.

Kesan Penambahan Zat Besi dan Pendidikan Pemakanan di Kalangan Remaja Wanita Kekurangan Zat Besi dan Anemia Kekurangan Zat Besi di Jalur Gaza-Palestine

ABSTRAK

Kekurangan zat besi (ID) merupakan salah satu masalah kekurangan zat makanan yang paling biasa di seluruh dunia dan telah yang menpengaruhi lebih daripada 2000 juta orang di seluruh dunia. Kekurangan zat besi anemia (IDA) adalah sangat biasa di negara-negara yang sedang membangun. Remaja perempuan merupakan populasi yang kelima besar daripada jumlah populasi wanita di seluruh dunia. Remaja adalah salah satu tempoh yang paling mencabar dalam pembangunan manusia. Keperluan pemakanan memuncul akibat perubahan yang mendadak. Tempoh pertumbuhan dan pembangunan ini dianggap sebagai masa terbaik untuk mengantara dan membantu dalam pembangunan fizikal dan mental, dan untuk mencegah anemia maternal pada masa depan. Anggaran kelaziman IDA di kalangan remaja adalah 30-55% di seluruh dunia. Zaman remaja adalah masa yang paling sesuai untuk menangani anemia. Bukan sahaja disebabkan oleh keperluan (pertumbuhan, persediaan untuk mengandung), tetapi sejumlah besar kedua-dua remaja lelaki dan perempuan boleh dicapai dengan mudah jika kehadiran sekolah atau penyertaan dalam aktiviti kumpulan lain adalah tinggi. Juga, remaja adalah terbuka kepada maklumat dan amalan baru kerana mereka sering mencabar untuk mencapai kecemerlangan fizikal atau akademik. Kajian ini bertujuan untuk menentukan prevalen anemia, ID dan IDA dan mengkaji keberkesanan suplemen zat besi dan pendidikan pemakanan pada hemoglobin dan aras ferritin di kalangan remaja perempuan yang berumur 15-19 tahun dan tinggal di jalur Gaza-Palestin. Kajian ini mengandungi dua fasa: fasa pertama ialah kajian keratan lintnag deskriptif, di mana sebanyank 374 pelajar perempuan yang berumur 15-19 tahun di Gaza jalur-Palestin akan dipilih secara rawak. Populasi kajian dalam kajian ini adalah pelajar perempuan yang mendaftar di sekolah-sekolah menengah. Lima buah sekolah menengah perempuan akan dipilih secara rawak daripada lima negeri di jalur Gaza. Selepas itu, satu atau dua kelas dari setiap gred akan dipilih secara rawak juga dari setiap sekolah. Dengan menggunakan senarai nama pelajar, subjek akan dipilih mengiukut nombor ganjil dalam rekod sekolah. Fasa kedua adalah percubaan kawalan rawak (fasa pengantara) yang akan dibahagikan kepada tiga kumpulan; kumpulan yang pertama akan mengambil pil zat besi, kumpulan kedua akan diberi pendidikan pemakanan dan kumpulan yang terakhir akan dijadikan sebagai kumpulan kawalan. Data akan dikumpulkan melalui borang soal selidik, kaedah antropometri, pengiraan darah lengkap dan analysis aras ferritin serum. Semua remaja wanita yang mengalami ID dan anemia kekurangan zat besi akan dipantau untuk menilai keberkesanan suplemen zat besi dan pendidikan pemakanan. Pakej statistik bagi sains sosial (IBM-SPSS) versi 22 akan digunakan untuk analisis data.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Iron deficiency (ID) is the most common form of malnutrition worldwide, affecting more than 2 billion people globally. Iron deficiency anemia (IDA) is highly widespread in developing countries but also remains a problem in developed countries. ID is not the only cause of anemia, but where anemia is prevalent, ID is the most common cause (WHO 2011).

The most commonly used definition of anemia come from the Centers for Disease Control and Prevention (CDC) in Atlanta USA and the World Health Organization (WHO). WHO defines anemia as hemoglobin concentration cut-offs at 12.0 g/dl for non-pregnant women, and 11.0 g/dl for preschool children and pregnant women (Benoist et al. 2008).

The importance of controlling anemia as being one of the worldwide developments. In addition, it highlighted the universal prevalence of anemia in non-pregnant women to be at 30.2%, and the universal prevalence of anemia in pregnant women is 41.8% (Benoist et al. 2008).

Adolescence is a critical stage in life for physical growth and sexual development. A previous study on teenage pregnancy and adverse birth outcomes, concluded that adolescents who become pregnant increase the risk of adverse birth outcomes and fetal mortality (Chen et al. 2007). Adolescents considered a nutritionally vulnerable segment of the population. The accelerator growth coupled with marginal nutrient intake increases the risk of nutritional deficiencies in adolescent population (Hettiarachchi et al. 2006).

Most of adolescents are suffering from ID with its adverse effects on health. ID and IDA in adolescence is a major public health problem (WHO 2011). Adolescents are tomorrow's adult population and their health and well-being are crucial (Shekhawat et al. 2014).

Adolescence have very high iron requirements, and the iron demand of individual children during period of rapid growth is highly variable and may exceed mean estimated requirement. The growth spurt in adolescents female usually occurs in early adolescence before menarche, but growth contributes post-menarche at slower rate. Moreover, the addition of menstrual iron loss to the iron demand for growth leads to particularly high iron requirements for post-menarche adolescent female (Caballero 2009). Anemia is not used as an indicator for body mass index; therefore, obese adolescents may be at risk of anemia. Overweight children and adolescents are more than two times more likely than those with normal weight to be deficient of iron (Lecube et al. 2006).

Anemia and iron deficiency anemia are an indicators of both poor nutrition and poor health (Bhanushali et al. 2011), and it has been associated with impaired cognitive function, decreased attention, inability to concentrate, and memory loss (Bourre 2006).

The risk factors of IDA are low intake of meat and meat products, frequent dieting, vegetarian female, skipping meal and heavy menstrual periods (Frith-Terhune et al. 2000).

Achievement scores of adolescents students with anemia were much lower than healthy students. Students who are not healthy may experience different symptoms. Anemia and iron deficiency anemia are one of the common problems among students,

which can have a negative impact on their academic performance and productivity. Brain enzymes are the first body function, which affected by iron deficiency, which are related to behaviour and cognition. The consequences of iron deficient patient in infant related to cognition is irreversible. But in later stages of life has undesirable consequences on mental and academic activity (Soleimani & abbaszadeh 2011).

Anemia reduces the learning power, which cause academic dropout among student (Soleimani & abbaszadeh 2011). The adolescent may suffer from poor intelligent quotient (IQ), impaired physical work, and decreased cognitive function due to iron deficiency. All adolescent female should know about the importance of foods rich in iron and functions of iron in human body. These can approved by nutritional education and weekly iron supplementation. The same study concluded that major risk factors for iron deficiency anemia are dietary habits and menorrhagia among Saudi women of childbearing age (Al-Quaiz 2001).

A previous study entitled "Effectiveness of daily and weekly iron and folic acid supplementation in anemic adolescent girls" found that there was significant decline in the iron deficiency anaemia from 65.3% to 54.3%. The same study concluded that weekly supplementation of iron among adolescents female should be started before they become pregnant (Mehta 1998). Other study in Iran tackled adolescents female concluded that weekly iron supplementation recommended to iron deficient adolescents (Akramipour et al. 2008).

1.2 PROBLEM STATEMENT

Iron deficiency anemia is the most common nutritional disorder in the world, constituting a public health condition of epidemic proportions. It particularly affects women in the reproductive age group and young children in tropical and subtropical regions (Kurz & Galloway 2000).

The nutritional status of the population of the Eastern Mediterranean Region is suboptimal, suggesting that for several nutrients including iron, iodine, zinc, calcium, vitamin A, vitamin D and folate. IDA is a serious public health problem in all countries of the region (Bagchi 2008).

Adolescent female constitute about 1/5th of total female population in the world (Kulkarni & Durge 2011). Adolescent is one of the most challenging period in human development. The sudden changes create nutritional needs. As a period of growth and development, is considered the best time to intervene, to assist in physical and mental development, and to prevent later maternal anemia. Moreover, the requirements for some nutrients are higher in adolescent than in other stages of life (Olumakaiye et al. 2010).

During adolescence, the requirement for iron is double as compared to younger age group. Iron requirement increases two to three folds from a pre-adolescent level of approximately 0.7-0.9 mg/day to 1.40-3.27 mg/day iron in adolescent female. In menstruation, there is iron loss monthly from 12.5-15 mg or 0.4-0.5 mg daily of iron during menstruation period. Therefore, in female, following the growth spurt, the risk of ID continues to be a public health concern through the entire reproductive age (WHO 2011).

A previous study in the north Gaza conducted by Abudayya et al. came out with the prevalence of anemia among adolescent males and females aged 12-15 years at 49.7% and 51.3% respectively (Abudayya et al. 2007). The same study showed that diseases in Palestine nowadays are related to nutrition. Moreover, nutrition during adolescence plays an important role during the life cycle, because the adolescents' stage requires higher amounts of micronutrients like iron. Another study concluded that prevalence rates of anemia among pregnant Palestinian women are more than twice as high as those observed in Europe (Abudayya et al. 2007; Khader et al. 2009) Despite the magnitude of the problem, no strategies known at the Palestinian Ministry of Health to tackle iron deficiency anemia in adolescent female.

IDA is estimated that 20% of maternal deaths are directly related to anemia and 50% of maternal deaths are associated with it (Anand et al. 2014). Many girls are already anemic by the time they become pregnant (16-55%), and that pregnancy is a short period of time in which to reduce preexisting anemia, especially when many woman do not seek prenatal care until the second or third trimester. Thus, they are concluded that emphasis needs to be placed on pre-pregnancy programs to increase body iron stores (Meier et al. 2003).

A recent study in India, reported that 90% of anemia occurring in developing countries significantly affecting morbidity, mortality and national development rates (Anand et al. 2014).

Prevalence estimates of ID among female adolescent range from 9-40%, depending on the context. Meanwhile, prevalence estimates of IDA among adolescents is 30–55% worldwide (Hermoso et al. 2011). The greatest percentage of people affected is in Asia, where 68% of non-pregnant and 56.1% of pregnant women are anemic (Benoist et al. 2008).

In the Gaza strip, data on malnutrition and anemia focus on children younger than 5 years and pregnant women as well. Knowing that according to Palestinian central bureau of statistics (PCBS), the total population of the Gaza strip is 1.76 million, the estimated number of female secondary students is 45019. It compromises 2.6% of the total populations (PCBS 2014). The present study designed to determine the prevalence of anemia, ID and IDA and to study the effectiveness of iron-supplementation and nutritional education on hemoglobin and ferritin levels among female aged 15-19 years living in the Gaza strip-Palestine.

1.3 JUSTIFICATION OF THE STUDY

Anemia is a widespread public health problem associated with an increased risk of morbidity and mortality. Anemia is a disease with multiple causes, both nutritional (vitamins and minerals deficiencies) and non-nutritional (infection) that frequently might appear (WHO 2008). Unfortunately, there has been little documented progress in the universal fight against anemia and data from WHO showed that 818 million children under the age of five and women affected by anemia, about one million of them die every year mainly in developing countries (WHO 2008).

Anemia also impairs educational achievement and economic productivity, costing the government and families enormous amount of money to treat related illnesses. Descriptive calculations for 10 developing countries recommend that the median value of physical productivity losses annually due to ID is around US$2.32 per capita, or 0.57% of gross domestic product (GDP). Median total losses (physical and

cognitive combined) are $16.78 per capita, 4.05% of GDP. Clearly, implications of economic of IDA are also massive (Anand et al. 2014).

Adolescents need to different foods, particularly iron will increase, but the need is much higher among females. Anemia and iron deficiency anemia are an indicators of both poor nutrition and poor health (Bhanushali et al. 2011), and it has been associated with impaired cognitive function, decreased attention, inability to concentrate, and memory loss. The relationship between iron status and cognitive performance is currently attracting interest (Bourre 2006). However, intelligence Quotient (IQ) will be lower among iron deficient anemic patients. Learning abilities will then decrease during this condition and so will be with achievement (Soleimani 2011).

Adolescence is an opportune time for interventions to address anaemia. Not only is there a need (growth, preparation for pregnancy), but large numbers of both boys and girls can be reached easily if school attendance or participation in other group activities is high. Also, adolescents are open to new information and new practices since they are often striving for physical or academic excellence (WHO 2011).

Strategic focus on prevention of IDA among adolescents is more important from the point of view of productivity gains from improved physical capacity; productivity gains from increased cognitive ability; and (for adolescent female) improved pregnancy out comes and intergenerational benefits (WHO 2011).

In north Gaza strip, the prevalence of anemia among adolescent male and female (12-15 years old) was 49.7% and 51.3% respectively (Abudayya et al. 2007). Which reflect the magnitude of the problem and highlights the urgent need for an action. Anemia remains common in many countries of the world and it can be uprooted through effective interventions (Kraemer & Zimmermann 2007). As far as the researcher knowledge, no such reliable data on prevalence of IDA in late adolescent population in Gaza are not available. Recent study in Palestine, reported the scarcity of studies regarding the prevalence of anaemia among Palestinian adolescents (Mikki et al. 2011)

Many professionals may use the results of this study as basic data to build up strategies for nutritional intervention. In addition, it could represent a directional clue for many investigations in this field.

Up to recently, little was known about nutrition of adolescents, particularly in low- and middle-income countries. There is a dearth of data on adolescents nutritional status in developing countries (WHO 2005).

This study will be carried out to evaluate the effectiveness of iron supplementation and nutritional education among ID and IDA female adolescents in the Gaza strip-Palestine.

Given data, that adolescent period is a critical time for major changes in the human body, most adolescents during the period of puberty gain 20% of their adult height and 50% of their adult weight and skeletal mass (Marian et al. 2007).

Adolescent females represent a group at high risk of iron deficiency both in developing and industrialized countries of the world. A study in Malaysia reported that iron and folate intervention most effective and should serve as a prototype for future studies aimed at eliminating iron deficiency (Tee et al. 1999).

However, to the best of the researcher knowledge, no nutrition intervention program to treat ID and IDA among adolescents has ever been carried out in Gaza strip. Moreover, after searching published scientific studies, the novelty of the study comes from its investigation of the roots of occurrence and health impact of IDA among future mothers followed by an intervention trial that will tackle the problem. It is important to consider treatment before anemia develops as women get married and become pregnant without being aware if they are anemic or not. Since the adolescents is one of the most susceptible groups to nutritional deficiencies and need more nutritional care for suitable growth, the international health and nutrition institutes recommends more focus and studies about adolescents' nutritional assessment, so this study was designed to focus on the following objectives.

1.4 OBJECTIVES

1.4.1 General Objective

To determine the prevalence of anemia, ID and IDA and to study the effectiveness of Fe-supplementation and nutritional education on hemoglobin and ferritin levels among female aged 15-19 years living in the Gaza strip-Palestine.

1.4.2 Specific Objectives

1. To determine the effects of SES factors on hemoglobin and ferritin levels.
2. To identify the risk factors associated with anemia, ID and IDA.
3. To determine the relationship between iron supplementation and nutritional education onmalonyldialdehyde (MDA) level.
4. To evaluate the efficacy of iron-supplementation and nutritional education intervention among iron deficient and iron deficient anemic adolescents.
5. To evaluate the sustainability of follow up intervention on Hb and ferritin levels.

1.5 RESEARCH QUESTIONS

1- What is the prevalence of anemia, ID and IDA among adolescents' students?
2- What are identify the risk factors associated with anemia, ID and IDA?
3- What is the relationship between iron supplementation and nutritional education on malonyldialdehyde (MDA) level?
4- What is the efficacy of iron supplementation and nutritional education intervention among iron deficient and iron deficient anemic adolescents?
5- What is the difference of sustainability of follow up intervention on Hb and ferritin levels?

1.6 STUDY HYPOTHESES

There is no difference between Iron supplementation and nutritional education intervention to improve haemoglobin and ferritin levels.

1.7 CONCEPTUAL FRAMEWORK

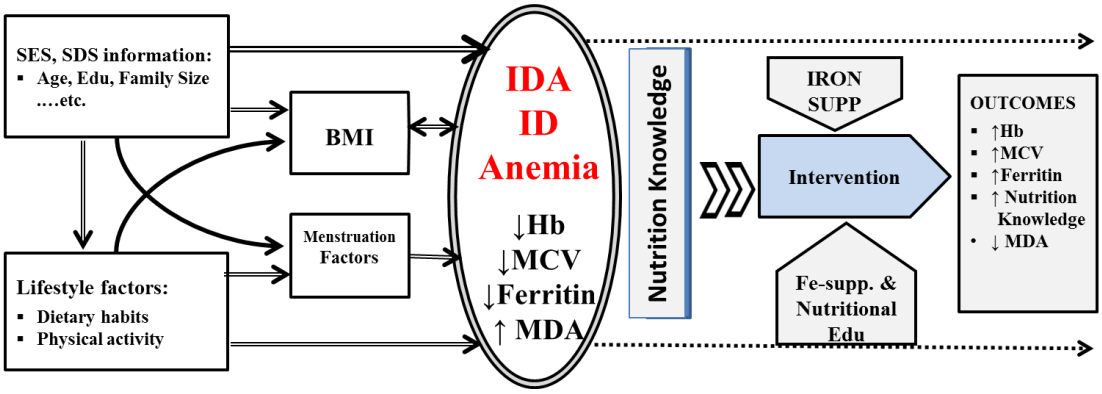


Figure 1.1: Conceptual framework

Anemia, ID, and IDA which is as a result of low haemoglobin, MCV and ferritin levels has many factors associated with it. The haemoglobin and ferritin levels affected directly or indirectly by many factors. As displayed by the above framework in the figure (1.1), socioeconomic status and demographic region (parents' education, employment, family income, family size etc.) are associated of anemia, IDA and ID either positively or negatively. There is an interplay relationship between socioeconomic status, life style factors (dietary habits and physical activity), BMI and menstruation which all lead to anemia and IDA. However, these relationships could be positive or negative, meaning one factor could lead to increase or decrease of another factor.

The life style factor (dietary habits and physical activity) has a direct relationship with anemia and IDA. The relationship between BMI (weight and height) by dividing to 4 groups (underweight, normal weight, overweight and obese) is a two way or a feedback which is not directly.

On the right side of the conceptual framework deal with the intervention that will be carried out on subjects with IDA. The first part has to do with developing a questionnaire and two parts of intervention (Iron supplementation and iron supplementation with nutritional education) will be employed.

Iron supplementation is to administer iron for 3 months while iron supplementation with nutritional education will be 3 months also. At the end of the

intervention, outcomes like haemoglobin, MCV and ferritin, nutrition knowledge will be measured to ascertain which of these interventions give a better effect to the outcomes.

After a follow up will be done all subjects 3 months after the end of the intervention exercise to check the blood and nutrition knowledge has helped to preclude ID and IDA.

CHAPTER 2

REVIEW OF LITERATURE

In this chapter definition, classifications of anemia among adolescents are presented, then prevalence of anemia, and IDA; locally, regionally, and internationally are discussed. Moreover, the risk factors associated with anemia and intervention are explored.

2.1 DEFINITION OF ANEMIA

Anemia is the condition of having less than the normal number of red blood cells or less than the normal quantity of hemoglobin in the blood. The oxygen-carrying capacity of the blood is therefore, decreased (Patterson 2010). Iron deficiency, which is one of the most prevalent nutritional problems worldwide, affects approximately one third of the world's population (Sim et al. 2014) and almost half of the world's children may have IDA (Powers & Buchanan 2014).

2.2 PREVALENCE OF ANEMIA AND IDA

Prevalence estimates of iron deficiency (ID) among female adolescent range from 9-40%, depending on the context. Meanwhile, prevalence estimates of IDA among adolescents is 30‑55% worldwide (Hermoso et al. 2011). The greatest percentage of

people affected is in Asia, where 68% of non-pregnant and 56.1% of pregnant women are anemic (Benoist et al. 2008). In India, the prevalence of anemia among adolescent females aged 10-19 year in Nagpur, India was 35.1% (Sanjeev & Vasant 2008).

Iron deficiency and its associated anemia problems are widespread among women, especially those of childbearing age, and among children. Data on anemia rates among pre-schoolers, pregnant women and women of childbearing age from 1995 to 2001 showed no improvement in the overall situation among the member states of Arabic region. The same study showed that the prevalence rates for anemia are high, ranging between 30% and 55% among adolescents in Egypt, Saudi Arabia and Yemen (Bagchi 2008).

The prevalence of anemia in Saudi Arabian adolescent female was 21.8%, but in Kuwaiti the prevalence of anemia among adolescents was 30%, and 25% of their anemic was iron deficient anemia, on another hand the prevalence in Seri Lanka was 58.1% (Jackson & Al-Mousa 2000; Abalkhail & Shawky 2002). While the prevalence of anemia in Iranian female students was 30%, 21.5% of adolescent females suffered from IDA (Soleimani & abbaszadeh 2011).

In Gaza, the level of anemia among schoolchildren 6-18 years old was estimated at 32.3% in 2009. Those with lower level of education had a prevalence of 49% as compared to 24.5% among those with higher levels of education (WHO 2009). Recently, a study targeting female adolescent aged 15-19 years concluded that the prevalence of anemia among them was 33.3% in Gaza strip (Jalambo et al. 2013).

2.3 RISK FACTORS

Lower socioeconomic groups were less likely to consume fruits or vegetables, and consumed fewer varieties than higher socio-economic groups. One of the most essential factors that influence iron intake is the poor socioeconomic factors that cut iron intake from meat, fish, poultry and other animal sources. The higher intake of iron from all animal sources, as meat, fish, poultry increases with household incomes (Giskes et al. 2002). Female adolescents may be more affected by dietary inadequacies than male, particularly in iron, and professionals advocate iron supplementation as a short-term

solution, for only large increases in household income could allow a higher intake of non-staples to meet iron requirements (WHO 2005).

On the other hand, anemia is highly associated with poverty. People in lower socioeconomic groups have double the risk of those who are middle or upper class. It has been indicated that heavy menstruation for longer than five days is an important risk factor of anemia in female adolescents (Bhargava et al. 2001).

Prevalence of anemia was found lower in nuclear families than in extended families. Further, the higher the numbers in the family, the higher the prevalence of anemia. As both quality and quantity of food consumed get affected by the number of family members especially with limited monthly income (Gupta & Kochar 2009).

Lowest prevalence of anemia was reported among adolescents with university education 42.2%, while the highest prevalence was found among adolescents with low education 52.2%; this result means that the estimated risk of anemia increased significantly with decreased level of education. The risk of anemia decreased as the educational level increased. Adolescent from the low socioeconomic class had 1.4 times the risk of anemia than those from the high class (El-Sahn et al. 1999).

A study entitled "prevalence and risk factors of anemia among women of reproductive age in Bursa, Turkey" concluded that the prevalence of anemia among family members up to four was 32.9%, while it was 32.8% among family members with five or more. On the other hand, the prevalence of anemia according to average monthly income was 32.9% among families with less than 500 EURO, while it was 32.5% among families with 500 EURO or more (Pala & Dundar 2008).

2.4 DIETARY RISK FACTORS

The major determinant of anemia, particularly in developing countries, is inadequate dietary consumption. Many people are dependent on plant-based food in which iron absorption is poor and several substances in the diet interfere with this. It is known that iron requirement increases during pregnancy. Also rapid growth during infancy and childhood increases iron requirements. Iron requirements increase considerably during

puberty; in girls, the onset of menstruation imposes a double burden (Michael et al. 2004).

A study in Benin showed that adolescent girls aged 12 to 17 years, with a low meat and fruits consumption less than 4 times a week were more susceptible to iron deficiency than those who consumed such food more than 4 times a week (Alaofe et al. 2007). In concordance with previous studies, less than 2% of adolescents consumed an adequate amount of all food groups, and that almost 20% of girls did not consume an adequate amount of portions of food groups (Strauss 1999).

Higher hemoglobin level was observed among students who frequently ate meat (≥ twice per week) as compared to those who infrequently ate meat (2-3 three times per month). On the other hand, the frequent consumption of citrus fruits and green leafy vegetables were also associated significantly with higher hemoglobin concentration as compared to infrequent consumption of these determinants. Moreover, Consumption of milk or dairy products was not associated significantly with hemoglobin levels. The same study found that tea drinking was associated with lower hemoglobin level among adolescent students. About 11.5% reduction in hemoglobin concentration was observed in heavy tea drinkers (>5 cups per day) compared to non-tea drinkers (Sirdah 2008). A recent study conducted in Kuwait and Palestine concluded that negative correlation exists between anemia and frequency of tea and coffee consumption (Ahmed & Al-Sumaie 2010; Jalambo et al. 2013).

2.5 CONSEQUENCES OF ANEMIA

The consequences of anemia have bad effects on the future of anemic people. Iron deficiency has detrimental effect on work capacity, learning ability and resistance to disease. Anemia consequences include impairment in cognitive (performance and behavior) and in women more pregnancy complications (Gupta & Kochar 2009).

A study in the United States reported that babies whose mothers had anemia in pregnancy during their first trimester had high rates of cardiovascular morbidities and mortalities in their adults life compared to babies whose mother did not have anemia in pregnancy (Adebisi & Strayhorn 2005).

The risk of anemia appears as early as childhood for both boys and girls after whom it subsides for boys but remains for girls because of menstrual blood loss. Anemia affects mental development and learning capacity. Poor dietary habits and lack of nutritional awareness among the youth are the reasons behind the prevalence of Anemia. Among these dietary habits, is daily breakfast intake with quality and not only quantity of food being important (Abalkhail & Shawky 2002).

There appear to be good evidence that cognitive impairment occurs in iron deficient children. The rapid growth of the brain in the first few years of life may make it more vulnerable to iron deficiency. There also appears to be evidence that the changes may not be totally reversible even when iron deficiency is completed corrected (Harris 2007).

Brain enzymes are the first body function, which affected by iron deficiency, which are related to behaviour and cognition. The consequences of iron deficient patient in infant related to cognition is irreversible. But in later stages of life has undesirable consequences on mental and academic activity (Soleimani & abbaszadeh 2011).

Adolescent who have received less iron, the scores was lower in IQ test (Pollitt 1993). Moreover, other studies reported that low serum iron, decreased IQ, precision, concentration and learning in school age children, and iron supplementation could increase their scores of intelligence and academic achievement (Benton & Roberts 1988; Soleimani & abbaszadeh 2011).

2.6 INTERVENTION

2.6.1 SUPPLEMENTATION

Where the prevalence of anemia is high among many vulnerable groups, it is recommended that iron supplementation (plus folate in girls and women) be universally implemented in pregnant women, under-five children, and girls and women from 10-49 years of age. Supplementation should be considered for girls where anemia is widespread, before the first pregnancy (WHO 2005). Several recommendations have been made to improve effectiveness of iron supplementation programs, including

schools and youth groups are among the community-based structures that could be used for delivery of iron supplements to adolescent (WHO 2005).

In Malaysia, hemoglobin and ferritin concentrations were shown to increase significantly in adolescent girls following weekly supplementation with iron combined or not with folate. However, these findings may in part reflect better compliance with weekly than daily supplementation, and there are conflicting results. For instance, in a study on comparative efficacy of weekly and daily supplementation in iron deficient but non-anaemic and non-pregnant women, it was found that there was no specific absorption advantage to weekly over daily dosage, while the latter also fell short of requirements for those situations in which iron supplementation is widely used (WHO 2005).

The results of a total of 22 completed trials of iron supplementation, including nine studies with adolescent groups showed a positive effect with weekly supplements in five out of nine studies. The meta-analysis concluded that supplementation can have an effect on anemia prevalence and that the daily and weekly approaches may have similar effect. The same study concluded that weekly supplementation was recommended only in situations where there is a strong guarantee of supervision and good compliance (WHO 2011).

2.6.2 Nutritional Education

The presence of nutritional education into formal education is recommended strategies, mainly because the students obtain and fix the information in an easy, exciting, and permanent way (García-Casal et al. 2011). Nutritional education intervention have an impact improving nutritional health in students. The nutritional education intervention produced a significant improvement in the hemoglobin and ferritin levels (Falahi et al. 2010) as well as significant reduction in iron deficiency prevalence among school children (García-Casal et al. 2011).

Kulkarni and his colleagues recommended that nutrition education along iron prophylaxis should be implemented among adolescents' females. Regular nutritional education sessions should be carried out to increase awareness in adolescent girls regarding anemia (Kulkarni et al. 2012).

CHAPTER 3

METHODOLOGY

This chapter deals with the subjects and methods of this study. The following methods, study design, study area, study population, eligibility criteria, sampling, data collection, and questionnaires will be employed for this study. Additionally, a pilot study will be carried out to illustrate the method of analysis, ethical consideration and finally the limitation of the study.

Before starting the data collection process, pilot study will be carried out to check applicability, identify problems in the questionnaire and test data collection for validity and reliability. Sample of 38 female students as 10 percent of the total sample size will be chosen randomly from remedial classes and little modification of questionnaire questions may be done when needed. All the items in the questionnaire will be written in Arabic language and will be accepted by the target population.

The study has two phases: the first phase will be cross sectional descriptive study, which will enroll randomly 374 female student aged 15-19 years in the Gaza strip-Palestine. The second phase is randomized controlled trial (intervention phase) that will be divided into three groups; the first group for iron supplementation, the second for iron supplementation with nutritional education intervention and the last as control group. Data will be collected by questionnaires, anthropometric measurements, and blood analysis; complete blood counts (CBC), serum ferritin, CRP, ESR, and MDA

will be analysed. All ID and iron deficient anemic female adolescents will be monitored to evaluate the effectiveness of iron supplement and nutritional education.

3.1. STUDY AREAS

The Gaza strip is located on the coast of the Mediterranean Sea from the west, Egypt from the south. The occupied Palestinian lands "Israel" from the north and east. Gaza strip is very crowded place with an area of 365 km2. According to PCBS in 2014, the population in Gaza strip was 1.76 million, accounting to about 4822 inhabitants/Km², making it one of the most density-populated places on earth. It comprises five governorates, which are the North governorate, Gaza governorate, the Middle governorate, Khanyounos governorate and Rafah governorate in the south. The mean of the Gazian family size was 6.0 in 2014 (PCBS 2014).

The study will cover all the five governorates in the Gaza Strip, (North Gaza, Gaza city, Middle area governorate, Khanyonos governorate and Rafah governorate) as shown in the figure 3.1.

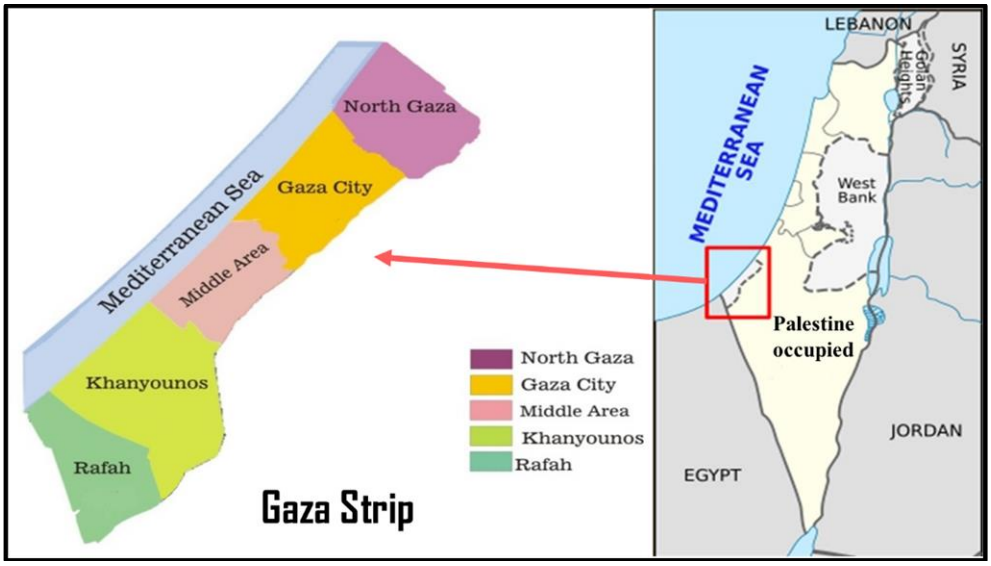


Figure 3.1: Map of Gaza Strip

3.2. PHASE ONE

3.3.1 Study Design

Phase one is a quantitative descriptive study using cross sectional design. The target group will be female adolescents aged 15-19 years enrolled in the secondary schools in the Gaza strip-Palestine.

Based on eligibility criteria (inclusion and exclusion criteria), 374 students will be selected from the list of names records in classes of schools. The questionnaires was developed and will be modified by the researchers after implementation the pilot-tested study on a group of 38 students in secondary schools, and then will be revised to ensure the questions' simplicity and compatibility with the research objectives. All items in the questionnaire will be written in Arabic language, and will be considered appropriate by the target population. Trained researchers (two nurses) are responsible for collecting all socio-economic, demographic, dietary, health data, anthropometric measurements and blood samples.

3.3.2 Study Population

The study population in the study is female students enrolled in the secondary schools. Five female secondary schools will be selected randomly from five governorates in the Gaza strip. In each school, one to two classes of each grade will be selected randomly as well. According to the list of the student's names, the subjects will be selected upon odd number in the school records (interval equal 2).

3.3.3 Eligibility Criteria

Inclusion Criteria

1. Female students.
2. Aged 15-19 years.
3. Stayed in Gaza strip more than one year.

Exclusion Criteria

1. Female students with history of chronic diseases.
2. Female students with history of any blood diseases.
3. Married students.
4. Pregnant, Lactating.
5. Students with disabilities.

3.3.4 Sample Size Calculation of Phase 1

The sample size calculation in the present study by two ways, the first by formula equation to calculate the sample size and the second by Epi Info software ™ 7.0.

For cross sectional part, the sample size will be chosen to obtain the estimated prevalence of anemia with a 95% confidence interval. The expected prevalence of anemia to be used is about 30% (Abudayya et al. 2007; Jalambo et al. 2012). To calculate the sample size n, which satisfies the relation given above, the formula below will be used (Charan 2013):

$$n = \frac{(Z_{\alpha/2})^2 \ (P)\ (1-P)}{e^{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The reliability coefficient ($Z_{\alpha/2}$) = 1.96

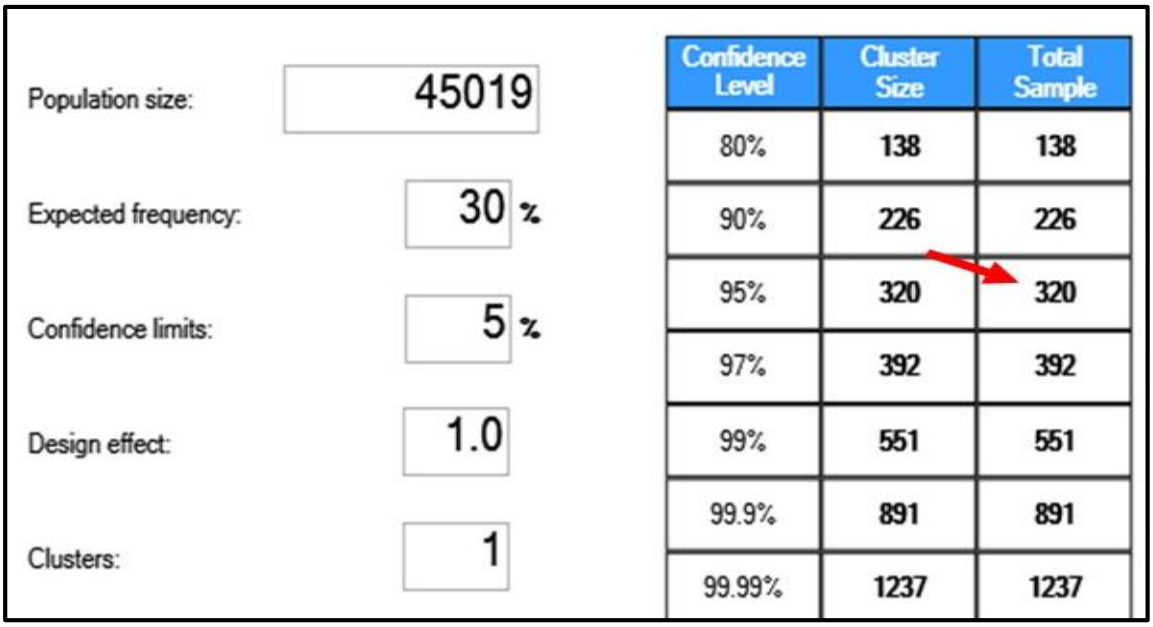
Estimated prevalence of Anemia (P) = 0.30

Estimated prevalence of non-anemic (1-P) = 0.70

The margin of error (e) = 0.05

$$n = \frac{(1.96)^2 \ (0.30)\ (1-0.30)}{(0.05^{2}} \quad =322 \text{ adolescent students}$$

The sample size required for prevalence is **322** female students. The predicted response rate is 87% according to previous studies done among adolescents in Gaza Strip.
N+ response rate (86%) (Abudayya et al. 2007; Jalambo et al. 2012) = **374** students



Confidence Level	Cluster Size	Total Sample
80%	138	138
90%	226	226
95%	320	320
97%	392	392
99%	551	551
99.9%	891	891
99.99%	1237	1237

Figure 3.2: Population survey of description study for simple random sampling according to Epi Info ™ 7.0

3.3.5 Sampling Methodology

According to statistics of the Ministry of Education (MOE), study population is comprised of 45,019 female secondary school students aged 15-19 years enrolled in grade 10th ,11th and 12th of secondary schools in all the 5 governorates in the Gaza strip (MOE 2014). Table 3.1, 3.2 and 3.3 illustrates the total number of female students distributed over the five governorates, their number in each grade, and number of samples to be selected from each grade of each governorate respectively.

Table 3.1: Number of study population in the governorates in the Gaza strip

	North GAZA	Gaza City	Middle Area	Khanyounis	Rafah	Total (N)
GRADE X	3198	5957	2754	3335	2121	17365
GRADE XI	2480	4744	2147	2176	1429	12976
GRADE XII	2189	5606	2102	3205	1576	14678
TOTAL (N)	7867	16307	7003	8716	5126	**45019**

The study population will represent five secondary schools; the schools will be selected randomly from each governorate, taking into consideration, the available records in the Ministry of Education in the Gaza strip, in an attempt to obtain a representative sample for the overall population of the five governorates as shown as in figure 3.3.

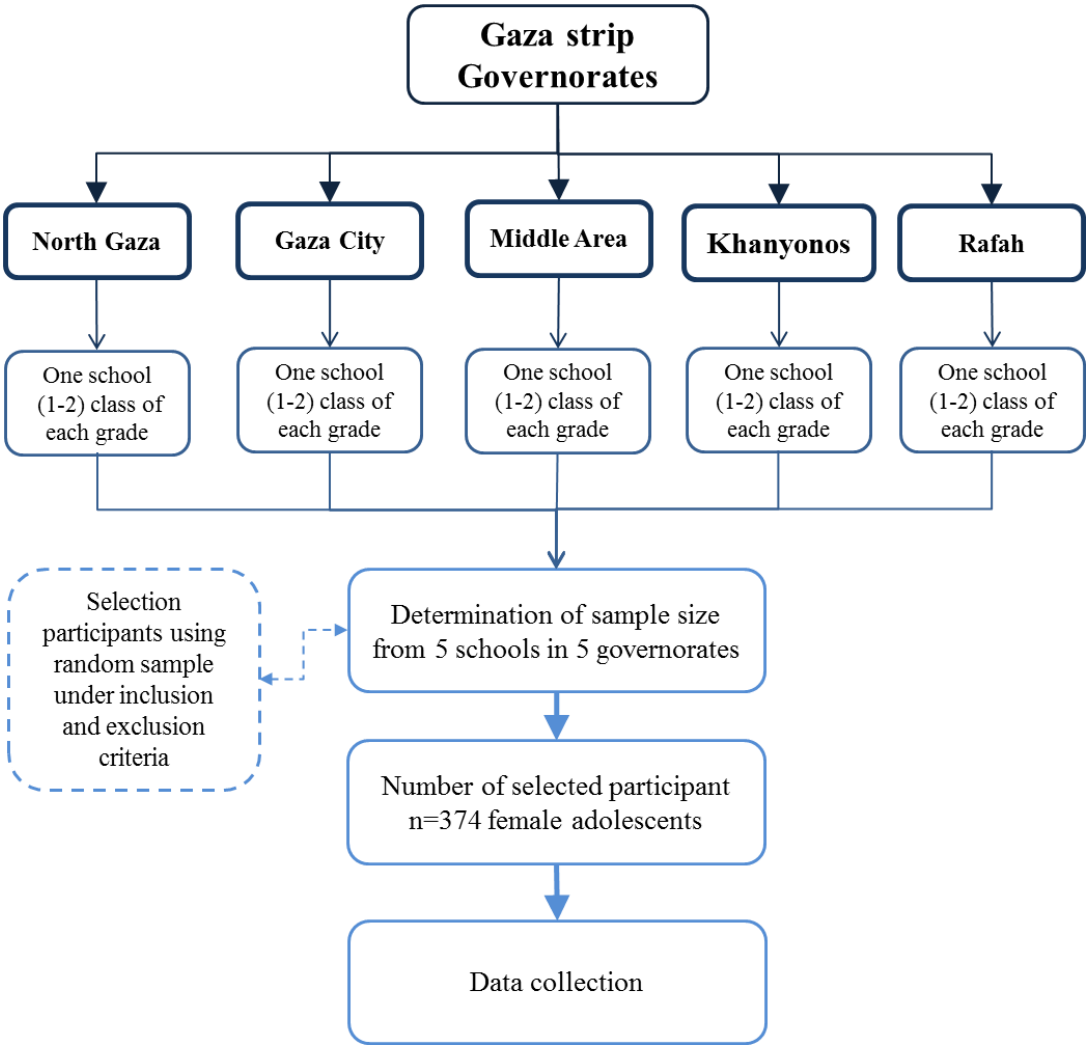


Figure 3.3: Sampling methods flow chart

Table 3.2 illustrates the number of the study sample that was calculated using equation 1 for sample size (322 students) and Epi info (320 students) with the response rate factor, and the selection of the target 374 students that matched each governorate overall student proportion.

Table 3.2: Sample proportion for each governorate

Governorates	Population No.	% of Total	Sample No.
North GAZA	7867	17.5	65
Gaza City	16307	36	133
Middle Area	7003	15.5	59
Khanyounos	8716	19.5	73
Rafah	5126	11.5	44
Total	45019	100	**374**

Table 3.3: sample size for each grade students in each governorate

	North GAZA	Gaza City	Middle Area	Khanyounis	Rafah	Total (N)
GRADE X	25	48	24	27	17	141
GRADE XI	22	39	18	20	14	113
GRADE XII	18	46	17	26	13	120
TOTAL (N)	64	133	58	72	43	**374**

3.3.6 Study Tools and Instruments of Phase One

Two trained nurses' assistants (interviewers) will be collected the research data questionnaires through face-to-face, grouped interviews with the participant inside the library of the school. The questionnaires consisted of four types of questionnaires; the first questionnaire will be filled by the parents of the participants, and consisted of items regarding to socio-economic and demographic characteristics of the households. The second questionnaire focused on evaluation of dietary habits and physiological status will be distributed among the study sample to be filled in through self-administration. Under the researcher guidance and two assistant nurses supervision, the entire questionnaire will be filled and collected. The data obtained from the dietary habits will be used to estimate the average consumption frequency of meat, fish, poultry, citrus fruits and tea, all of which affect the hemoglobin concentration level. The third

questionnaire focused on evaluation of physical activity pattern. The fourth questionnaire to evaluate the scores of the participants according to intelligence Q questionnaire.

1. Socioeconomic and Demographic Characteristics

Parents will be asked questions for information such as their education level, occupation, employment status, salary income, the number of family members contributing to the household income, household size, and household expenditure on food. The educational level of the parents will be classified as: low (illiterate or less than secondary school education; medium (secondary school education); or high (college or university education). Residence will be classified as city, camp or rural. In addition, the questionnaire will be asked the birthdate and health status of the participant.

2. Dietary Habits: Food Frequency Questionnaire (FFQ)

The Food Frequency Questionnaire (FFQ) used to record all the usual dietary intake of the participants. The FFQ consisted of 17 food items without portion sizes that commonly consumed by Palestinian people in Gaza strip, representing the major food groups: cereals and cereal products, meat and meat products, fish, fruits, vegetables, legumes and nuts, milk and dairy products, and beverages. Jalambo et al. 2013 validated this questionnaire (Jalambo et al. 2013). The food items questions are: "How often do you drink/eat the following items?" The response categories in the food frequency questionnaire will be as times/week, and these response categories will be: times/day –times/week –once/week – times/month, and seldom/never (Annex B).

3. Anthropometric Measurements

Nutritional status assessment using anthropometry is a simple, and yet extremely useful initial approach to adolescent nutrition, along with physical examination if in a clinical setting (WHO 2005).

Anthropometric measurements, with two basic variables (height and weight) and a single derived variable (body mass index) will be used in the present study. The body weight of each subject will be measured by using a calibrated scale (Seca model

750 1017009, Germany. The body mass index for age (BMI-age) will be computed using world health organization (WHO) software Anthro plus program version 1.0.4 for aged 5-19 years to monitor the growth of adolescent school-age. Duplicate measurements of weight and height of the students will be taken and the mean of value will be determined.

Students will be weighed barefooted to the nearest 0.5 kg; standing height also will be measured without shoes to the nearest 0.5 cm with the use of stadiometer (Seca body meter 206, Germany), keeping the shoulders in relaxed position and the arms hanging freely. Cut-off values that will be used for classification of the anthropometric indicators according to (WHO 2008) are described in table (3.4).

Table 3.4: Anthropometric cut-off points according to WHO

Indicators	Anthropometric variables	Cut-off values
Thinness	Low BMI for age	< 5th percentile
Normal	Normal BMI for age	5th ≤ BMI < 85th percentile
Overweight	High BMI for age	85th≤ BMI < 95th percentile
Obesity	High BMI for age	≥ 95th percentile

Source: WHO 2008

4. Hematological and Biochemical Assessment

One hematological (CBC) and 4 biochemical (serum ferritin) variables will be measured. About 6 ml of venous blood will be drawn (2 ml in ethylene diamine tetraacetic acid (EDTA) tube and 4 ml in serum tubes) will be collected from each student to perform a complete blood count (CBC), CRP, ESR, MDA and to determine serum ferritin. All samples will be placed in tubes rack and packaged in an appropriate container with ice. The sample will be analysed same day in the laboratory within three hours from the time of collection.

Anemia status will be assessed by measuring hemoglobin (Hb) concentration using a Sysmex XE-2100 hematology analyser. Calibration of the device will checked daily using control solutions provided by the manufacturer. The result will be recorded as hemoglobin in grams per deciliter. each sample will be analysed in duplicate. Anemia

will be defined according to WHO guidelines as Hb < 12.0 g/dl, mild anemia as Hb 9.0-< 12.0 g/dl, moderate anemia as Hb <90 g/dL, and severe anemia as Hb < 70 g/dl.

Iron status will be assessed by measuring serum ferritin and determined by enzyme linked immunosorbent assay (ELISA) by (Human Ferritin ELISA Kit-Sigma-Aldrich-Germany) in duplicate and the average results will be recorded (Annex C).

Iron deficiency for serum ferritin <15 µg/L, Iron depletion borderline for serum ferritin between 15 and ≤ 20 µg/L, Iron repletion for serum ferritin >20 µg/L. Iron deficiency will be defined as two or more indicators below normal. Table 3.6 and 3.7 describes the definition and multiple variable model used to explain the presence of iron deficiency and iron deficiency anemia.

Table 3.5: Multiple variable model for determination of ID and IDA

	Normal	**Depleted iron stores**	**Iron deficiency**	**Iron deficiency anemia**
Serum iron	N	↓	↓	↓ ↓
Haemoglobin	N	N	N	↓

Adapted from Yip & Dallman, 1996

Table 3.6: Operational Definitions of Iron status:

Subject Groups	**Parameters**
Group 1: Iron Sufficient	Serum Ferritin > 20 µg/L Hemoglobin: normal ≥ 12.0 g/dl
Group 2: Iron Deficient not Anemic	Serum Ferritin < 20 µg/L Hemoglobin: normal ≥12.0 g/dl
Group 3: Iron Deficient Anemic	Serum Ferritin < 12 µg/L Hemoglobin < 12.0 g/dl

Adapted from Tobin and Beard 1997

5. Physical Activity Pattern

To evaluate the physical activity pattern among adolescents, the present study will be used physical activity questionnaire for adolescents (PAQ-A) (Annex D). In 2007, Maher et al. reviewed about 30 physical activity surveys, and they found that physical

activity questionnaire for adolescents (PAQ-A) was the best and a widely used (Maher et al. 2007). The same study selected this questionnaire on the basis of its relatively high validity and its seven days reference frame (Maher et al. 2007). PAQ-A is self-administered tool consisting of eight items which evaluate physical activity at different times during the day (e.g. lunchtime at school, physical education classes, after school, evenings, and weekends) in the preceding 7 days. Each item contains five response options, which are scored on the basis of the frequency or intensity with which it is undertaken (where 1=minimal activity, and 5=high level of activity). An overall physical activity score is calculated from the average of all item scores, with a higher overall physical activity scores indicating a greater physical activity level (Kowalski et al. 2004).

At the completion of the data collection and analyses, all participants will receive a comprehensive summary of individual results, as well as values and normal ranges for blood parameters that will be measured. Participants will be encouraged to contact the researcher for clarification of results or for answers to any questions related to the project.

3.4 PHASE TWO

The second phase of the study is randomized controlled trial. This experimental study will be conducted on 126 iron deficient and iron deficient anemic female students, aged 15-19 years, in the five governorates in the Gaza strip. The sample size divided to three parallel groups; iron supplementation group, iron supplementation with nutritional education and the last as control group without intervention.

The students and their parents will be informed of the study protocol. The students will be enrolled after fulfilling the criteria of phase one the study and getting the consent form signed from their parents. The intervention will be implemented from September 2015 to April 2016. Three assessments will be conducted: a baseline (September 2015, before the intervention), first post-intervention (January 2016, after three months), and second post-intervention which is follow up period (April 2016, after six months).

Figure (3.4) shows that the intervention part flow chart that will be carried out on subjects with ID and IDA. The first part has to do with developing a questionnaire and two parts of intervention (Iron supplementation and iron supplementation with nutritional education) will be employed.

Weekly iron supplementation (Feroous Fumarate 200 mg) will be administered for 3 months while the nutritional education will be 3 months also. The percentage of iron in ferrous fumarate 200 mg is 33%, which mean contain 66 mg elemental iron per tablet. The guidelines used by WHO of iron supplements to treat IDA recommended 60 mg iron daily. The same guidelines reported that the efficacy of once or twice/weekly supplementation in adolescents group appears promising, and the operational efficiency of intermittent dosing regimens is being evaluated (Stoltzfus et al. 1998)

The end of the intervention, outcomes like haemoglobin, MCV, CRP,ESR, MDA and ferritin levels, and nutrition knowledge will be measured to ascertain which of these interventions give a better effect to the outcomes.

After a follow up will be done all subjects 3 months after the end of the intervention exercise to check the blood and nutrition knowledge has helped to preclude ID and IDA.

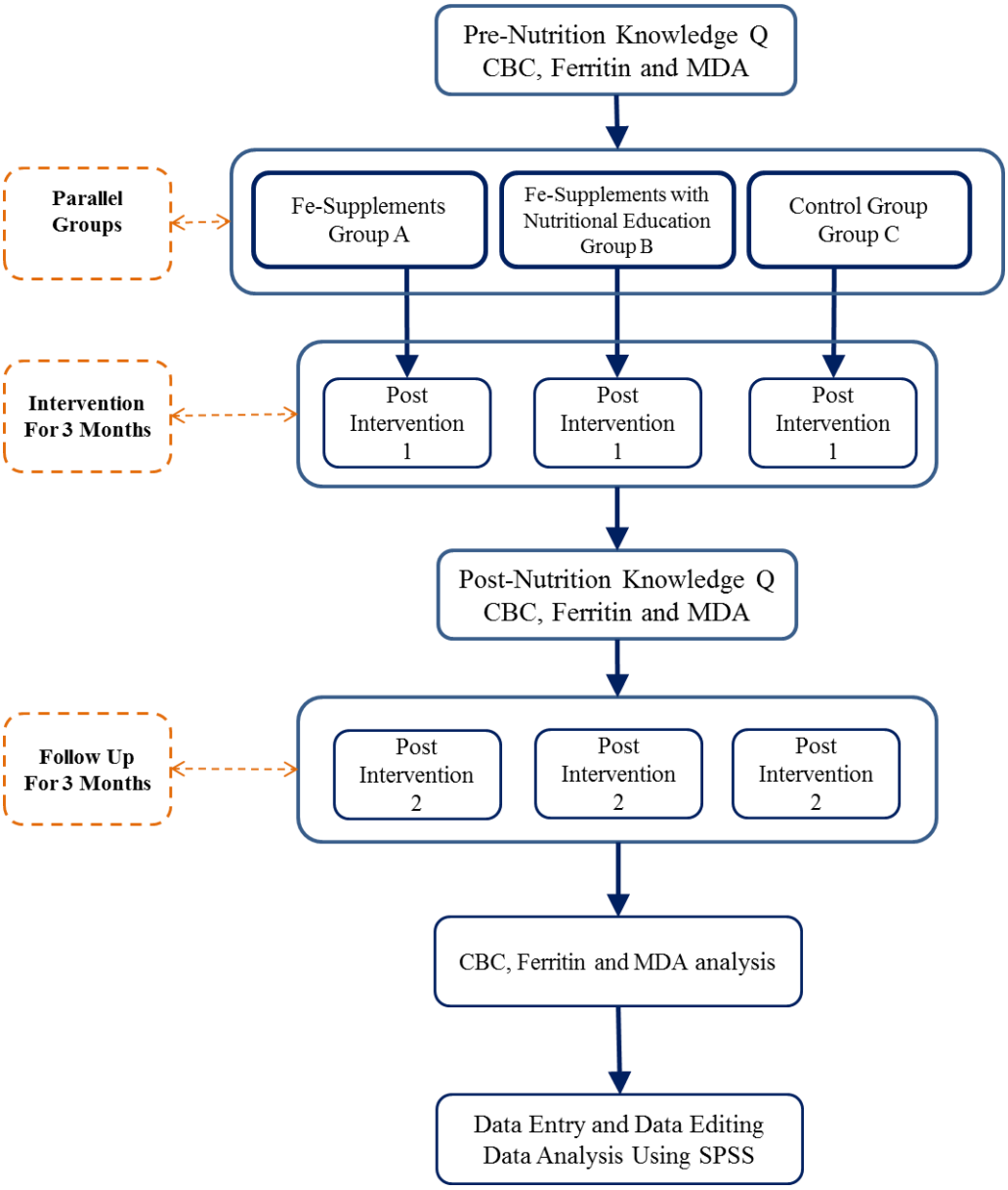


Figure 3.4: Intervention Program Flow Chart

3.4.1 Study Design

Phase two is randomized controlled trial (RCT) study. This part is the intervention part that will be divided (iron deficient and iron deficient anemic students) into 3 groups, group A for iron supplementation, group B for iron supplementation with nutritional education intervention and the last Group C as control group.

3.4.2 Study Population

The study population of phase two in the present study is iron deficient and iron deficient anemic female students who will be extracted in the phase one.

3.4.3 Eligibility Criteria

3.4.3.1 Inclusion Criteria

1. Students who diagnosed iron deficient and iron deficient anemic.
2. Students who iron deficient non-anemic, mild anemic or moderate iron deficient anemic.

3.4.3.2 Exclusion Criteria

1. Student who diagnosed anemic but have normal ferritin level.
2. Students who iron deficient severe anemic (Hb. < 7.0 g/dl).
3. White blood cells (WBC) above normal range.
4. Students who are diagnose underweight.
5. Students who have anemia either than iron deficiency anemia.
6. Students who are under medication.

3.4.4 Sample Size Calculation of Phase 2

Randomised controlled trial proposes to assess the effectiveness of iron supplementation in reducing anemia. A previous study showed that iron supplementation reduced anemia by 1.23g/dl (Yusoff et al. 2012) after 3 months with a standard deviation 1.195. The mean haemoglobin difference is 0.77 g/dl as compared to the expected. Consider a dropout rate of the participants is 10%. (Sakpal 2010)

Level of significance = 5%, Power = 80%, Type of test = two-sided

Formula of calculating sample size is

$n = [(Z_{\alpha/2} + Z_{\beta})^2 \times \{2(ó)^2\}]/ (\mu1 - \mu2)^2$ ……..……(2)

where

n = sample size required in each group,

μ1 = mean change in haemoglobin after 3 months by iron supplements = 1.23 g/dl,

μ2 = mean change in haemoglobin after 3 months by iron supplements = 2 g/dl,

μ1-μ2 = clinically significant difference = 0.77

ó = standard deviation = 1.195

$Z_{\alpha/2}$: This depends on level of significance, for 5% this is 1.96

Z_{β}: This depends on power, for 80% this is 0.84

Based on the above formula, the sample size required per group is 38. Hence, the total sample size required is 38*3 groups= 114. Considering a dropout rate of 10% total sample size required is ≈126 participants (42 in each group).

3.4.5 Study Tools and Instruments of Phase Two

Two trained nurses' assistants (interviewers) will be collected the research data questionnaires through face-to-face, grouped interviews with the participant in each group of the intervention groups; group A, group B and group C.

1. Hematological and Biochemical Assessment

One hematological (CBC) and one biochemical (serum ferritin) variables will be measured. About 6 ml of venous blood will be drawn (3 ml in ethylene diamine tetraacetic acid (EDTA) tube and 3 ml in serum tubes) will be collected from each student to perform a complete blood count (CBC) and to determine serum ferritin. Fresh juices will be provided for the participant once data collection is completed. All samples will be placed in tubes rack and packaged in an appropriate container with ice. The sample will be analysed same day in the laboratory within three hours from the time of collection.

Anemia status will be assessed by measuring hemoglobin (Hb) concentration using a Sysmex XE-2100 hematology analyzer; Calibration of the device will checked daily using control solutions provided by the manufacturer. The result will be recorded as hemoglobin in grams per deciliter, each sample will be analysed in duplicate. Anemia will be defined according to WHO guidelines as Hb < 12.0 g/dl, mild anemia as Hb 9.0-< 12.0 g/dl, moderate anemia as Hb <90 g/dL, and severe anemia as Hb < 70 g/dl.

C-reactive protein (CRP) is a sensitive marker of systemic inflammation that synthesized by the liver. CRP is a nonspecific acute-phase reactant that has traditionally used to detect acute injury infection and inflammation (Backes et al. 2004). C-reactive protein (CRP) will be measured from the serum of participants as an indicator of infection or inflammation, which can affect hemoglobin and serum ferritin concentrations, by latex-enhanced immunonephelometry on a BN II Analyzer (Dade Behring, Newark, DE). A cutoff value of > 6 mg/L will be used (Schneider 1973).

The American health association (AHA) and the center for disease control and prevention (CDC) in 2003 established the risk categories. The level of high sensitive C-reactive protein (Hs-CRP) should be less than 1.0 mg/l to reduce inflammatory risk. Average risk level (1.0 to 3.0 mg/l). A very high level of Hs-CRP (more than 10.0 mg/l) indicates an infection status, and the test should be repeated after 2 weeks of infection treatment (Pearson et al. 2003). Moreover, in stable very high level of Hs-CRP for a while, it suggests that the condition is more likely to be form of chronic rather than acute inflammation (Ishii et al. 2012).

Iron status will be assessed by measuring serum ferritin and determined by enzyme linked immunosorbent assay (ELISA) by (Human Ferritin ELISA Kit-Sigma-Aldrich-Germany) in duplicate and the average results will be recorded (Annex C).

Malonyledialdehyde (MDA) will be measured by thiobarbituric acid (TBA) method seems to be most suitable method because of its high sensitivity (Kei 1978).

2- Knowledge Questionnaire

The knowledge questionnaire will be used to evaluate the knowledge in management of iron deficiency anaemia among adolescent female (Sichert-Hellert et al. 2011). A planned teaching programme will be prepared on anemia, iron deficiency anaemia, and risk factors that causes its.

3- Nutritional Education Tools

Education materials will include lectures, mass media, brochures, and pamphlets. During nutritional education lecture, the researcher will present nutritional topics such as food groups, iron absorption enhancers and inhibitors, good sources of iron and ways to improve absorption of iron from foods in simple ways and words.

At the completion of the data collection and analyses, all participants will receive a comprehensive summary of individual results, as well as values and normal ranges for blood parameters that will be measured. Participants will be encouraged to contact the researcher for clarification of results or for answers to any questions related to the project.

3.5 MONITORING

Monitoring procedures will be implemented throughout the intervention program to ensure that the participants receive iron supplementation weekly for group A, and nutrition education sessions for group B, and record any notes receive from the participant. At the end of the intervention, compliance with iron supplementation and nutritional education will be assessed as a ratio of total days of students' attendance during the three months of the intervention period.

The monitoring tools will be as the following records:

1. Supplementation checklist; this checklist will indicate the number of the total number of iron pills intake by particular participants.

   $$\text{Compliance \% (Group A)} = \frac{\text{Total no. of iron pills intake by participants}}{\text{Total no. of supposed no. of pills}} \times 100\%$$

2. Attendance records; this sheet will be used for the subjects in the nutritional educational group B to indicate the number of session attended by a particular students.

   $$\text{Compliance \% (Group B)} = \frac{\text{Total no. of sessions attendant by participants}}{\text{Total no. of sessions delivered}} \times 100\%$$

3- Weekly health recall; The study monitoring the changes observed during the intervention, as well as side effects, such as constipation, diarrhea, nausea or vomiting, abdominal or stomach pain cramping (continuing) or soreness

3.6 CONTEXT VALIDITY INDEX

The present study questionnaire will be designed for the purpose of this study, after reviewing many studies related to the research objectives. Experts working in different related fields of the questionnaire items will evaluate the validity of the questionnaire. All of them will reviewed the questionnaire and put their comments. According to their suggestions and advice, the researcher will modify some of the questionnaire items, to become more suitable.

3.7 STUDY PROTOCOL

The field assessment will be carried out during September 2015 and April 2016 in the governorates of the Gaza strip. The researcher will collect data with two trained assistant nurses to avoid embarrassment for sensitive questions that may lead to bias results. The study group will include male and female students from 15-19 years of age attending secondary schools in the five governorates. The total sample size will be calculated according to Epi Info ™ program, 2014 version 7.1.4.0 as 320 students, with an expected frequency of 30%, a worst acceptable frequency of 25% and a confidence level of 95 % (Annex A). The sample selected will be representative of the 10^{th}, 11^{th} and 12^{th} grade students in 5 governorates (North Gaza, Gaza, Middle area, Khanyonous and Rafah governorates), using stratified single-stage probability proportional-to-size sampling within each governorate in which the class will be the primary sampling unit. The Palestinian Ministry of Education will provide a list of year 2015-2016 students in these three grades. The present study estimation on the prevalence of anemia and IDA was based on previous study data from the regional area and other data available in Palestine.

3.8 ETHICAL CONSIDERATION

1. Approval letter from Faculty of Health Science "FSK" (UKM)
2. Ethical approval from Helsinki committee (Annex F)
3. Informed consent from Student's parents (Annex E)
4. An official letter of request will be obtained from Ministry of Education
5. An official letter of request will be obtained from Ministry of Health

3.9 STATISTICAL ANALYSIS

Statistical package for social science (IBM-SPSS) version 22 will be used for analysis of data, which will be conducted as follow:

1. Review of the filled questionnaire, Coding the questions, Data entry, Data cleaning, and coding variable.
2. Descriptive statistics including frequencies, percentages, mean, median, confidence interval (CI), interquartile range (IQR) and standard deviation.

3. Chi-square distribution to determine the association between categorical variable like: parents education, parent employment, family size …etc.
4. Univariate analysis, after assumption of normality, parametric test like t-tests, one way ANOVA, and non-parametric tests like; Mann-Whitney test, Kruskal Wallis test to compare continuous variables like hemoglobin, ferritin, and IQ.
5. Bivariate analysis after assumption of normality to identify the relationships between continuous variable like; Spearman, Pearson and simple linear regression.
6. Multivariate analysis; Odd ratio OR CI determine by conditional logistic regression of association of risk factors associated with anemia and IDA.

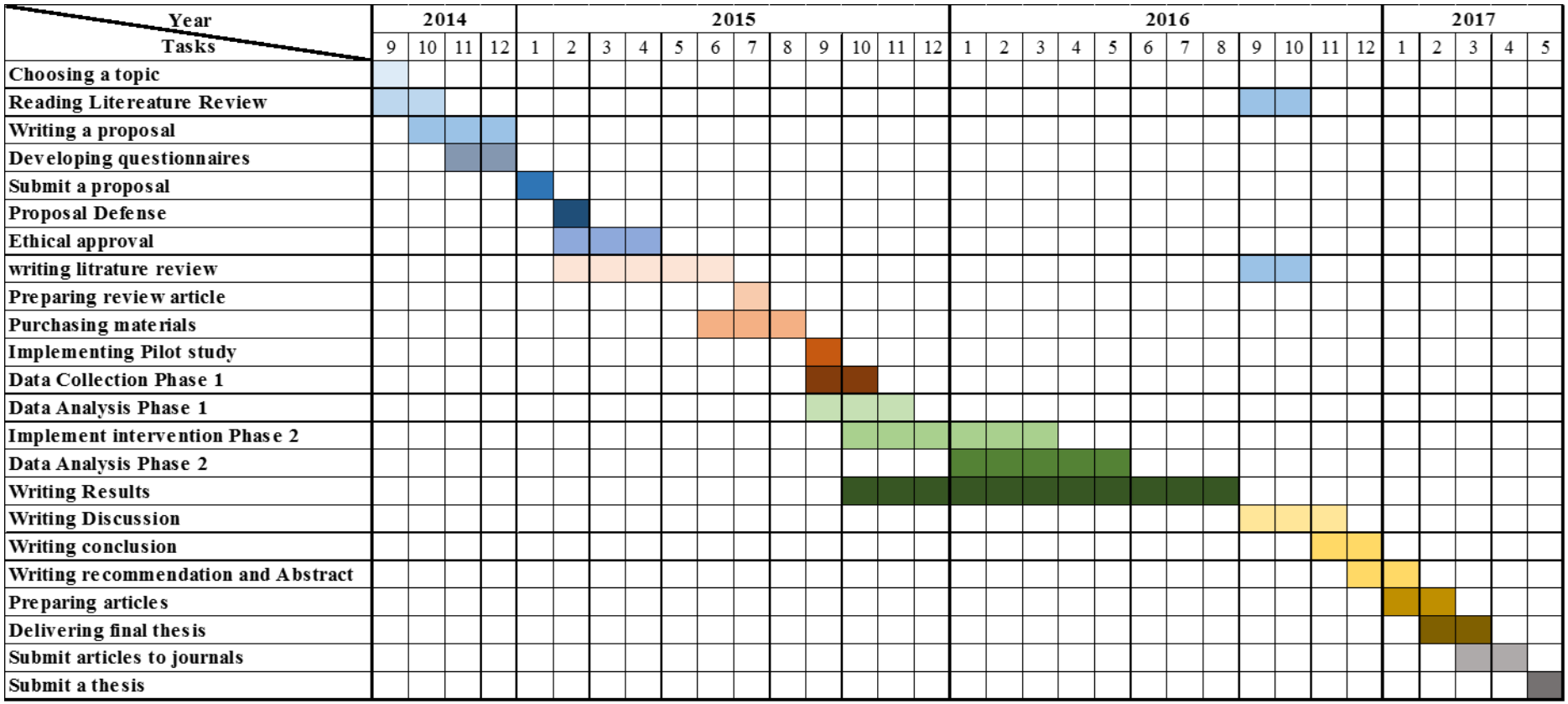
3.10 STUDY STRENGTH

The strength of this study has several strength, because this study is the first to study effects of iron supplementation and nutritional education among female adolescents in Gaza strip-Palestine. In addition, this study will use biochemical tests: Hemoglobin, MCV and serum ferritin to identify the iron deficiency anemia that is used for the intervention.

Serum ferritin testing will be tested for all study samples (n=374), which is not common in the local previous studies among the same age group in Gaza due to its high cost.

This study will determine the prevalence of anemia, iron deficiency, and iron deficiency anemia. In contrast, other studies just determined the prevalence of anemia among adolescents (Abudayya et al. 2007; Sirdah 2008; Jalambo et al. 2012)

3.11 GANTT CHART



3.12 BUDGET

Item	Unit	Expected unit Cost ($)	Amount	Expected total Cost ($)
EDTA tube	piece	0.2	700	140
Serum tube	piece	0.2	700	140
Sterile gloves	Pair	0.5	350	175
Syringe and needle.	Piece	0. 1	700	70
CBC analysis	Result	3.0	700	2100
Serum ferritin analysis	Result	8	700	6500
CRP	Result	5	400	2000
ESR	Result	1	400	400
MDA	Result	3	250	750
Posters, brochures and Pamphlet	------	-----	---	300
Container bag (Rack).	Piece	10	1	10
Cold keeping container	Piece	4	1	4
11- Alcohol 70%.	Liter	4	1	4
Distilled water	Liter	4	20	80
Cotton bag	Bag 100g	1	2	2
Paper A4, and Photocopying	Pack (500 pcs)	60	5	300
Researcher assistant (Nurse)	Nurse	200*4 months	2	1600
Transportation	Per a day	20	150 d	3000
Total				$17,575

ANNEXES

ANNEX A: SAMPLE SIZE CALCULATION BY EPI INFO SOFTWARE

Population survey or descriptive study
For simple random sampling, leave design effect and clusters equal to 1.

Population size:	82547
Expected frequency:	30 %
Confidence limits:	5 %
Design effect:	1.0
Clusters:	1

Confidence Level	Cluster Size	Total Sample
80%	138	138
90%	227	227
95%	321	321
97%	394	394
99%	554	554
99.9%	900	900
99.99%	1253	1253

ANNEX B: QUESTIONNAIRE

Name: …….			Date:		
Part I: Socioeconomic and demographic status					
1. Locality (governorate)	□ North Gaza	□ Gaza	□Middle area	□Khanyounis	□ Rafah
2. Birth date: …….					
3. Tel/Mobile:					
4. No. of member inside house:					
5. The house :	□ Owned	□ Rented			
6. Education level of mother	□ Primary	□ Preparatory	□ Secondary	□ Diploma/ University	
7. Education level of father	□ Primary	□ Preparatory	□ Secondary	□ Diploma/ University	
8. Father job:	□ Employee	□ Not employee			
Job types:	□ UNRWA	□ Governmental	□ Private	□ Farmer	
9. Mother job:	□ Employee	□ Not employee			
Job types:	□ UNRWA	□ Governmental	□ Private	□ Farmer	
10. Are the parents relatives?	□ Yes		□ No		
11.Monthly expenditure NIS :	……. (NIS)				
12. Monthly Income (NIS):	□ < 1000	□ 1001-2000	□ 2001-3000	□ > 3000	
13. Does income go with the family requirements of food?			□ Yes	□ No	
14. Does the family receive financial of food supports?			□ Yes	□ No	
15. Does the student suffer from any of these diseases? (Cardiac, Hypertension, Liver, Kidney, Thalasemia or Blood disease)			□ Yes	□ No	
16. Are present any other disease?			□ Yes	□ No	

If answer yes: mention?

Part II: Anthropometric measurements
17. Weight (KG et al.) ……..
18. Height (Cm) ……

Part III: Dietary Habits					
Food frequency	Once daily	2-3 weekly	Once weekly	once / 2 weeks	Rare /never
19. Milk					
20. Dairy products					
21. Egg					
22. Red meat(e.g. beef)					
23. White meat(e.g. chicken)					
24. Fish					
25. Liver					
26. Legumes					
27. Fruit					
28. Vegetables					
29. Beverages					
30. Natural juices					
31. Starch food					
32. Sweet /candies					
33. Tea					
34. Crackers					

35. Chips					
36. Tea drinking □ within meals □ directly after meal □ ≥2hr. after meal					
Cups volume □ small □ big Cups No. ……..					
37. Crackers □ Directly after meal □ ≥2hr. after meal					
38. How many times did you eat meal? □ one □ two □ three or more					
39. Do you skip breakfast? □ Yes □ No					
If answer yes, how many skipped? …….					
Causes of skipping? □ on a diet □ no time □ no appetite					
40. Average of academic achievement in the past semester?					
□ 50-59.9% □ 60-69.9% □ 70-79.9% □ ≥ 80%					

Part IV: Physiological status			
42. Menstruation period cycle	□ Regular		□ Irregular
43. Menstruation period	□ 3-5 days	□ 5-7 days	□ > 7 days

44. At what age did you start to menstruate periods? …….
45. When was the date of your previous period? ……...
46. If you are take any medications, can you please mention them:
……..

Part V: Biochemical test
47. Hemoglobin level …….
48. MCV……
49. Serum ferritin……
50. CRP ……
51. MDA……
52. ESR ……..

ANNEX C: FERRITIN ELISA KIT

SIGMA-ALDRICH sigma-aldrich.com

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.0 Revision Date 22.08.2014
Print Date 27.12.2014
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : FTL/Ferritin, Light Polypeptide ELISA Kit

Product Number : SE120054
Brand : Sigma
REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Israel Ltd.
3 PARK RABIN, PLAUT
76100 REHOVOT
ISRAEL

Telephone : +499728948
Fax : +499728948

1.4 Emergency telephone number

Emergency Phone # :

This is a summary MSDS for a kit, for the full MSDS for each of the components listed in section 16 please visit our website.

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.
This substance is not classified as dangerous according to Directive 67/548/EEC.

2.2 Label elements

The product does not need to be labelled in accordance with EC directives or respective national laws.

2.3 Other hazards - none

SECTION 3: Composition/information on ingredients

Refer to component MSDS

SECTION 4: First aid measures

Refer to component MSDS

SECTION 5: Firefighting measures

Refer to component MSDS

SECTION 6: Accidental release measures

Refer to component MSDS

SECTION 7: Handling and storage

7.1 Precautions for safe handling

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place. Store in cool place.

Recommended storage temperature 2 - 8 °C

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

Refer to component MSDS

SECTION 9: Physical and chemical properties

Refer to component MSDS

SECTION 10: Stability and reactivity

Refer to component MSDS

SECTION 11: Toxicological information

Refer to component MSDS

SECTION 12: Ecological information

Refer to component MSDS

SECTION 13: Disposal considerations

Refer to component MSDS

SECTION 14: Transport information

14.1 UN number

ADR/RID: 3316 IMDG: 3316 IATA: 3316

14.2 UN proper shipping name

ADR/RID: CHEMICAL KIT
IMDG: CHEMICAL KIT
IATA: Chemical kit

14.3 Transport hazard class(es)

ADR/RID: 9 IMDG: 9 IATA: 9

14.4 Packaging group

ADR/RID: II IMDG: II IATA: II

14.5 Environmental hazards

ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user

No data available

SECTION 15: Regulatory information

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

Safety, health and environmental regulations/legislation specific for the substance or mixture

No data available

Chemical Safety Assessment
For this product a chemical safety assessment was not carried out

TION 16: Other information

Kit Components:

Ferritin Standard 1, 0 ng/mL	SIGMA	SE120054B	-
Ferritin Standard 2, 10 ng/mL	SIGMA	SE120054C	-
Ferritin Standard 3, 50 ng/mL	SIGMA	SE120054D	-
Ferritin Standard 4, 150 ng/mL	SIGMA	SE120054E	-
Ferritin Standard 5, 400 ng/mL	SIGMA	SE120054F	-
Ferritin Standard 6, 800 ng/mL	SIGMA	SE120054G	-
Stop Solution	SIGMA	SESTOP3	-
TMB Substrate	SIGMA	SETMB2	-
Incubation Buffer	SIGMA	SEIB1	-
Ferritin Enzyme Conjugate	SIGMA	SE120054A	-
Wash Concentrate 20X	SIGMA	SEWASH2	-

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

ANNEX D: MEASURING OF PHYSICAL ACTIVITY

Physical Activity Questionnaire (High School)

Name:_____ Age:_____

Sex: M_____ F_____ Grade:_____

Teacher:_____

We are trying to find out about your level of physical activity from ***the last 7 days*** (in the last week). This includes sports or dance that make you sweat or make your legs feel tired, or games that make you breathe hard, like tag, skipping, running, climbing, and others.

Remember:

3. There are no right and wrong answers — this is not a test.
4. Please answer all the questions as honestly and accurately as you can — this is very important.

---

1. Physical activity in your spare time: Have you done any of the following activities in the past 7 days (last week)? If yes, how many times? (Mark only one circle per row.)

	No	1-2	3-4	5-6	7 times or more
Skipping	❍	❍	❍	❍	❍
Rowing/canoeing	❍	❍	❍	❍	❍
In-line skating	❍	❍	❍	❍	❍
Tag	❍	❍	❍	❍	❍
Walking for exercise	❍	❍	❍	❍	❍
Bicycling	❍	❍	❍	❍	❍
Jogging or running	❍	❍	❍	❍	❍
Aerobics	❍	❍	❍	❍	❍
Swimming	❍	❍	❍	❍	❍
Baseball, softball	❍	❍	❍	❍	❍
Dance	❍	❍	❍	❍	❍
Football	❍	❍	❍	❍	❍
Badminton	❍	❍	❍	❍	❍
Skateboarding	❍	❍	❍	❍	❍
Soccer	❍	❍	❍	❍	❍
Street hockey	❍	❍	❍	❍	❍
Volleyball	❍	❍	❍	❍	❍
Floor hockey	❍	❍	❍	❍	❍
Basketball	❍	❍	❍	❍	❍
Ice skating	❍	❍	❍	❍	❍
Cross-country skiing	❍	❍	❍	❍	❍
Ice hockey/ringette	❍	❍	❍	❍	❍
Other:					
_____	❍	❍	❍	❍	❍
_____	❍	❍	❍	❍	❍

2. In the last 7 days, during your physical education (PE) classes, how often were you very active (playing hard, running, jumping, throwing)? (Check one only.)

I don't do PE ..... ❍
Hardly ever ..... ❍
Sometimes ..... ❍
Quite often ..... ❍
Always ..... ❍

3. In the last 7 days, what did you normally do *at lunch* (besides eating lunch)? (Check one only.)

Sat down (talking, reading, doing schoolwork)..... ❍
Stood around or walked around ..... ❍
Ran or played a little bit ..... ❍
Ran around and played quite a bit ..... ❍
Ran and played hard most of the time ..... ❍

4. In the last 7 days, on how many days *right after school*, did you do sports, dance, or play games in which you were very active? (Check one only.)

None ..... ❍
1 time last week ..... ❍
2 or 3 times last week ..... ❍
4 times last week ..... ❍
5 times last week ..... ❍

5. In the last 7 days, on how many *evenings* did you do sports, dance, or play games in which you were very active? (Check one only.)

None ..... ❍
1 time last week ..... ❍
2 or 3 times last week ..... ❍
4 or 5 last week ..... ❍
6 or 7 times last week ..... ❍

6. *On the last weekend*, how many times did you do sports, dance, or play games in which you were very active? (Check one only.)

None ..... ❍
1 time ..... ❍
2 — 3 times ..... ❍
4 — 5 times ..... ❍
6 or more times ..... ❍

7. Which *one* of the following describes you best for the last 7 days? Read *all five* statements before deciding on the *one* answer that describes you.

F. All or most of my free time was spent doing things that involve little physical effort .....❍

G. I sometimes (1 — 2 times last week) did physical things in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics) .....❍

H. I often (3 — 4 times last week) did physical things in my free time .....❍

I. I quite often (5 — 6 times last week) did physical things in my free time .....❍

J. I very often (7 or more times last week) did physical things in my free time .....❍

8. Mark how often you did physical activity (like playing sports, games, doing dance, or any other physical activity) for each day last week.

	None	Little bit	Medium	Often	Very often
Monday	❍	❍	❍	❍	❍
Tuesday	❍	❍	❍	❍	❍
Wednesday	❍	❍	❍	❍	❍
Thursday	❍	❍	❍	❍	❍
Friday	❍	❍	❍	❍	❍
Saturday	❍	❍	❍	❍	❍
Sunday	❍	❍	❍	❍	❍

9. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)

Yes .....❍
No .....❍

If Yes, what prevented you? _____

ANNEX E: CONSENT FORM (PARENT'S RESPONDENT)

RESEARCH TITLE:

EFFECTS OF IRON SUPPLEMENTATION AND NUTRITIONAL EDUCATION AMONG ID AND IDA FEMALE ADOLESCENTS IN THE GAZA STRIP-PALESTINE

RESEARCHER: MARWAN O. A. JALAMBO

I'm.....Parent's of student ..... **Address**: .....

hereby voluntarily agree to take part in the research *(nutritional study, questionnaire study/ iron supplement and nutritional education).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent Information Sheet). I understand that my son/daughter have the right to withdraw from this research at any time without assigning any reason whatsoever. I also understand that this study is confidential and all information provided concerning my identity would remain private and confidential.

I* wish / do not wish to know the results of the tests performed on any samples taken from me.

Signature ……..……

(Parent's *Respondent*)

Date :……..

ANNEX F: HELSINKI COMMITTEE APPROVAL

المجلس الفلسطيني للبحث الصحي
Palestinian Health Research Council
تعزيز النظام الصحي الفلسطيني من خلال مأسسة استخدام المعلومات البحثية في صنع القرار
Developing the Palestinian health system through institutionalizing the use of information in decision making

Helsinki Committee
For Ethical Approval

Date: 02/02/2015 **Number:**

Name: Marwan O. A. Jalambo **الاسم: مروان عمر أحمد جلمبو**

We would like to inform you that the committee had discussed the proposal of your study about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:-

"Effect of Iron Supplementation and Nutritional Education among Iron Deficient Anemic Female Adolescents in the Gaza Strip-Palestine"

"تأثير مكملات الحديد والتثقيف التغذوي لدى المراهقات اللاتي يعانين من أنيميا عوز الحديد في قطاع غزة-فلسطين"

The committee has decided to approve the above mentioned research. Approval number PHRC/HC/3^/14 in its meeting on 02/02/2015

و قد قررت الموافقة على البحث المذكور عاليه بالرقم والتاريخ المذكوران عاليه

Signature

Member

Member

Chairman

Genral Conditions:-

١. Valid for 2 years from the date of approval.
٢. It is necessary to notify the committee of any change in the approved study protocol.
٣. The committee appreciates receiving a copy of your final research when completed.

Specific Conditions:-

The subject was approved following the World Medical Association Declaration of Helsinki-Ethical principles for medical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964 and amended by the 59th WMA General Assembly, Seoul, Korea, October 2008.

E-Mail:pal.phrc@gmail.com
Gaza - Palestine غزة - فلسطين
شارع النصر - مفترق العيون

REFERENCES

Abalkhail, B. & S. Shawky 2002. Prevalence of daily breakfast intake, iron deficiency anaemia and awareness of being anaemic among Saudi school students. *International Journal of Food Sciences and Nutrition* 53: 519-528.

Abudayya, A., M. Thoresen, Y. Abed & G. Holmboe-Ottesen 2007. Overweight, stunting, and anemia are public health problems among low socioeconomic groups in school adolescents (12-15 years) in the North Gaza Strip. *Nutrition Research* 27(12): 762-771.

Adebisi, O. Y. & G. Strayhorn 2005. Anemia in pregnancy and race in the United States: blacks at risk. *FAMILY MEDICINE-KANSAS CITY*- 37(9): 655.

Ahmed, F. & M. A. Al-Sumaie 2010. Iron deficiency and iron deficiency anemia among pregnant women in Kuwait. *The FASEB Journal*.

Akramipour, R., M. Rezaei & Z. Rahimi 2008. Prevalence of iron deficiency anemia among adolescent schoolgirls from Kermanshah, Western Iran. *Hematology* 13(6): 352-355.

Al-Quaiz, J. M. 2001. Iron deficiency anemia. A study of risk factors. *Saudi medical journal* 22(6): 490-496.

Alaofe, H., J. Zee & H. T. O'Brien 2007. Iron status of adolescent girls from two boarding schools in southern Benin. *The FASEB Journal* 21: 687.1.

Anand, T., M. Rahi, P. Sharma & G. Ingle 2014. Issues In Prevention Of Iron Deficiency Anemia In India. *Nutrition* 30: 764–770

Anand, T., M. Rahi, P. Sharma & G. K. Ingle 2014. Issues in prevention of iron deficiency anemia in India. *Nutrition* 30(7-8): 764-70.

Backes, J. M., P. A. Howard & P. M. Moriarty 2004. Role of C-reactive protein in cardiovascular disease. *Annals of Pharmacotherapy* 38(1): 110-118.

Bagchi, K. 2008. Nutrition in the eastern Mediterranean region of the World Health Organization. *East Mediterr Health J* 14: S107-S113.

Benoist, B. d., E. McLean, I. Egll & M. Cogswell. 2008. Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia Ed.: World Health Organization.

Bhargava, A., H. E. Bouis & N. S. Scrimshaw 2001. Dietary intakes and socioeconomic factors are associated with the hemoglobin concentration of Bangladeshi women. *The Journal of nutrition* 131(3): 758-764.

Bourre, J.-M. 2006. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. *Journal of Nutrition Health and Aging* 10(5): 377.

Caballero, B. 2009. Guide to nutritional supplements Ed.: Academic Press.

Charan, J., & Biswas 2013. How to Calculate Sample Size for Different Study Designs in Medical Research? *Indian Journal of Psychological Medicine* 35(2): 121–126.

Chen, X.-K., S. W. Wen, N. Fleming, K. Demissie, G. G. Rhoads & M. Walker 2007. Teenage pregnancy and adverse birth outcomes: a large population based retrospective cohort study. *International journal of epidemiology* 36(2): 368-373.

El-Sahn, F., S. Sallam, A. Mandil & O. Galal 1999. Anaemia among Egyptian adolescents: prevalence and determinants. *Eastern Mediterranean health*

journal= La revue de sante de la Mediterranee orientale= al-Majallah al-sihhiyah li-sharq al-mutawassit 6(5-6): 1017-1025.

Falahi, E., M. Rashidi, F. Ebrahimzadeh, S. Karbasi & N. Shokrollahi 2010. Effect of nutritional education on iron-deficiency anemia in high schools girls. *Journal of Shahrekord University of Medical Sciences* 12(1): 37-45.

Frith-Terhune, A. L., M. E. Cogswell, L. K. Khan, J. C. Will & U. Ramakrishnan 2000. Iron deficiency anemia: higher prevalence in Mexican American than in non-Hispanic white females in the third National Health and Nutrition Examination Survey, 1988–1994. *The American journal of clinical nutrition* 72(4): 963-968.

García-Casal, M. N., M. Landaeta-Jiménez, R. Puche, I. Leets, Z. Carvajal, E. Patiño & C. Ibarra 2011. A program of nutritional education in schools reduced the prevalence of iron deficiency in students. *Anemia* 2011.

Giskes, K., G. Turrell, C. Patterson & B. Newman 2002. Socio-economic differences in fruit and vegetable consumption among Australian adolescents and adults. *Public health nutrition* 5(05): 663-669.

Gupta, N. & G. Kochar 2009. Pervasiveness Of Anemia In Adolescent Girls Of Low Socio-Economic Group Of The District Of Kurukshetra (Haryana). *The internet journal of nutrition and wellness* 7(1).

Hermoso, M., V. Vucic, C. Vollhardt, A. Arsic, B. Roman-Viñas, I. Iglesia-Altaba, M. Gurinovic & B. Koletzko 2011. The effect of iron on cognitive development and function in infants, children and adolescents: a systematic review. *Annals of Nutrition and Metabolism* 59(2-4): 154-165.

Hettiarachchi, M., C. Liyanage, R. WickremasinghePhD, D. C. Hilmers & S. A. Abrams 2006. Prevalence and severity of micronutrient deficiency: a cross-sectional study among adolescents in Sri Lanka. *Asia Pacific journal of clinical nutrition* 15(1): 56-63.

Ishii, S., A. S. Karlamangla, M. Bote, M. R. Irwin, D. R. Jacobs Jr, H. J. Cho & T. E. Seeman 2012. Gender, obesity and repeated elevation of C-reactive protein: data from the CARDIA cohort. *PloS one* 7(4): e36062.

Jackson, R. T. & Z. Al-Mousa 2000. Iron deficiency is a more important cause of anemia than hemoglobinopathies in Kuwaiti adolescent girls. *The Journal of nutrition* 130(5): 1212-1216.

Jalambo, M. O., A. Hamad & Y. Abed 2012. Anemia and risk factors among female secondary students in the Gaza Strip. *Journal of Public Health* 21(3): 271-278.

Jalambo, M. O., A. Hamad & Y. Abed 2013. Anemia and risk factors among female secondary students in the Gaza Strip. *Journal of Public Health* 21(3): 271-278.

Kei, S. 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica chimica acta* 90(1): 37-43.

KG, N., H. JS, K. JM, A. P & W. M. 2004. Overweight children and adolescents: a risk group for iron deficiency. *Pediatrics* 114(1): 104-108.

Khader, A., H. Madi, F. Riccardo & G. Sabatinelli 2009. Anaemia among pregnant Palestinian women in the Occupied Palestinian Territory. *Public health nutrition* 12(12): 2416-2420.

Kowalski, K. C., P. R. Crocker & R. M. Donen 2004. The physical activity questionnaire for older children (PAQ-C) and adolescents (PAQ-A) manual. *College of Kinesiology, University of Saskatchewan* 87.

Kraemer, K. & M. B. Zimmermann. 2007. Nutritional Anemia Ed. Basel, Switzerland: Sight and life press

Kulkarni, M. V. & P. Durge 2011. Reproductive health morbidities among adolescent girls: Breaking the silence. *Ethno Med* 5(3): 165-168.

Kulkarni, M. V., P. Durge & N. Kasturwar 2012. Prevalence of anemia among adolescent girls in an urban slum. *Natl J Community Med* 3: 108-111.

Kurz, K. M. & R. Galloway 2000. Improving adolescent iron status before childbearing. *The Journal of nutrition* 130(2): 437S-439S.

Lecube, A., A. Carrera, E. Losada, C. Hernández, R. Simó & J. Mesa 2006. Iron deficiency in obese postmenopausal women. *Obesity* 14(10): 1724-1730.

Maher, C. A., M. T. Williams, T. Olds & A. E. Lane 2007. Physical and sedentary activity in adolescents with cerebral palsy. *Developmental Medicine & Child Neurology* 49(6): 450-457.

Marian, M. J., P. Williams-Mullen & J. M. Bowers. 2007. Integrating therapeutic and complementary nutrition Ed.: CRC Press.

Mehta, M. 1998. Effectiveness of daily and weekly iron and folic acid supplementation in anemic adolescent girls. *Bombay: United Nations Children's Fund*: 21-5.

Meier, P. R., H. J. Nickerson, K. A. Olson, R. L. Berg & J. A. Meyer 2003. Prevention of iron deficiency anemia in adolescent and adult pregnancies. *Clinical medicine & research* 1(1): 29-36.

Michael, G. J., M. M.Barrie, K. M. John & A. Lenore. 2004. Public Health Nutrition Ed. Oxford. UK: Back well Science LTD, .

Mikki, N., H. Abdul-Rahim, H. Stigum & G. Holmboe-Ottesen 2011. Anaemia prevalence and associated sociodemographic and dietary factors among Palestinian adolescents in the West Bank. *EMHJ* 17(3).

MOE. 2014. Statistics Guide for the Ministry of Education. http://www.mohe.gov.ps/ShowArticle.aspx?ID=335 [30-Nov.-2014

Olumakaiye, M., T. Atinmo & M. Olubayo-Fatiregun 2010. Food consumption patterns of Nigerian adolescents and effect on body weight. *Journal of nutrition education and behavior* 42(3): 144-151.

Pala, K. & N. Dundar 2008. Prevalence & risk factors of anaemia among women of reproductive age in Bursa, Turkey. *Indian Journal of Medical Research* 128(3): 282.

Patterson, C. 2010. Cure Your Self of Cancer from Desperation to a healthy Body, Mind and Spirit Ed. Hardy, Virginia: Paterson Press International.

PCBS. 2014. Population in Gaza strip. http://www.pcbs.gov.ps/site/lang__en/881/default.aspx#Population

Pearson, T. A., G. A. Mensah, R. W. Alexander, J. L. Anderson, R. O. Cannon, M. Criqui, Y. Y. Fadl, S. P. Fortmann, Y. Hong & G. L. Myers 2003. Markers of inflammation and cardiovascular disease application to clinical and public health practice: a statement for healthcare professionals from the centers for disease control and prevention and the American Heart Association. *Circulation* 107(3): 499-511.

Powers, J. M. & G. R. Buchanan 2014. Diagnosis and Management of Iron Deficiency Anemia. *Hematology/Oncology Clinics of North America*.

Sakpal, T. V. 2010. Sample Size Estimation in Clinical Trial. *Perspectives in Clinical Research* 1(2): 67-69.

Sanjeev, C. & D. Vasant 2008. A study of anemia among adolescent females in the urban area of Nagpur. *Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine* 33(4): 243.

Schneider, R. 1973. Developments in laboratory diagnosis. *Sickle cell disease: diagnosis, management, education and research. St Louis: Mosby*: 230-43.

Shekhawat, S., P. Gupta, M. Gupta, M. Kakkar, M. Mathur, N. Ahaluwalia & P. Pahal 2014. A STUDY OF NUTRITIONAL AND HEALTH STATUS OF ADOLESCENT GIRLS (10-19 YEARS) IN JAIPUR CITY. *Journal of Evolution of Medical and Dental Sciences* 3(16).

Sichert-Hellert, W., L. Beghin, S. De Henauw, E. Grammatikaki, L. Hallström, Y. Manios, M. I. Mesana, D. Molnár, S. Dietrich & R. Piccinelli 2011. Nutritional knowledge in European adolescents: results from the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study. *Public health nutrition* 14(12): 2083-2091.

Sim, C.-S., Y. Kim, H. Lee, C.-Y. Park, J.-O. Ham & B.-K. Lee 2014. Iron deficiency increases blood lead levels in boys and pre-menarche girls surveyed in KNHANES 2010–2011. *Environmental research* 130: 1-6.

Sirdah, M. 2008. Determinants of hemoglobin level in adolescence students at Gaza Strip, Palestine. *International Journal of Health Research* 1(4).

Soleimani, N. 2011. Relationship between anaemia, caused from the iron deficiency, and academic achievement among third Grade high school female students. *Procedia-Social and Behavioral Sciences* 29: 1877-1884.

Soleimani, N. & N. abbaszadeh 2011. Relationship Between Anaemia, Caused from the Iron Deficiency, and Academic Achievement Among Third Grade High School Female Students. *Procedia - Social and Behavioral Sciences* 29: 1877-1884.

Stoltzfus, R. J., M. L. Dreyfuss & W. H. Organization. 1998. Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia Ed.: Ilsi Press Washington^ eDC DC.

Strauss, R. S. 1999. Self-reported weight status and dieting in a cross-sectional sample of young adolescents: National Health and Nutrition Examination Survey III. *Archives of pediatrics & adolescent medicine* 153(7): 741-747.

Tee, E.-S., M. Kandiah, N. Awin, S.-M. Chong, N. Satgunasingam, L. Kamarudin, S. Milani, A. E. Dugdale & F. E. Viteri 1999. School-administered weekly iron-folate supplements improve hemoglobin and ferritin concentrations in Malaysian adolescent girls. *The American journal of clinical nutrition* 69(6): 1249-1256.

WHO 2005. Nutrition in adolescence: issues and challenges for the health sector: issues in adolescent health and development.

WHO. 2008. Worldwide prevalence of anaemia 1993-2005 : WHO global database on anaemia. / Edited by Bruno de Benoist, Erin McLean, Ines Egli and Mary Cogswell http://apps.who.int/iris/bitstream/10665/43894/1/9789241596657_eng.pdf?ua=1 [20/9/2014

WHO. 2009. Gaza Health Assessment. http://unispal.un.org/UNISPAL.NSF/0/5E423F675C1CA8A28525761C00651777

WHO 2011. IRON PREVENTION OF DEFICIENCY ANAEMIA PREVENTION OF DEFICIENCY ANAEMIA IN ADOLESCENTS IN ADOLESCENTS IRON

Yusoff, H., W. Wan Daud & Z. Ahmad 2012. Nutrition education and knowledge, attitude and hemoglobin status of Malaysian adolescents. *Southeast Asian Journal of Tropical Medicineand Public Health* 43(1): 192.