An-Najah National University Faculty of Graduate Studies

Determination of the normal level of vitamin B12 among Palestinian Adolescents (10-18 years old) in north West Bank

By Amany Yaseen

Supervised by Dr. Ayman Hussein

Submitted in Partial Fulfillment of the requirements for the Degree of Master in Public Health, Faculty of Graduate Studies, at An-Najah National University, Nablus, Palestine.

Determination of the normal level of vitamin B12 among Palestinian Adolescents (10-18 years old) in north West Bank

By

Amany Yaseen

This thesis was defended successfully on 10/8/2010 and approved by

Defense Committee members

1. Dr. Ayman Hussein (Supervisor)

2. Dr. Bashar Al-Karmi (External Examiner)

3. Dr. Adham AboTaha (Internal Examiner)

Signature

20.01

Dedication

This thesis is dedicated to my father, my beloved mother, brothers, sisters, daughters, lovely son and all my family members for his continuous encouragement throughout the course of my research.

In addition I would like to express my love and gratitude to my husband Muthanna for his endless assistance and limitless effort.

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my supervisor:

Dr. Ayman Hussein

for his continuous support, patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing my thesis. I could not have imagined a better supervisor and mentor for my Master study.

Amany Yassin

"Determination of the normal level of vitamin B12 among Palestinian Adolescents (10-18 years old) in north West Bank"

أقر بأن ما اشتملت عليه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزئ منها لم يقدم من قَبْل لنيل أية درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researchers own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name:	اسم الطالب:
Signature:	التوقيع:
Date:	التاريخ:

Abbreviations

MMA	Methylmalonic Acid					
IF	Intrinsic Factor					
MTHF	Methyltetrahydrofolate					
Нсу	Homocystiene					
BMD	Bone mineral density					
CbLD	Cobalamin Deficiency					
NTDs	Neural Tube Defects					
DNA	Deoxyribo Nucleic Acid					
dTMP	Thymidine monophosphate					
ALA	Aminolaevulinic Acid					
NADH	Nicotinamide adenine dinucleotide					
AD	Alzheimer's disease					
TC	Transcobalamin					
PCV	Packed Cell Volume					
MCV	Mean Corpuscular Volume					
CBL	Cobalamin					
BMI	Body mass index					
CVD	Cardio Vascular Disease					
THF	Tetrahydrofolate					
СоА	Coenzyme					
dTMP	Deoxyribose thymidine monophosphate					
dTDP	Deoxyribose thymidine diphosphate					
dUM	Deoxyribose uracil monophosphate					
DHF	Dihydro pholate					

List of Contents

Subject	Page No.
Dedication	III
Acknowledgement	IV
اقرار	V
Abbreviations	VI
List of contents	VII
List of tables	VIII
List of figures	VIII
List of appendices	VIII
Abstract in English	IX
Chapter One: Introduction	1
1. Introduction	2
1.1 Structure of B12 vitamin	2
1.2. Synthesis of B12 vitamin	3
1.3. Source of B12 vitamin	4
1.4. Function of B12 vitamin	4
1.5. Absorption of B12 vitamin	9
1.6. Anaemia	10
1.7. Types of Anemia	11
1.8. Vitamin B12 Deficiency	13
Chapter Two: Literature Review	16
2. Literature Review	17
2.1 Statement of the Problem	22
Chapter Three: Methodology and Procedures	23
3.1. Study area & study population	24
3.1.1. Inclusion Criteria	25
3.1.2. Exclusion Criteria	25
3.2. Data collection	26
3.3. Questionnaire	28
3.3.1. Validity	29
3.3.2. Ethical consideration	29
3.3.3. Measurement procedures	29
3.3.4. Scales	30
3.3.5. Data analysis	30
Chapter Four: Results	31
4. Results	32
Chapter Five: Discussion and recommendation	38
5.1. Discussion	39
5.2. Recommendations	40
References	42
APPENDICES	50
الملخص باللغة العربية	ب

VIII

List of Tables

No.	Table Name	Page
Table 1.	Prevalence of Vitamin B12 among different population	18
	around the World	
Table 2.	Participants distribution in Tulkarm and Qalqilia districts	24
Table 3.	Levels of Vitamin B ₁₂ , MCV, RBC and Hemoglobin among study participants	32
Table 4.	Levels of vitamin B12, Hb and MCV and their association to males students	33
Table 5.	Levels of vitamin B12, Hb and MCV and their association to females students	33
Table 6.	The frequencies of MCV (fL), according to the participants	33
Table 7.	Levels of RBC, Hb and MCV and their association to females students	34
Table 8.	mean and standard deviation of vitamin B12, MCV, Hb, RBC levels	34
Table 9.	Demographic data and it's association to Vitamin B_{12} level	35
Table 10.	Vitamin B ₁₂ level and Food Habits among participants	36
Table 11.	Vitamin B ₁₂ level and Sport Habits among participants	37

LIST OF FIGURES

No.	Figure					
Figure (1)	Figure The chemical structure of vitamin B12.					
Figure (2)	The biochemical reactions of vitamin B12 in humans. Ado vitamin B12 deoxyadenosylcobalamin; CoA, coenzyme A; THF. Tetrahydrfolate					
Figure (3)	The biochemical of vitamin B12 in human					
Figure (4)	The biochemical basis of megaloblastic anaemia caused by vitamin B12 or folate deficiency.					
Figure (5)	The absorption of dietary vitamin B12 after combination with intrinsic factor (IF) through the ileum.					
Figure (6)	Map of Palestine showing Tulkarm and Qalqilia districts.	24				

LIST OF APPENDICES

No.	Appendix	Page
Appendix A	Questionnaire in English language	51
Appendix B	Questionnaire in Arabic language	53
Appendix C	Consent form	55
Appendix D	Statistical Results	56
Appendix E	Request approval from the ministry of education to allow taking samples of blood from students	82

Determination of the normal level of vitamin B₁₂ among Palestinian Adolescents (10-18 years old) in north West Bank

By

Amany Yaseen

Supervised by Dr. Ayman Hussein

Abstract

Objectives: This study aimed to determine the normal level of vitamin B_{12} among Palestinian adolescents (10-18 years old) in north West Bank and to assess associated sociodemographic variables.

Methodology: A cross-sectional study was conducted by collecting data from 404 adolescents (10-18 years old) from regions of North West Bank. CBC as well as vitamin B12 tests were conducted alongside a designed questionnaire to obtain different demographic and other factors from participants.

Results: The data of this study showed that 43.3% (88/203) of boys and 44.8% (90/201) of girls have vitamin B12 level of \leq 200 pg/mL. However, only 10 of those have MCV level \geq 92 fl indicating that 94.4% of those with vitamin B12 level < 200 pg/mL cannot be considered having vitamin B12 deficiency. Examination of the school children under study showed that they had no symptoms associated with vitamin B12 deficiency. These results suggest that the normal vitamin B12 level among Palestinian adolescents have to be investigated.

Demographic data and food habits analysis among participants showed that there are no significant association between vitamin B12 level and the following variables (p-value> 0.05): place of residence, family income, number of family members, food habits.

Chapter one

Introduction

1. Introduction:

Vitamin B_{12} (cobalamin) is the name for a class of chemically-related compounds, all of which have vitamin activity. It is normally involved in the metabolism of every cell of the body. It is important for the normal functioning of the brain and the nervous system, and for the formation of the blood, but also fatty acid synthesis and energy production.

Hydrochloric acid in the stomach releases vitamin B_{12} from protein during digestion. Once released, vitamin B_{12} combines with a glycoprotein called Intrinsic Factor (IF) before it is absorbed into the blood stream.

Vitamin B_{12} deficiency results in serious diseases. Historically, vitamin B_{12} was discovered from it's relationship to the disease pernicious anemia.

Vitamin B_{12} is not made by plants or animals and can be synthesized only by a few species of microorganisms. The severe pernicious anemia results from the inability of individual to produce a sufficient amount of (IF), a glycoprotein essential for vitamin B_{12} absorption in the intestine. Vitamin B_{12} is synthesized by intestinal bacteria, or obtained from diet at digestion of food .The vitamin B_{12} is an essential water soluble vitamin that is commonly widespread in foods of animals origin, especially meats, fishs, lamb's liver, kidneys, eggs and cheese; but vegetables and fruits are very poor source.

1.1. Structure of B₁₂ vitamin:

vitamin B_{12} is the most chemically complex of all the vitamins. Vitamin B_{12} is a collection of cobalt and corrin ring molecule. Cobalt is bound to six

coordination sites: four by corrin ring, one die (CH₃) benzimidazole group, one variable group (X) which may be methyl hydroxyl or adenosyle.

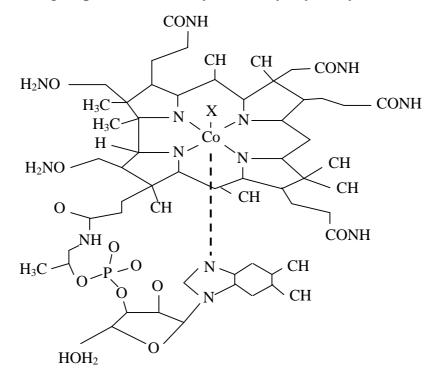


Figure 1. (The chemical structure of vitamin B₁₂)

1.2. Synthesis of B₁₂ vitamin:

All of the subtracts cobalt-corrin molecules from which vitamin B_{12} is made must be synthesized by bacteria. Vitamin B_{12} can't be made by plants or animals as only bacteria have the enzymes required for its synthesis. Species from several genera are known to synthesize vitamin B_{12} including: Aerobacter, Agrobacterium, Bacillus, Clostridum, Corynebterium, Flavobacterium, Micromonospora, Mycobacterium, Nocardia, Propionibacterium, Protaminobacter, Proteus, Pseudomonas, Rhizobaium, Salmonella, Serratia, Streptomyces, Streptococcus and Xanthomonas.

1.3. Source of B₁₂ vitamin:

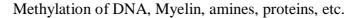
Vitamin B₁₂ is naturally found in foods of animals origin, including meat (especially liver and shellfish) and milk product⁽¹⁾. Animals, in turn must obtain it from bacteria, certain insects such as atermits contain vitamin B_{12} products by their gut bacteria. Plants only supply vitamin B_{12} to human when the soil containing vitamin B_{12} producing microorganisms has not been washed away from them. While lacto-ove vegetarians (lacto, as in lactose, which is what makes milks sweet as in lactose, ovo as in ova, the Latin word for egg, means vegetarians who consumes milk and egg), usually get enough vitamin B₁₂ through consuming dairy products. Vitamin B12 may be found to be lacking in those practicing vegan diets who don't use multivitamins supplements or eat vitamin B₁₂ fortified foods, such fortified breakfast cereals. vitamin B_{12} is available as a singly supplements or combination with other supplements. In some cases where digestive absorption is impaired, injection specify of vitamin B12 is used. Human need about one mg of vitamin B_{12} per day⁽²⁾. So it is very important to obtain at least 2-5 mg per day to ensure that the body will absorb at least one mg per day, and this is partially due to the efficiency of vitamin B_{12} absorption in the body $^{(3)}$.

1.4. Function of B₁₂ vitamin:

Vitamin B12 is normally involved in the metabolism of every cell in the body. However, many functions of vitamin B12 can be partially helped by sufficient quantities of folic acid (another B vitamin), since vitamin B_{12} is used to regenerate folate in the body. Most vitamin B12 deficient symptoms are due to folate deficiency, they include all the effects of pernicious and megaloblastic anemia, which are due to poor synthesis of

DNA, when the body doesn't have the proper supply of folic acid for the production of thymine. In humans there are two major consequences of vitamin B_{12} deficiency, Hematological and Neurological. These symptoms appear to reflect the significance of the two biochemical reactions where vitamin B_{12} is known to participate:

1- In the first reaction, methyl derivatives of vitamin B_{12} is required for the methionine synthesis reaction, which converts homocysteine to methionine. Fig2



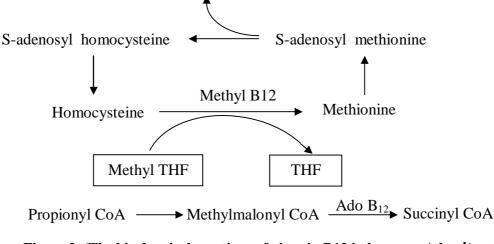
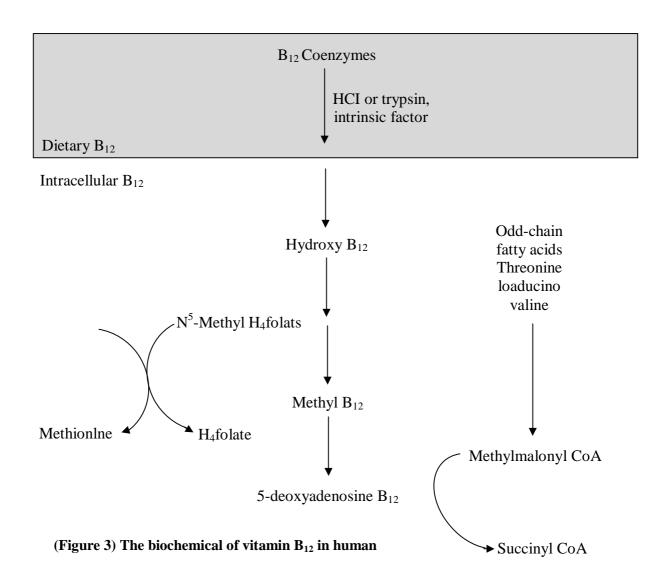


Figure 2. (The biochemical reactions of vitamin B12 in humans. Ado vitamin B_{12} deoxyadenosylcobalamin; CoA, coenzyme A; THF. Tetrahydrfolate).

2- In the second reaction, 5-deoxyadenosylcobalamin derivatives of the vitamin is required for the methylmalonyl CoA mutase reaction. (methylmalonyl CoA à succinyl CoA), a key reaction in the catabolism of some branched-chain amino acids and odd chain fatty acids. These neurological disorders seen in vitamin B₁₂ deficiency are due to progressive demyelination of the nervous tissue⁽¹⁾ Fig3.



It has been proposed that the methylmalonyl CoA that accumulated in vitamin B_{12} deficiency interferes with myelin sheath formation where methylmalonyle CoA can substitute for malonyl CoA, leading to synthesis of branched chain fatty acids, which might disrupts membrane structure⁽¹⁾. The megaloblastic anemia associated with vitamin B_{12} deficiency is thought to reflect the effect of vitamin B_{12} on folate metabolism. The vitamin B_{12} dependent methionine synthesis reaction (homocysteine + N⁵ methyl **à** THE methionine + THF) as show in figure 3.

Vitamin B_{12} appears to be the only major pathway by which N^5 methyltetrahydrofolate can return to the tetrahydrofolate. Essentially all of the folate becomes "trapped" as the N^5 methyl folate in case of vitamin B_{12} deficiency and therefore affect the cell ability to convert tetrahydrofolate to its polyglutaminated form. This also increases the requirement for folic acid because it is the polyglutaminated form of tetrahydrofolate that is retained in cells. Large amounts of supplemental folate can partially overcome the megaloblastic anemia associated with vitamin B_{12} deficiencies, but not the neurological problems⁽⁴⁾.

Vitamin B_{12} is also involved in the DNA synthesis and regulation. DNA is formed by polymerization of four deoxyribonucleoside triphosphates. Folate deficiency is thought to cause megaloplastic anaemia by inhibiting thymidylate synthesis, a rate-limiting step in DNA synthesis in which thymidine monophosphate (dTMP) is synthesized, as this reaction needs, 5,10 methylene THF polyglutamate as coenzyme (see fig. 4).

Lack of vitamin B12 prevents the demethylation of methyl THF, thus depriving cells THF and so the folate polygluttamate coenzymes⁽⁵⁾.

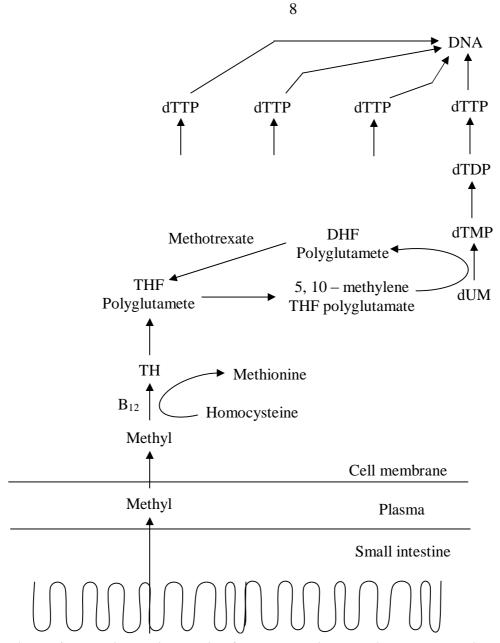


Figure 4. The biochemical basis of megaloblastic anaemia caused by vitamin B_{12} or folate deficiency. Folate is required in one of its coenzyme forms ,5,10-methylene tetrahydrofolate (THF) polyglutamate, in the synthesis of thymidine monophosphate from its precursor deoxyuridine monophosphate vitamin B_{12} is needed to convert methyl THF, which enters the cells from plasma. to THF, from which polyglutamate forms of folate are synthesized. Dietary folates are all converted to methyl THF (a monogulutamate) by the small intestine.

1.5. Absorption of B₁₂ vitamin:

Absorption of vitamin B_{12} is a complex process, subject to problems of several points. Vitamin B_{12} from animal food inters the stomach as part of animal protein and must first be liberated by pepsin and hydrochloric acid. Free vitamin B_{12} then attaches to R –protein (polypeptic binding protein which is released from the salivary cells, and the parietal cells that release hydrochloric acid. To be absorbed efficiently, vitamin B_{12} must attach to called Intrinsic Factor. This can't happen until the R-protein complexes are broken down by pancreatic enzymes. vitamin B_{12} then binds with (IF) and proceeds through the gut to the lower portion of the small intestine where the (IF) vitamin B_{12} complex attache to cell receptors, a process that involves $calcium^{(6)}$. The vitamin B_{12} IF complex remains undisturbed until the distal 30 cm of ileum, where it attaches to mucosal cell receptors (cubilin), which then binds to a second protein, amnioless which directs endocytosis of the cubilin IF- B_{12} complex in the distal ileum where B_{12} is absorbed and IF destroyed (Figure 5). The absorded vitamin B_{12} is bound to transport protein known as (TC). The transcobalamin is taken up by means of endocytosis and the cobalamin is liberated and then converted 2 forms, enzymatically into coenzyme methylcobalamin and adenosylcobalamin.

Vitamin B_{12} is absorbed into portal blood where it becomes attached to the plasma-binding protein transcubalumia which delivers vitamin B_{12} to bone marrow and other tissue. TC is thus essential plasma protein for transferring vitamin B_{12} into the cells of the body. Therefore, vitamin B_{12} deficiency can result from deficiency of any one of the following factors⁽⁴⁾:

1. pepsin.3. Intrinsic factor.5. Cell receptors.2. hydrochloric acid.4. Calcium.

- 6. R-protein.
- 7. Pancreatic enzyme.

Within the cells, enzyme liberate vitamin B_{12} from the protein complex and convert it to it's two coenzymes forms. Deficiency in the required enzyme can block this conversion.

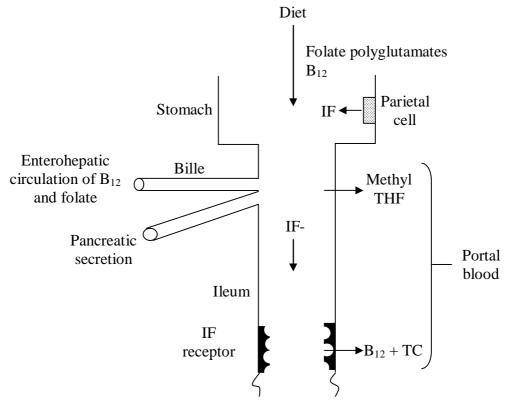


Figure 5. The absorption of dietary vitamin B_{12} after combination with intrinsic factor (IF) through the ileum. Folate absorption occurs through duodenum and jejunum after conversion of all dietary forms to methyltetrahydrofolate (methyl THF). TC , transcobalamin.

1.6. Anaemia:

It is defined as a reduction in the haemoglobin concentration of the blood. Although normal values can vary between laboratories, typical values would be less than 13 g/dl in adult male and less than 11.4 g/dl in adult female. From the age of 2 years to puberty, less than 11.0 g/dl indicate anaemia⁽⁵⁾. As newborn infants have a high haemoglobin level 14.0 g/dl is taken as the lower limit at birth⁽⁵⁾. Reduction of haemoglobin is usually accompanied by a fall in red cells count and packed cell volume (PCV) but these may be normal in some patients with subnormal haemoglobin levels (and therefore by definition anaemic)⁽⁵⁾. Alterations in total circulating plasma volume as well as the total circulating haemoglobin mass determine the haemoglobin concentration. Reduction in plasma volume (as in dehydration may mask anaemia or even cause (pseudo) polycythaemia; conversely, an increase in plasma volume (as with splenomegally or pregnancy) may cause anaemia even with normal red cell and haemoglobin mass⁽⁵⁾.

Iron deficiency is the most common cause of anaemia in every country of the world. It is the most important cause of a microcetic hypochromic anaemia, in which the two red cell indices MCV (Mean Corpusculor Volume) and MCH (Mean Corpuscular Haemoglobin) are reduced. This appearance is caused by defect in haemoglobin synthesis.

1.7. Types of Anemia:

Anaemia includes more than one type but most types related to vitamin B_{12} deficiency are:

- 1. Pernicious anemia: Which is caused by autoimmune attack on the gastric mucosa leading to atrophy of stomach. There is a chlorhydria and secretion of IF is absent or almost absent. Serum gastrin levels are raised. Helicobacter pylori infection may initiate an autoimmune gastritis which presents in younger subjects as iron deficiency and in the elderly as pernicious anemia.
- **2.** Megaloblastic anemia: This is a group of conditions in which the erthyroblasts in the bone marrow show characteristics abnormality –

maturation of the nucleus being delayed relative to that of the cytoplasm. The underlying defect accounting for the asynchronous maturation of the nucleus is defective DNA synthesis and in clinical practice, this is usually caused by deficiency of vitamin B12 or folate. Less commonly, abnormality of metabolism of these vitamins or these lesions in DNA synthesis may cause an identical hematological appearance. Dietary and metabolic aspects of two vitamins are reviewed before considering the anemia.

Causes of megaloblastic anaemia:

- Vitamin B₁₂ deficiency.
- Folate deficiency.
- Abnormality of vitamin B12 or folate metabolism.
- Other defects of DNA synthesis⁽¹⁾.

Other types of anemia:

- 3. Sideroblastic anaemia: This a refractory anemia with hypotchromic cells in the peripheral blood and increased marrow iron, it is defined by the presence of many pathological ring sideroblastic in the bone marrow.
- Malignancy (anemia of chronic inflammation): Hypochromic anemia includes lack of iron (iron deficiency) or of iron release from macrophases to serum.
- 5. Thalasaemia (α or β): Microcytic anemia include failure of globin synthesis.
- 6. Methaemoglobinaemia: This is a cynical state in which circulating haemoglobin is presented with iron in the oxidized (Fe^{+2}) instead of

usual FE^{+2} state. It may arise because of hereditary deficiency of reduced nicotinamide adenine dinucleotide. (NADH).

7. Polycythaemia: Occurs when the red blood cells give up O_2 less rapidly than normal.

1.8. Vitamin B₁₂ Deficiency:

Vitamin B_{12} deficiency can potentially cause severe and reversible damage, especially to the brain and nervous system, including fatigue and poor memory. Folate and vitamin B_{12} deficiency may play a role in the alterations in gene expression and increased DNA damage⁽³⁰⁾. The relationship between folate and vitamin B_{12} may provide insight carcinogenesis since both vitamins are involved in the synthesis, repair and methylation of DNA⁽⁸⁾. Studies have documented an inverse relationship between optimal intake of folate and vitamin B_{12} with several types of cancers⁽³¹⁾. Hyperhomocysteinemia due to folate and vitamin B_{12} deficiency may also cause depression⁽³²⁾, dementia and Alzheimer's disease⁽³³⁾.

Vitamin B_{12} deficiency has the following pathomorphology and clinical symptoms:

- 1. Pathomorphology: Spongiform state of neural tissue along with edema of fibers and deficiency of tissue. The myelin sheath decays along with the axial fibers. In later phase fibric sclerosis of nervous tissues occurs. Those changes apply to dorsal parts of the spinal cord and to pyridal track in lateral cord.
- 2. Clinical symptoms: vitamin B_{12} deficiency results in a serious disease. The severe disease in pernicious (that is "deadly") anaemia which occurs when a person lacks the (IF) and can't absorb vitamin

 B_{12} . The most common cause of pernicious anaemia is and autoimmune reaction that attacks and destroys the stomach cells that produce the (IF). The disease is characterized by:

- 1. immature abnormality large red blood cell (macrocytes) which are very inefficient at carrying oxygen.
- 2. white blood cells with abnormal nuclei.

It is characterized by atriad of symptoms:

- 1. Anemia with bone marrow promegaloblastosis (megaloblastic anaemia).
- 2. Gastrointestinal symptoms.
- 3. Neurological symptoms.

Each of these symptoms can occur either alone or along with others:

The neurological complications, consists of the following symptoms:

- 1. Impaired perception of deep touch, pressure and vibration, abolishment of sense of touch, very annoying and persistent pareshesias.
- 2. Ataxia of dorsal cord type.
- 3. Decrease of abolishment of deep muscle tendon reflexes.
- 4. Pathological reflexes, also severe paresis.

During the course of disease, mental disorder can occur which include: irritability, focus, concentration problems, depressive state with suicidal tendencies, and paraphrenia complex. These symptoms may not reverse after correction of hematological abnormalities and the chance of complete reversal, decrease with the length of the chance of complete reversal, decrease with the length of the time the neurological symptoms have been present.

Chapter two

Literature Review

2. Literature Review:

Vitamin B_{12} deficiency is more wide spread in several population than has been assumed so far⁽⁸⁾. Among the population groups at risk are older people, pregnant women and patients with renal or intestinal disease⁽⁸⁾. Prevalence of vitamin B_{12} deficiency in the general population has not been well established because a universally accepted normal vitamin B_{12} level has not been defined⁽⁹⁾.

The incidence of deficiency of this vitamin appears to increase with age.⁽¹⁰⁾

Even though there is a high prevalence of vitamin B_{12} deficiency among the elderly, it appears also in children, much of these deficiency in this vitamin is subclinical.

Many studies were performed in many countries of the world, these studies have shown that lack of vitamin B_{12} is associated with elderly especially over the age of fourty, and it was due mostly to number of reasons, the mostly important one is mal absorption⁽¹⁴⁾.

Epidemiological studies show a prevalence of Cobalamin deficiency of around 20% in the general population of industrial countries⁽¹⁵⁾. Table 1 summarizes the prevalence of vitamin B_{12} vitamin deficiency from a number of countries. The Framingham study demonstrated a prevalence of 12% among elderly people living in the community⁽¹⁰⁾. The cut off point for the vitamin B_{12} level seems to be different from one country to another. For example, the cutoff point in Canada to vitamin B_{12} level is less than 150 pmole/L⁽¹⁵⁾. In USA, the cutoff point is reported to be < 258 Pmol / L⁽¹⁰⁾. However, a high cutoff (500 Pg/ml) was suggested in Japan⁽¹⁹⁾.

			the w	UTTU .		
Country, Reference	Age (Year, mean or range)	n	Sex	Cutoff point	Vitamin B ₁₂ in Deficiency % (range or mean)	Comments
Canada, Green et al, 1998 ⁽³⁴⁾	16-19 y	105	F	< 148 Pmol / L	3.9%	
Canada, Gupta et al, $2004^{(35)}$	18-84y	988	MF	< 132 Pmol / L	22-46%	44% men, South Asians
Israel, Figlin et al, 2003 ⁽³⁶⁾	> 69y	749	MF	< 221 Pmol / L	37%	Folic acid, Hcy, and MMA measured
Jordan, For a and Mohammad, 2005 ⁽³⁷⁾	19-50y	216	MF	< 222 Pg / L	48.1%	Folic acid measured
Spain, Planells et al, 2003 ⁽³⁸⁾	25-60y	3528	MF	< 200 Pg / L	11%	B6, folic measured, Mediterranean population
UK, Eastley et al, $2000^{(39)}$	75.6	1432	MF	< 210 Pg / L	88%	Elderly subjects
UK, Starr et al, 2004 ⁽⁴⁰⁾	11-79y	470	MF	< 200 ng / L	25%	Folic acid measured
US, Hao et al, 2007 ⁽⁴¹⁾	35-64y	2407	MF	< 185 Pmol / L	59%	Chinese adults
US, McLean et al, 2007 ⁽⁴²⁾	5-14y	503	MF	< 148 Pmol / L	40%	Kenyan Children
US, Tucker et al, 2000 ⁽⁴³⁾	65-83y	2999	MF	< 148 Pmol / L	9%	

 Table 1: Prevalence of vitamin B₁₂ among different population around the World

Economic status, age, and dietary choices, can influence the occurrence of vitamin B_{12} deficiency⁽¹⁶⁾. Generally when anemia is present, Vitamin B_{12} levels are measured, but one of the evidence suggests that while symptoms of vitamin B_{12} deficiency might be subtle, it can still cause metabolic and neurologic abnormalities such as hyper homocysteinemia, cognitive function decline or depression⁽¹⁶⁾.

In Spain in October 2009 Fernadez, Monzon – et. aL, found in their study that anemia and Vitamin B_{12} deficiency are frequent finding in most diseases which cause mal-absorption⁽¹⁷⁾.

In Amman city, capital of Jordan, Mahmoud Abu-Samak et al⁽²⁷⁾. found in his research that was made among Jordanian youth (aged 18-24 years) from Amman city. This study have shown correlation between body mass index (BMI) and serum vitamin B12 levels, of an students (120 males), 16% had vitamin B₁₂ deficiency (< 200 pg / ml) and 65% of them were overweight. This present finding suggest that over weight youth are a risk group for vitamin B₁₂ deficiency.

There are several studies that have shown relationship between Vitamin B_{12} deficiency and many other diseases, like depression, dementia, neuropathy, anemia, mental impairment cancer and $CVD^{(13)}$. Because of the possible health benefits of folate and Vitamin B_{12} in preventing these diseases⁽¹⁸⁾, it is important to determine the dietary intakes and serum levels of these vitamins in young adults⁽¹³⁾.

The prevalence of vitamin B_{12} deficiency in the general population has not been well established because universally accepted normal vitamin B_{12} has not been defined⁽²⁶⁾. People in the United Kingdom had lower mean vitamin B12 levels than other European countries (357 pmol/L)⁽⁴⁵⁾. In Asia, Indians had lower vitamin B_{12} levels than Chinese or Malayans⁽²⁸⁾. All Indians had lower limits than other European countries⁽²⁸⁾. In contrast, the lower limit of normal vitamin B_{12} in Japan is 500-550 pg/ml and this may explain the lower rates of Al-Zahimer's dementia in that country⁽¹⁹⁾.

A study carried out by Incercick et.al⁽¹⁸⁾. showed that at an advanced stage of the lack of vitamin B_{12} , the presence of oral signs and symptoms including glossitis, recurrent oral ulcer, oral candidacies appearance are associated. In Denmark there was a study done by Roed – et. al⁽²⁰⁾. on two children suffered from anemia and delayed development, due to vitamin B_{12} deficiency caused by strict maternal vegan diet during pregnancy⁽²⁰⁾. This study has shown a strong relationship between vegetarian women and the lack of vitamin B_{12} in their children⁽²⁰⁾.

Bone fractures and vitamin B_{12} deficiency are common in vegetarian⁽²¹⁾. In Germany; Herman and others⁽²²⁾ found a strong relationship between low level of vitamin B_{12} and bone fractures.

Large numbers of studies in different populations were conducted to assess the level and severity of association between vitamin B_{12} deficiency and depressive disorders as BMD (Bone Mineral Density) which was conducted in older American, this study revealed that Hcy and vitamin B_{12} status indicators are associated with BMD⁽²³⁾ and multiple sclerosis, this was the conclusion of a study made in London⁽²⁴⁾.

Other study in Turkey showed that there was an association between vitamin B_{12} deficiency and eye movement disorder⁽²⁵⁾.

Several studies were conducted in Japan (which has a lower limit of normal vitamin B_{12} is 500-550 pg/ml), one study found that vitamin B_{12} deficiency can develop one year after total gastrectomy and causes the expected symptoms. Because vitamin B_{12} treatment increases serum vitamin B_{12} concentration and leads to rapid solution of the symptoms, it should be prescribed routinely to patients undergoing total gastrectomy⁽³⁾.

2.1. Statement of the Problem:

The normal blood level of vitamin B_{12} ranges between 200 and 600 Pg/ml (148-442 pmol/L)⁽⁴⁶⁾.

Unfortunately, there are no published studies about vitamin B_{12} status in Palestine which suffers from difficult economic situation. It is very vital from a community health point of view to know the prevalence of vitamin B_{12} deficiency in our population, especially among adolescents, and young individual (10-18) years old because in this age they overcome the childhood age and begin the age of maturity. In this age which is more critical for growing for both males and females and menstrual cycle in females, so they must be aware of vitamin B_{12} deficiency that causes severe diseases especially anaemia.

Knowing the prevalence of vitamin B_{12} deficiency in this early age, we can develop a program to treat deficient individual by giving them the special medication protocols. By this way, the vitamin B12 prevention cost is much lower than complication treatment.

In this study based on the above assumption.

The aims of the study are:

1. To find the normal level of vitamin B_{12} among Palestinian adolescents.

2. To find out the prevalence of vitamin B_{12} level among these adolescents in two districts of north West Bank (Tulkarm and Qalqilia.

Chapter Three

Methodology and Procedures

3.1. Study area & study population:

This cross-sectional study has been done between September 2008 & June 2009.The study has been done in Tulkarm and Qalqilia districts of West Bank, Palestine (figure 6). Tulkarm & Qalqilia represent 7.1% (2.8% represented Qalqilia, 4.3% represented Tulkarm) of the total population of West Bank⁽²⁹⁾. The number of schools in Tulkarm district is 137 and the number of schools in Qalqilia district is 78.



Figure 6. (Map of Palestine showing Tulkarm and Qalqilia

districts.)

The number of students in Tulkarm and Qalqilia districts are 23,205 and 14,821 respectively⁽²¹⁾.

Table 1: summarizes the sample distribution that has been selected randomly. The type of the sample is a stratified. The sample size (404) is divided into two strata from both districts based on the total number of population in each district.

Tuble2. Tuttelpunds distribution in Tunkurin und Quiquid districts						
Governorate	Gender	10-12	12 > -14	14>-16	16 > -18	Total
	Utilidei	Years	Years	Years	Years	
Tulkarm	Male	36	32	32	32	132
	Female	32	32	32	32	128
Qalqilia	Male	18	18	18	18	72
	Female	18	18	18	18	72

Table2: Participants distribution in Tulkarm and Oalgilia districts

The sample size was determined using RaoSoft Sample Size Calculator, according to the following equation:

$$n = \frac{1.96^2 \times (1-p)}{p \times e^2}$$

Where 1.96^2 is statistical parameter corresponding to the confidence level of 95%.

P: is the expected prevalence: 0.35

 ϵ : relative precision = 0.20

According to the above equation, the sample should be 404 students. The researcher increased the sample size up to 410. Six cases were eliminated due to inaccurate measures, so the sample size therefore is 404.

3.1.1. Inclusion Criteria:

Students aged from (10-18) years old male and female, who reside in the area for at least the past three years.

3.1.2. Exclusion Criteria:

Students who suffer from any chronic disease, and are not on any medication that affect their hematological status including vitamin B_{12} treatment or iron supplementation.

3.2. Data collection:

Data about hematological parameter (e.g vitamin B_{12} & CBC) were collected from participants a long side a questionnaire that has been performed by interviewing the respondent.

To control bias, all the blood tests were performed 24 hours after sample collection by the same technician. The steps that followed by the medical staff to collect the samples from participants were summarized as follows:

- 1. About 5 ml of blood samples were withdrawn from the students who have enrolled in the study.
- 2. About 4 ml of blood samples were put in plain tubes.
- 3. About 1 cm of blood samples were put in pediatric EDTA tubes and analyzed directly for CBC parameter.
- 4. Plain tubes samples were centrifuged to insure that serum was separated.
- Separated serum, then placed in refrigerator for 24 hours before measuring vitamin B12 level using Tosso 600 11Hormone machine, (Shiba – Koen First Bldg, Minato – Ku, Tokyo, Japan).

Principle and country Blood cells (CBC) Couller Actd:

it is detecting principle (Resistance detection) as follows:

Cells are diluted in electrically conductive diluents large differences between conductivity or resistance of cells and diluents. Blood cells in diluents are aspirated through a transducer aperture. Inside outside. The transducer there are electrodes with constant D.C electric current flowing from internal to external electrode.

When a blood cell passes through the aperture electrical resistance increase between electrodes this change the voltage between electrodes which is proportional to the resistance change.

The volume of the cell passing through the aperture is proportional to this voltage change.

Voltage change are amplified, wave shaped, sent to the Temperature compensations circuit.

Principle of AIA – 600 II Tosoh:

The AIA-600 II is capable of performing three methods of immunoassay: an immunoenzymatic (IEMA) or Saud which immunoassay a competitive binding (EIA) immunoassay and a two – step immunoenzymatic immunoassay.

An Antigen – Anti body reaction begins by combining a patient sample, control calibrator with a diluents in on immunoreactions test cup from the AIA-Pack neaget pack reagat service In the IEMA assay during the incubation period the Abs attach to two distinct epitopes on the Ag being measured forming a sandwich.

In the EIA assay during incubation, Ag in the patient sample competes with enzyme labeled Ag for a limited number of Ab binding sites.

In all methods, specimens are incubated at 37 o with Ab bound to the surface of magnetic beads separation of the bound Ab from the free Ab is achieved by washing the beads with a wash solution that removes any unbound conjugates.

After washing, a substance, 4 methylumbelliferyl phosphate (4 - nup) is added to the test cup. Magnetic beads is then measured using fluorescent rate method.

All samples were performed along with control samples. Our control samples included normal control, and abnormal- pathological control samples. Control samples were run on a daily basis. External quality control was performed monthly in co operation with (Almarkaz Alsihi) center.

3.3. Questionnaire:

Demographic data about participants such as (gender, place of residence, sport habits, food type, food supplements, parental education, income ..etc).

In addition to CBC and vitamin B12 tests, the researcher designed a questionnaire to collect personal data about the participants. (Appendix 2)

3.3.1. Validity:

To ensure that the contents of the questionnaire were valid, a pilot study was conducted by distributing the questionnaire, to 20 people (2 teachers, 2 parents, 16 students). comments from participants were taken.

3.3.2. Ethical consideration:

All participants were asked to sign consent form agreement to declare their acceptance to take part in this study. (Appendix 1)

All participants were informed about the results of their vitamin B12 status and CBC results along with advice for further follow up if needed.

3.3.3. Measurement procedures:

The researcher followed certain procedures to achieve the goals of the study:

- Preparing the study instruments.
- Taking Ministry of Education and parent's approval, to conduct the required tests.
- Taking CBC and vitamin B₁₂ samples from the participants, with a supervision of a qualified team.

- Data entry to SPSS, to analyze data.
- Writing results and suggest recommendations.

3.3.4. Scales:

The researcher depends on international scales, to classify the results of vitamin B_{12} and CBC. The classification was as follows ⁽⁵⁾:

Variables (unit)	Normal level	Ref.
B12 (pg/mL)	> 200	44
Hg (g/dL)	Male ≥ 12	5
	Female ≥ 11.5	5
MCV (f L)	≤ 92	5
RBC ($*10^{6}$ / ul)	≥ 3500	5

3.3.5. Data analysis:

Data were analyzed using the statistical package for the social sciences (SPSS). Frequency, percentage and graphs were used to describe data. Chi-square test (χ^2) was used to examine relations between different variables.

Chapter Four

Results

4. Results:

The results of this study showed that 43.3% (88/203) of boys and 44.8% (90/201) of girls have vitamin B_{12} level less than 200 pmole/L based on the classification of the WHO (Table 3). Other hematological parameters are summarized in the table. For example, 17.2% (35/203) of boys and 18.9% (38/201) of girls have anemia.

	Male		Fe	Female To		Total		Total		
	Ν	%	Ν	%	Ν	%	value			
B ₁₂ level										
< 200 (pg/ml)	88	43.3	90	44.8	178	44.1	0.850			
$\geq 200 \text{ (pg/ml)}$	115	56.7	111	5.2	226	55.9				
MCV level (fl)							0.438			
< 92	199	98.03	195	97.02	394	97.52				
≥ 92	4	1.97	6	2.98	10	2.48				
RBC level										
< 3.5 (x10^6/ul)	0	0%	1	0.5%	1	0.2%	0.996			
\geq 3.5 (x10^6/ul)	203	100%	200	99.5%	403	99.8%				
Hgb level										
< 12 (g/dl)	35	17.2	38	18.9	73	18.1	0.760			
\geq 12 (g/dl)	168	82.8	163	81.1	331	81.9				

Table 3: Levels of vitamin B12, MCV, RBC and hemoglobin among
study participants

* Statically significant at ($\alpha = 0.05$)

Tables 3 and 4 summarize the levels of vitamin B12, hemoglobin and MCV among boys and girls involved in this study, respectively. Of the 88 boys having vitamin B12 level (<200 pmole/L), only 17 (19.3%) had Hb level (<12 g/L). However, 10 of them had MCV level >92 fl (Table 2 & 3). Of the 90 girls having Vitamin B12 level <200 pmol/L 6 (1.5%) of them had MCV level> 92 fl. And only 4 school boys (1%) had MCV above 92 fl. Therefore 10 of the 404 participants (2.5%) have actually vitamin B12 deficiency when both parameters vitamin B12 and MCV taken into consideration. Table 5 shows the number of school children with various MCV levels. There are 290 (71.1%) students with MCV level between 80 and 92.

B ₁₂	Hg (g / dl)			MCV (fl)				
(pg/ml)					<u> 92</u>	>92		
< 200	No).	%	No.	%	No.	%	
	<12	17	19.3%	17	19.8%	0	0	
	>12	71	80.7%	69	80.2%	4	0.02	
Total		88		86				
≥ 200	<12	18	15.6%	18	15.9%	0	0	
	>12	97	84.4%	95	84.1%	0	0	

Table 4: Levels of vitamin B12, Hb and MCV and their association to males students

Table 5: Levels of vitamin B12, Hg and MCV and their association tofemales students

B12	Hg (g / dl)			MCV (fl)					
(pg/ml)				<	≤ 92		2		
	No	•	%	No.	%	No.	%		
< 200	<11.5	12	13.33%	12	13.6%	0	0		
	>11.5	78	86.67%	76	86.4%	6	0.03		
Total		90		88					
<u>> 200</u>	<11.5	6	5.41%	12	11.7%	0	0		
	>11.5	105	94.59%	91	88.3%	0	0		

Table 6: The frequencies of MCV (fL), according to the participants

	Sex of participants							
	Schoo	ol girls	Schoo	ol boys				
MCV level	Number	%	Number	%				
<80	40	19.9	59	29.06				
80-91	155	77.12	140	68.97				
<u>> 92</u>	6	2.98	4	1.97				
Total	201	100	203	100				

		Age (years)				
Data	10-	10-12		> 12		
	Number	%	Number	%		
MCV level						
< 92 (fl)	39	27.1	60	23.1	0.438	
\geq 92 (fl)	105	72.9	200	76.9		
RBC level						
< 3.5 (x10^6/ul)	1	0.7	0	0	0.764	
\geq 3.5 (x10^6/ul)	143	99.3	260	100.0		
Hg level						
< 12 (g/dl)	35	24.3	38	14.6	0.022*	
\geq 12 (g/dl)	109	75.7	222	85.4		

Table 7: Levels of RBC, Hb and MCV and their association to females students

Table 8: mean and standard deviation of vitamin B₁₂, MCV, Hb, RBC levels

levels							
Mean <u>+ 25</u> D	Min-Max						
223 <u>+</u> 218	54 - 605						
82 <u>+</u> 13.2	58 - 98						
13.47 <u>+</u> 11.8	6 - 18						
4.5 <u>+</u> 0.8	4 - 5						
	Mean <u>+ 25 D</u> 223 <u>+</u> 218 82 <u>+</u> 13.2 13.47 <u>+</u> 11.8						

Table 9: shows the association between vitamin B12 level anddemographic data.

Demographic data	< 200 Pg / ml		≥ 200 Pg / ml		P-Value	
Demographic data	Number	%	Number	%	I - value	
Place of residence						
Tulkarem	121	45.38	146	54.6	0.545	
Qalqilia	57	41.6	80	58.4		
Age						
10-12	<u>66</u>	45.8	78	54.2	0.667	
> 12	112	43.1	148	56.9		
Mother education						
Low educated	58	32.6	73	32.3	0.952	
High educated	120	67.4	153	67.6		
Father education						
Low educated	42	23.6	52	23.0	0.984	
High educated	136	76.4	174	77.0		
Income						
≤ 1000 NIS	10	5.60	16	7.10	0.696	
> 1000 NIS	168	94.4	210	92.9		
Number of brothers						
< 3	92	51.7	119	52.7	0.926	
\geq 3	86	48.3	107	47.3		
Number of sisters						
< 3	80	44.9	116	51.3	0.240	
≥ 3	98	55.1	110	48.7		

Table 9: Demographic data and it's association to Vitamin B₁₂ level

* Statically significant at ($\alpha = 0.05$)

Note: low educated means education less than Tawjihi (General Secondary certificate).

Results showed that there is no significant association between vitamin B_{12} level and where students live or whether their parents have low or high education. Income or number of brothers or sisters are also have no significant association to vitamin B_{12} level where p-value is > 0.05 (Table 8).

The food type (animal or plant source) has been also analyzed as shown in table 5. Similarly, no significant association has been found between level of vitamin B12 and food habits shown in table 5 where p. values are > 0.05.

	< 200	units	≥ 200	units		
Food Habits	(p m	ol / L)	(p mo	ol / L)	P-Value	
	Ν	%	Ν	%		
Food type						
Plant food	9	5.10	9	4.00	0.634	
Animal food	2	1.10	1	0.40	0.034	
Plant and animal	167	93.8	216	95.6		
Plant food type						
Vegetables only	4	2.20	4	1.80	0.668	
Fruits only	2	1.10	1	0.40	0.008	
Vegetables and fruits	172	96.7	221	97.8		
Vegetables type						
Cooked	16	9.00	9	4.00	0.102	
Uncooked	7	3.90	12	5.30	0.102	
Cooked and uncooked	155	87.1	205	90.7		
Fruits type						
Fresh	126	70.8	144	63.7	0.164	
Canned	52	29.2	82	36.3		
Animal food type						
Fish	89	50.0	134	59.3	0.099	
Birds	78	43.8	85	37.6	0.099	
Others	11	6.20	7	3.10		
Food complements						
No	167	93.8	219	96.9		
Iron bills	2	1.10	3	1.30	0.140	
Vitamins	6	3.40	1	0.50		
Fish oil	3	1.70	3	1.30		

Table 10: Vitamin B12 level and Food Habits among participants

* Statically significant at ($\alpha = 0.05$)

The sport habits of students has been also investigated. There is no association between vitamin B_{12} and Sport Habits (table 10).

	< 200) units	≥ 200	units	
Sport Habits	(p mol / L)		(p m	ol / L)	P-Value
	Ν	%	Ν	%	
Sport activities practicing					
yes	157	88.2	204	90.3	0.613
No	21	11.8	22	9.70	
Sport type					
Running	54	30.3	74	32.7	
Fitness	28	15.7	26	11.5	
Swimming	10	5.60	18	8.00	0.756
Weight Lifting	2	1.10	2	0.90	
Football	60	33.7	72	31.9	
Others	24	13.5	34	15.0	
Sport time					
Daily	51	28.7	61	27.0	
Once a week					0.024
Twice a week	45	25.3	54	23.9	0.934
Undetermined	18	10.1	26	11.5	
	64	36.0	85	37.6	

 Table 11: Vitamin B₁₂ level and Sport Habits among participants

* Statically significant at ($\alpha = 0.05$)

Chapter Five

Discussion and Recommendation

5.1. Discussion:

The world health organization has determined the lowest level of vitamin B_{12} to be < 150-221 pmole/L (200-300 pg/ml)⁽⁴⁴⁾. The cut-off 150 pmol/L represents the serum concentration below which clinical symptoms of deficiency (e.g. neurological, cognitive and hematological) start to appear. Below 221 pmol/L biochemical signs of inadequacy start to occur, including elevated MMA and homocysteine⁽⁴⁴⁾. However, the average level of the vitamin varies from country to another ^(15, 10, 3). For example Canada was reported to have the lowest vitamin B12 level in the World ⁽¹⁵⁾. In contrast United Kingdom has the highest vitamin B12 level among its European countries. But in our study we use vitamin B12 level of 200 pmol/L as our lower normal level. This level was adopted from the reagent in use.

The results of this study show that nearly 40% of school students involved in this work have vitamin B_{12} level below the WHO recommended levels. However, other parameters such Hb level and MCV level do not support that these students have vitamin B_{12} deficiency since only 10 (2.5%) of them had MCV level >92 fl. Additionally, there was no symptoms associated with vitamin B_{12} deficiency on any of the participants. Thus, our conclusion would be that the normal level of vitamin B12 among Palestinian adolescents is comparable to other countries. The mean value of vitamin B_{12} vitamin among our participants has been found 223 (55-525) Pg /mL. This cut-off point is in the range recommended by WHO⁽⁴⁴⁾. It's worth mentioning that there is no correlation between vitamin B_{12} and MCV levels. The demographic data as well as food habits or sport activities of participants have been analyzed. There was no significant association between students of vitamin B_{12} level <200 pg/ml and those with vitamin B12 levels more than 200 pg/ml. The explanation of later results is supportive to our conclusion that vitamin B12 level seen in current investigation is normal.

Since there is no symptom, regarding vitamin B_{12} deficiency have been seen in our participants, the high prevalence of vitamin B_{12} deficiency can't be explained. In the vitamin B_{12} determination test, an internal control has been used. Ten samples have been also measured in another laboratory in the country to validate our findings and the results were almost similar.

Data about demographic or well as food as sport habits of the study participants have been collected. No significant association has been found to the high prevalence of vitamin B_{12} level "deficiency". The later result may indicate that this finding (40% of vitamin B_{12}) is not real. Our conclusion is that a physician should be careful in interpreting vitamin B12 level.

The researcher observed also that there was no relation between each of sex, gender, place of residence, parent's education, type of food and level of vitamin B_{12} . As there were no symptoms of vitamin B12 "deficiency" we assume that our findings reflect the normal vitamin B12 level in the region - West Bank of Palestine, and this is comparable with levels found in other parts of the world.

5.2. Recommendations:

In the light of the results shown by the study, the researcher recommended that:

- 1. To expand the investigation to other regions of Palestine in order to determine the normal level of vitamin B_{12} among Palestinians.
- 2. To carry the study on different age groups in the country.
- 3. To involve more health institutions with the cooperation of Ministry of Health in educating people about the nature of vitamin B12, deficiency symptoms and methods of prevention and deficiency treatment.
- 4. Educating mothers regarding the importance of balanced food habits in preventing vitamin B12 deficiency.

References

References:

- Davlin M. Th. Biochemistry with clinical correlation. 5th Vitamin B₁₂ (Cobalamin) contains cobalt in Tetrapyrron rings. 635; Wileyliss 2000. USA.
- Herpert V., Biagauette J. Call for endorsement of petition to the Food and Drug Administration to always add vitamin B₁₂ to any folate fortification or supplement. American journal clinical Nutrition,1997; 65, pp-375.
- Adachi S., Kawamoto T.m Otska M., Todoroki T., Fukao K. *Enternal vitamin B₁₂ Supplements Reverse Postgastrectomy B₁₂ Deficiency*, Annals of Surgery Journal. 2000; 232: 99-201.
- Nelson D. L., Cox. M. M. 3rd ed. Oxidation of propionyl CoA produced by B₁₂ oxidation of food number fatty acid; 609; Mdision Avenue. 2000. USA.
- Hoffbrand A. V., Moss P. A.H, Pettit J. E. 5th ed. Anaemia and clinical features of anaemia; 20-223; Blackwell publishing. 2005. Oxford.
- Gielchinsky Y., Elstein D., Green R., Miller W., Elstein Y., Algur N. High prevalence of low serum vitamin B12 in a multi-ethic Israeli population. British Journal of hematology. 2001; 115: 707-709.
- Hoffbrand A.V, Moss P. A. H., Pettit J. E. 5th ed. *Megaloblastic anaemia*; 42-45; Blackwell publishing. 2005. Oxford.

- Wolfgang H, habil and Rina Obeid. *Causes and Eanly Diagnosis of vitamin B₁₂ Deficiency*. Deutsches Arzteblatt International, 2003, 105: 680-685.
- Satly F, Mary G and Enig. Vitamin B₁₂ Vitat nutrient for good theath, the Weston A. Price Foundation for wise tradition in Food.
 Farming and the Healing Arts, 2005, 27 Wednesday, 27 July, 21:10.
- Linden banm J, Rosen verg IH, Wilson P.W.F, stabler S. P, Allen R.u. *Prevalence of cobalamin deficiency in Framingham elderly patient*. American journal of clinical Nutrition, 1994, (60): 2-4.
- Emmanuel A, Noureddine H. L, Esther N, Georges K, Maher B. A6, Anne E.P, Marie N.D, Fredric M, Jean L.S and Jean F. *Vitamin B*₁₂ (*cobalamin*) *deficiency in elderly Patient*. Canadian Medical Association, 2004, 171 (3).
- 12. Milly R. H, W. *Clinical review: vitamin B₁₂ and health.* Canadian Family physician. 2008, 54:526-41.
- 13. Susan. H, Leonard. L, Rajaethinam. R. P. Vitamin B_{12} deficiency and depression in the elderly. 2009, H (5).
- Ferradez Banares, F, Monzon. H, Forne. M. A short review of malapsortion and anemia. World journal Gastroenterat. 2009, L5 (37): 4644-52.
- 15. Emmanuel A, Noureddinc H.L, Esther, Georges. K, Maher/B. Abd, Anne. E. f, Marie. N. D, Frederic. M, Jean. L. S, Jean. F. B. *Vitamin*

*B*₁₂ (*Cobalamin*) deficiency in elderly patients. Canadian Medical Association, 2004, 171 (3).

- Milly. R. H, Walid A. Vitamin B₁₂ and health. Candian Metical Association, 2004, 171 (3).
- 17. Fernadez. B. F, Monzon. H, Forne. M. Ashort review of *malalosorption and anmia.* 2009, 15 (37): 4644-52.
- 18. Incercilk, F, Herguner. M.O, Altunbasak. S, Leblebisatan. G. *Neurologic finding of Nutritional vitamin* B_{12} *deficiency in children*. Turk journal pediatric, 2010, 52 (1): 17-21.
- Lindenbaum, John. Prevalence of cobalamin deficiency in the Framingham elderly population. American journal of clinical Nutrition. 1994; 69: 2-11.
- Roed. C, Skovby, F, Lurd. A. M. Sereve Vitamin B₁₂ deficiency in infants breastfed by vegan. Ugesker Laeger, 2009; 171 (93): 30 99-101.
- Wolfgang H, Rina O, Heik Sc, Ulrich H, Jungen G, Maraga S. Nayyar A and Markus H. *Enhanced bone metabolism in vegeteiansthe role of vitamin B*₁₂ *deficiency*. Clinical chemistry lab. Med. 2009; 24 (11): 1381-1387.
- 22.Heffbrand A. V, Moss. P. A. H. Pettit J. E, 5th ed. Anaemia and clinical features of anaemia, 20-223, Black well publishing. 2005. Oxford.
- 23. Tucker KL, Hannan MT, Qiaon, Jacques PF, Selhnbt, Cupples LA, Kiel DP. *Low plasma vitamin* B_{12} *is associated with lower BMD:*

the Framingham Osteoporosis study. Journal Bone miner Res. 2005; 20 (1): 152-8.

- 24. Nijst. T. Q, Wevers, R. A., Schoordewaldt H. C., Hommes. O. R., dehaan. A. F. vitamin B_{12} and folate concentration in serum and cerebrospinal fluid of neurological patients with special reference to multiple sclerosis and dementia. 2006; 98 (1) : 67-72.
- 25. Akdol. G., Yener G. G., Ada E., Halmagyi. G. M. Eye movement disorders in vitamin B12 deficiency: two new cases and a review of the literature. European Journal of neurology. 2007; 14(10): 1170-2.
- Anil K., Alkarim P., Aparava U. *Prevalence among South-Asians* at a Toronto Clinic. Canadian Family Physician. 2004; 50: 743-747.
- 27. Mahmoud .A .S, Rula. Kh, Mahmmad. A. H, Malak .J, Mohammed.
 F. *Relationship of vitamin B12 deficiency with overweight in mole Jordanian youth.* Journal of applied science. 2008, 8 (17_ 3060 – 3063.
- Hughes K., Ong C. Homocysteine, folate. vitamin B12 and cardiovascular risk in Indians, Malayans and Chinese in Singapore. Epidemiol Community Health Journal. 2000; 54: 31-40.
- 29. http://www.pcbs.gov.ps/Portals/_PCBS/Downloads/book1665.pdf
- 30. Choi SW, Mason JB. Folate and carcinogenesis. An integrated schemc. Journal Nutrition. 2000; 130:129-132.

- 31. Xhang SM, Willett WC, Selhub J. Serum total homocysticuce levels plasma folate, vitamin B₆, vitamin B₁₂, homocystience, and risk of breast cancer. Journal nat cancer international. 2003; 59:373-380.
- 32. Tiemeier H, Van Tuj HR, Hefman A. Vitamin B₁₂, folate and homocystience in depression: the Rotterdam study. American Journal Psychiatry. 2002; 159: 2099 – 2101.
- 33.Clark R, Smith AD, Jabst KA. Folate Vitamin B₁₂, and serum total homocystiemcs levels in confirmed Alzheimer disease. Arch neurological 1998; 55: 1446-1455.
- 34. Green TJ, Allen OB, and O'Connor DL. (1998). A three-day weighed food record and a semi quantitative food-frequency questionnaire are valid measures for assessing the Scarlett JD, Read H, and O'Dea. (1992). Protein-bound cobalamin absorption declines in the elderly. American Journal of Hematology, 39, 79-83.
- 35. Gupta AK, Damji A, Uppaluri A. (2004): Vitamin B₁₂ deficiency, prevalence among south Asians at a Toronto clinic. Canadian Family Physician, 50, 743-747.
- 36. Figlin E, Cheret A, Shahar A, Shpilberg O, Zivelin A, Rosenberg N, Simoni F, Gadoth N, Sela B, Seligsohn U. (2003). *High prevalence of vitamin B₁₂ and folic acid deficiency in elderly subjects in Israel.* British Journal of Hematology, 123, 696-701.

- 37. For a MA, MA Mohammad. (2005). High frequency of suboptimal serum vitamin B₁₂ level in adults in Jordan. Saudi Medical Journal, 26 (10), 159-165.
- Planells E, Sa'nchez C, Montellano MA, Mataix J and Llopis J. (2003). Vitamins B₆ and B₁₂ and folate status in an adult Mediterranean population. European Journal of Clinical Nutrition, 57, 777-785.
- 39. Eastley R, G K Wilcock, AR Bucks. (2000). Vitamin B₁₂ Deficiency in Dementia and Cognitive Impairment: The Effects of Treatment on Neuropsychological Function. International Psycho geriatrics, 15, 226-233.
- 40. Starr JM, A Pattie, MC Whiteman, IJ Deary. (2005). Vitamin B₁₂, Serum Folate, and Cognitive Change between 11 and 79 Years.
 Journal of Neurology Neurosurgery and Psychiatry: 76, 291-292.
- 41. Hao L, Jing M, Jianghui Z, Meir JS, Yihua T, Walter CW, and Zhu L (2007): *Vitamin B₁₂ Deficiency is prevalent in 35 to 64 Year-Old Chinese Adults*. Journal of Nutrition, 137, 1278-1285.
- 42. McLean ED, Allen LH, Neumann CG, Peerson JM, Siekmann JH, Murphy SP, Bwibo NO, and Demment MW. (2007) Low Plasma vitamin B₁₂ in Kenyan School Children Is Highly Prevalent and Improved by supplemental Animal Source Foods. Journal of Nutrition, 137, 676-682.
- 43. Tucker KL (2000). Are you vitamin B₁₂ deficient?. Agricultural Research Magazine, August 2000 issue.

44. Lindsay. H. Allen: Vitamin B₁₂ fortification. World Health Organization. 30 March, 3 April 2008.

http://www.sph.emory.edu/wheatflour/atlanta08/CDC_B12_32408.pdf

- 45. Anil K., Alkarim P., Aparava U. *Prevalence among South Asian* at a Toronto Clinic. Canadian Family Physician. 2004; 50: 743-747.
- 46. Has. R, Larsen Msc ch. Vitamin B₁₂, summaries of the latest research concerning vitamin B₁₂. international health news. 9/20/2010.

Appendices

Appendix A English questionnaire

A special questionnaire to examine B₁₂

Dear reader, I hope you to answer the following questions for the field of my research, we also confirm that for the purposes of scientific research no more and tick the box on the right answer with thanks.

1. Name:
2. Gender: Male Female
3. Birth date: / /
4. Place of residence:
5. No. of brothers:
6. No. of sisters:
7. Level of education of father: \Box Elementary of less \Box Secondary \Box Two years diploma
□ Bachelor □ Post graduate
8. Level of education of mother: 🗌 Elementary of less 🗌 Secondary 📋 Two years diploma
□ Bachelor □ Post graduate
9. The level of family income: \Box 1000 NIS or less \Box 1000-2000 NIS \Box 2000-3000 NIS
□ 3000-4000 NIS □ 4000NIS or more
10. Do you suffer from one disease of anemia: 🗌 Yes 📋 No
If you have a disease, please specify: and duration of cases of this
disease
11. Do you have a family member suffering from a diseases for anemia \Box Yes \Box No
If the answer is yeas, please specify the type of disease and duration of
injury by
12. Do you eat vegetarian food? Yes No
What kind of food you eat? \Box Vegetarian \Box Animal \Box Vegetarian and animal
What kind of vegetarian food you eat? \Box Vegetables \Box Fruits \Box Vegetables & fruits
Do you take: \Box Cooked vegetables \Box Fresh vegetables \Box Cooked and fresh vegetables
Do you take: 🗌 Fresh fruit 🗌 Canned fruit such as juices
13. Do you eat animal food? \Box Yes \Box No
Do you take the: \Box Meat of fish \Box Meat of birds \Box Other types of meat
14. Do you take nutritional supplements? 🗌 Iron tables 🗌 Vitamins 🗌 Fish oil
15. Do you have sport activity? 🗌 Yes 📋 No

If the answer is yes, then what is the type of sport?

□ Jogging □ Fitness □ Swimming □ Lifting weights

☐ Football ☐ Other types

If you play sports, do you play sports: \Box Daily \Box Once a weak \Box Twice a weak

 \Box Non specific

With thanks

Prepared By: Amani Yaseen

Appendix B Arabic questionnaire

استبانة خاصة ببحث الفيتامين بــ12

عزيزي القارئ أرجو منك الإجابة على الأسئلة التالية الخاصة بمجال بحثي وهو الفيتامين بـــ12 مع التأكيد أنه لأغراض البحث العلمي لا أكثر ووضع إشارة في مربع الإجابة الصحيحة مع الشكر:

الاسم:
 الجنس: [ذكر]أنثى.
 تاريخ الميلاد: / / /
 مكان السكن: / / /
 مكان السكن:
 عدد الأخوة الذكور:
 عدد الأخوة الإناث.
 عدد الأخوة الإناث.
 مستوى تعليم الأب:] إعدادي فأقل] ثانوي] سنتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] سنتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] سنتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] سنتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] سنتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] مستوى تعليم الأم:]
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] منتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] منتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] منتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] منتين دبلوم
 مستوى تعليم الأم:] إعدادي فأقل] ثانوي] منتين دبلوم
 مستوى دخل العائلة:] 1000 شيكل فأقل] ثانوي] منتين دبلوم
 مستوى دخل العائلة:] 1000 شيكل فأقل] 100 -0000 شيكل
 مستوى دخل العائلة:] 1000 شيكل فأقل] 100 -0000 شيكل
 مستوى دخل العائلة:] 1000 شيكل مأقل] 100 -0000 شيكل
 مستوى دخل العائلة:] 1000 شيكل فأقل] 100 -0000 شيكل

واذا كنت مصاباً يرجى تحديد نوع المرض: ومدة الإصابة بهذا المرض

- - ما هو نوع الغذاء الذي تتناوله: 🗌 نباتي 🔄 حيواني فقط 🔄 حيواني ونباتي

هل تتناول: 🗌 فواكه طازجة 🔄 فواكه معلبة مثل العصائر

13 هل نتناول غذاء حيواني: 🗋 نعم 🛛 لا

هل تتناول لحوم أسماك لحوم طيور أنواع لحوم أخرى.....

14 هل تتناول مكملات غذائية 🛛 أقراص حديد 🗆 فيتامينات 🗋 زيت سمك

15 هل تمارس نشاط رياضي 🛛 نعم 🔄 لا

مع الشكر

مقدمة الاستبانة أمانى ياسين

Appendix C Consent form

Consent form:

We are the parents ofborn in the year.....born in the year.....born in the sample far vitamin B12 measurement and CBC analysis. Understand that the results of this work is part of scientific research done by student (Amany Ghalib Yassin) to obtain a Master degree of Public Health from An—Najah National University Signature of parent

Appendix D

Statistical Results

Statistical Results

Hypothesis one says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and gender.

For testing hypothesis one, the researcher conducted Chi Square test and the results of this analysis are shown in table (1).

Table (1): Results of Chi Square for relation between B12 and gender

	B12 pmol/L				Chi square	Sig*
Gender	< 200	200-300	> 300	D.F	Chi square value	
Male	88	69	46			0.26
Female	90	59	52	2	1.161	0.36 0

* Statically significant at ($\alpha = 0.05$).

Table (1) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and gender.

Results pertinent to hypothesis two :

Hypothesis two says : there is no significant relation at the level ($\alpha = 0.05$) between MCV, and gender.

For testing hypothesis one, the researcher conducted Chi Square test and the results of this analysis are shown in table .

	MCV fL				Chiaguana	
Gender	< 80	80 - 92	> 92	D.F	Chi square value	Sig*
Male	59	140	4			
Female	40	155	6	2	4.799	0.091

Table (2): Results of Chi Square for relation between MCV and gender

* Statically significant at ($\alpha = 0.05$)

Table indicates that there is no significant relation at the level ($\alpha = 0.05$) between MCV, and gender.

Results pertinent to hypothesis three :

Hypothesis three says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and Place.

For testing hypothesis one, the researcher conducted Chi Square test and the results of this analysis are shown in table (3).

	I	B12 pmol/L					
Place	< 200	< 200	< 200	D.F	Chi square value	Sig*	
Qalqilia	59	40	38		0.102	0.940	
Tulkarem	119	78	70	2	0.125		

Table (3): Results of Chi Square for relation between B12 and Place

* Statically significant at ($\alpha = 0.05$)

Table (3) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and place.

Results pertinent to hypothesis four :

Hypothesis four says : there is no significant relation at the level ($\alpha = 0.05$) between Hgb, and B12 according to males.

For testing hypothesis one, the researcher conducted Chi Square test and the results of this analysis are shown in table (4).

	Нg	gb g/dL				<u></u>	
B12	< 12	12-16	>16	D.F	Chi square value	Sig*	
< 200	17	68	3				
200-300	14	52	3	4	3.170	0.530	
> 300	4	40	2				

 Table (4): Results of Chi Square for relation between Hgb and B12

 according to males

* Statically significant at ($\alpha = 0.05$)

Table (4) indicates that there is no significant relation at the level ($\alpha = 0.05$) between Hgb, and B12 according to males.

Results pertinent to hypothesis five:

Hypothesis five says : there is no significant relation at the level ($\alpha = 0.05$) between Hgb, and B12 according to normal scale for both males and females.

For testing hypothesis five, the researcher conducted Chi Square test and the results of this analysis are shown in table (5).

Hgb g/dL			Chi square	G • *		
B12	Males (12-16) Females (11.5-15) D.F		D.F	value	Sig*	
< 200	68	77				
200-300	52	57	2	0.100	0.951	
> 300	40	48				

Table (5): Results of Chi Square for relation between Hgb and B12

* Statically significant at ($\alpha = 0.05$)

Table (5) indicates that there is no significant relation at the level ($\alpha = 0.05$) between Hgb, and B12 according to normal scale for both males and females .

Hypothesis 6 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and income.

For testing hypothesis 6, the researcher conducted Chi Square test and the results of this analysis are shown in table (6).

	B12 pmol/L				Chi square	
Income	< 200	200-300	> 300	D.F	value	Sig*
≤ 1000 NIS	10	12	4			
1001-2000	64	43	37			
2001-3000	66	45	36	8	4.602	0.799
3001-4000	26	15	13			
> 4000	11	12	8			

Table (6): Results of Chi Square for relation between B12 and income

* Statically significant at ($\alpha = 0.05$)

Table (6) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and income.

Hypothesis 7 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and Anemia.

For testing hypothesis 7, the researcher conducted Chi Square test and the results of this analysis are shown in table (7).

Anemia	B12 pmol / L				Chi square	<i>.</i>
	< 200	200-300	> 300	D.F	value	Sig*
Yes	1	1	0		0.700	0.007
No	176	126	98	2	0.722	0.697

Table (7): Results of Chi Square for relation between B12 and Anemia

* Statically significant at ($\alpha = 0.05$)

Table (7) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and Anemia.

Hypothesis 8 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and family members infected with anemia.

For testing hypothesis 8, the researcher conducted Chi Square test and the results of this analysis are shown in table (8).

Table (8): Results of Chi Square for relation between B12 and family							
members infected with anemia							

family members infected with anemia	B12 pmol / L				Chi square	
	< 200	200-300	> 300	D.F	value	Sig*
Yes	6	8	2		a 5 01	0.074
No	165	116	86	2	2.591	0.274

* Statically significant at ($\alpha = 0.05$)

Table (8) indicates that there is no significant relation at the level (α = 0.05) between B12, and family members infected with anemia.

Hypothesis 9 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and Vegetarian.

For testing hypothesis 9, the researcher conducted Chi Square test and the results of this analysis are shown in table (9).

Vegetarian	B12 pmol/L				Chi square	
	< 200	200-300	> 300	D.F	value	Sig*
Yes	164	120	95	2	1.012	0.404
No	6	5	1	2	1.812	0.404

Table (9): Results of Chi Square for relation between B12 and vegetarian

* Statically significant at ($\alpha = 0.05$)

Table (9) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and vegetarian.

Hypothesis 10 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and food kind.

For testing hypothesis 10, the researcher conducted Chi Square test and the results of this analysis are shown in table (10).

Table (10): Results of Chi Square for relation between B12 and food kind

	B12 pmol/	L			Chi square	Sig*
Food kind	< 200	200-300	> 300	D.F	value	
Vegetarian	8	7	0			
Animal	2	1	0	4	6.327	0.176
Vegetarian and animal	167	119	97			

* Statically significant at ($\alpha = 0.05$)

Table (10) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and food kind.

Hypothesis 11 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and Vegetarian.

For testing hypothesis 11, the researcher conducted Chi Square test and the results of this analysis are shown in table (11).

	B12 pmol/L				Chi square	
Vegetarian	< 200	200-300	> 300	D.F	value	Sig*
Vegetables	4	0	0			
Fruits	2	1	0	4	6.141	0.189
Vegetables & fruits	172	124	97			

Table (11): Results of Chi Square for relation between B12 andVegetarian

* Statically significant at ($\alpha = 0.05$)

Table (11) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and Vegetarian.

Hypothesis 12 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and vegetables kind.

For testing hypothesis 12, the researcher conducted Chi Square test and the results of this analysis are shown in table (12).

B12 pmol/L Chi square D.F Sig* vegetables kind value 200-300 < 200 > 300 9 Cooked 1 3 7 9 4 3 6.696 0.153 Fresh Cooked & fresh 155 114 91

Table (12): Results of Chi Square for relation between B12 andvegetables kind

Table (12) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and vegetables kind.

Hypothesis 13 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and fruits kind.

For testing hypothesis 13, the researcher conducted Chi Square test and the results of this analysis are shown in table (13).

 Table (13): Results of Chi Square for relation between B12 and fruits

 kind

fruits kind	B12 pmol/L			DE	Chi square	d . *
	< 200	200-300	> 300	D.F	value	Sig*
Fresh	121	74	63			0.110
Canned fruits as juice	52	52	30	2	4.274	0.118

* Statically significant at ($\alpha = 0.05$)

Table (13) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and fruits kind.

Hypothesis 14 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and animal food.

For testing hypothesis 14, the researcher conducted Chi Square test and the results of this analysis are shown in table (14).

Animal food	B12 pmol/L			DE	Chi square	G. *
	< 200	200-300	> 300	D.F	value	Sig*
Yes	159	115	91		0.010	0.604
No	11	9	4	2	0.910	0.634

Table (14): Results of Chi Square for relation between B12 and animal food

* Statically significant at ($\alpha = 0.05$)

Table (14) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and animal food.

Hypothesis 15 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and animal food kind.

For testing hypothesis 15, the researcher conducted Chi Square test and the results of this analysis are shown in table (15).

Table (15): Results of Chi Square for relation between B12 and animal food kind

Animal food kind	B12 pmol/L				Chi square	g. *
	< 200	200-300	> 300	D.F	value	Sig*
Fish	78	70	49			
Birds	78	45	40	4	6.886	0.142
Others	4	2	5			

Table (15) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and animal food kind.

Hypothesis 16 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and food complements.

For testing hypothesis 16, the researcher conducted Chi Square test and the results of this analysis are shown in table (16).

 Table (16): Results of Chi Square for relation between B12 and food
 complements

food complements	B12 pmol/L				Chi square	d . *
	< 200	200-300	> 300	D.F	value	Sig*
Iron tablets	2	0	3			
Vitamins	6	1	0	4	5.845	0.211
Fish oil	3	1	2			

* Statically significant at ($\alpha = 0.05$)

Table (16) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and food complements.

Hypothesis 17 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and sport practicing.

For testing hypothesis 17, the researcher conducted Chi Square test and the results of this analysis are shown in table (17).

	B12 pmol/	B12 pmol/L			Chi square	
sport practicing	< 200	200-300	> 300	D.F	Chi square value	Sig*
Yes	157	113	88			
No	21	14	8	2	0.799	0.671

 Table (17): Results of Chi Square for relation between B12 and sport

 practicing

* Statically significant at ($\alpha = 0.05$)

Table (17) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and sport practicing.

Hypothesis 18 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and sport type.

For testing hypothesis 18, the researcher conducted Chi Square test and the results of this analysis are shown in table (18).

 Table (18): Results of Chi Square for relation between B12 and sport

type

	B12 pmc	B12 pmol/L			Chi square	с. *
sport type	< 200	200-300	> 300	D.F	value	Sig*
Running	35	25	29		12.624	
Fitness	28	15	11			0.245
Swimming	10	7	11			
Weight lifting	2	2	0	10		
Football	60	48	24			
Others	24	18	16			

Table (18) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and sport type.

Hypothesis 19 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and times of sport practicing.

For testing hypothesis 19, the researcher conducted Chi Square test and the results of this analysis are shown in table (19).

 Table (19): Results of Chi Square for relation between B12 and times

 of sport practicing

times of sport practicing	B12 pmol/L				Chi square	
	< 200	200-300	> 300	D.F	value	Sig*
Daily	34	22	18	6	2.841	0.828
Once a week	45	35	19			
Twice a week	18	13	13			
Undetermined	64	45	40			

* Statically significant at ($\alpha = 0.05$)

Table (19) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and times of sport practicing.

Results pertinent to hypothesis one :

Hypothesis one says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and gender.

For testing hypothesis one, the researcher conducted Chi Square test and the results of this analysis are shown in table (1).

	B12 pmol/L				Chi square	
Gender <	< 200	200-300	> 300	D.F	value	Sig*
Male	88	69	46			0.0.00
Female	90	59	52	2	1.161	0.360

Table (1): Results of Chi Square for relation between B12 and gender

* Statically significant at ($\alpha = 0.05$).

Table (1) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and gender.

Results pertinent to hypothesis two :

Hypothesis two says : there is no significant relation at the level ($\alpha = 0.05$) between MCV, and gender.

For testing hypothesis one, the researcher conducted Chi Square test and the results of this analysis are shown in table .

Table (2): Results of Chi Square for relation between MCV and gender

	MCV fL				Chi square	a. *
Gender	< 80	80 - 92	> 92	D.F	value	Sig*
Male	59	140	4		4.500	0.001
Female	40	155	6	2	4.799	0.091

Table indicates that there is no significant relation at the level ($\alpha = 0.05$) between MCV, and gender.

Results pertinent to hypothesis three :

Hypothesis three says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and Place.

For testing hypothesis one, the researcher conducted Chi Square test and the results of this analysis are shown in table (3).

 Table (3): Results of Chi Square for relation between B12 and Place

	B12 pmol/L				Chi square	
Place < 2	< 200	< 200	< 200	D.F	value	Sig*
Qalqilia	59	40	38		0.105	0.040
Tulkarem	119	78	70	2	0.125	0.940

* Statically significant at ($\alpha = 0.05$)

Table (3) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and place.

Results pertinent to hypothesis four :

Hypothesis four says : there is no significant relation at the level ($\alpha = 0.05$) between Hgb, and B12 according to males.

For testing hypothesis one, the researcher conducted Chi Square test and the results of this analysis are shown in table (4).

			0			
B12 Hgb g/d < 12	Hgb g/dL			Chi square	~	
	< 12	12-16	>16	D.F	value	Sig*
< 200	17	68	3			
200-300	14	52	3	4	3.170	0.530
> 300	4	40	2			

Table (4): Results of Chi Square for relation between Hgb and B12

according to males

* Statically significant at ($\alpha = 0.05$).

Table (4) indicates that there is no significant relation at the level ($\alpha = 0.05$) between Hgb, and B12 according to males.

Results pertinent to hypothesis five:

Hypothesis five says : there is no significant relation at the level ($\alpha = 0.05$) between Hgb, and B12 according to normal scale for both males and females.

For testing hypothesis five, the researcher conducted Chi Square test and the results of this analysis are shown in table (5).

B12	Hgb g/dL Males (12-16) Females (11.5-15)		D.F	Chi square value	Sig*
< 200	68	77			
200-300	52	57	2	0.100	0.951
> 300	40	48			

Table (5): Results of Chi Square for relation between Hgb and B12

Table (5) indicates that there is no significant relation at the level ($\alpha = 0.05$) between Hgb, and B12 according to normal scale for both males and females .

Hypothesis 6 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and income.

For testing hypothesis 6, the researcher conducted Chi Square test and the results of this analysis are shown in table (6).

	B12 pmol/L	B12 pmol/L				S :~*
Income	< 200	200-300	> 300	D.F	value	Sig*
≤ 1000 NIS	10	12	4			
1001-2000	64	43	37			
2001-3000	66	45	36	8	4.602	0.799
3001-4000	26	15	13			
> 4000	11	12	8			

Table (6): Results of Chi Square for relation between B12 and income

* Statically significant at ($\alpha = 0.05$).

Table (6) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and income.

Hypothesis 7 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and Anemia.

For testing hypothesis 7, the researcher conducted Chi Square test and the results of this analysis are shown in table (7).

Table (7): Results of Chi Square for relation between B12 and Anemia

	B12 pmol / L			5.5	0:-*	
Anemia	< 200	200-300	> 300	D.F	value	Sig*
Yes	1	1	0			0.005
No	176	126	98	2	0.722	0.697

* Statically significant at ($\alpha = 0.05$)

Table (7) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and Anemia.

Hypothesis 8 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and family members infected with anemia.

For testing hypothesis 8, the researcher conducted Chi Square test and the results of this analysis are shown in table (8).

Table (8) :Results of Chi Square for relation between B12 and family

family members infected with anemia	B12 pmol	/ L	DE	Chi square	C . *	
	< 200	200-300	> 300	D.F	value	Sig*
Yes	6	8	2		0.501	0.074
No	165	116	86	2	2.591	0.274

members infected with anemia

Table (8) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and family members infected with anemia.

Hypothesis 9 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and Vegetarian.

For testing hypothesis 9, the researcher conducted Chi Square test and the results of this analysis are shown in table (9).

 Table (9): Results of Chi Square for relation between B12 and vegetarian

	B12 pmol/L				Chi square	0:-*
Vegetarian	< 200	200-300	> 300	D.F	value	Sig*
Yes	164	120	95		1.010	0.404
No	6	5	1	2	1.812	0.404

* Statically significant at ($\alpha = 0.05$).

Table (10) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and vegetarian.

Hypothesis 10 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and food kind.

For testing hypothesis 10, the researcher conducted Chi Square test and the results of this analysis are shown in table (10).

	B12 pmol/L				Chi square	
Food kind	< 200	200-300	> 300	D.F	value	Sig*
Vegetarian	8	7	0			
Animal	2	1	0	4	6.327	0.176
Vegetarian and animal	167	119	97			

Table (10): Results of Chi Square for relation between B12 and food kind

* Statically significant at ($\alpha = 0.05$).

Table (10) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and food kind.

Hypothesis 11 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and Vegetarian.

For testing hypothesis 11, the researcher conducted Chi Square test and the results of this analysis are shown in table (11).

Vegetarian	B12 pmol/L				Chi square	G*
	< 200	200-300	> 300	D.F	value	Sig*
Vegetables	4	0	0			
Fruits	2	1	0	4	6.141	0.189
Vegetables & fruits	172	124	97			

 Table (11): Results of Chi Square for relation between B12 and

 Vegetarian

Table (11) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and Vegetarian.

Hypothesis 12 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and vegetables kind.

For testing hypothesis 12, the researcher conducted Chi Square test and the results of this analysis are shown in table (12).

vegetables kind	B12 pmol/L				Chi square	
	< 200	200-300	> 300	D.F	value	Sig*
Cooked	9	1	3			
Fresh	7	9	3	4	6.696	0.153
Cooked & fresh	155	114	91			

 Table (12): Results of Chi Square for relation between B12 and

 vegetables kind

* Statically significant at ($\alpha = 0.05$).

Table (12) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and vegetables kind.

Hypothesis 13 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and fruits kind.

For testing hypothesis 13, the researcher conducted Chi Square test and the results of this analysis are shown in table (13).

	B12 pmol/	L		D.F	Chi square value	Sig*
fruits kind	< 200	200-300	> 300			
Fresh	121	74	63			0.110
Canned fruits as juice	52	52	30	2	4.274	0.118

Table (13): Results of Chi Square for relation between B12 and fruits kind

* Statically significant at ($\alpha = 0.05$).

Table (13) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and fruits kind.

Hypothesis 14 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and animal food.

For testing hypothesis 14, the researcher conducted Chi Square test and the results of this analysis are shown in table (14).

Table (14): Results of Chi Square for relation between B12 and animal food

	B12 pmol/L				Chi square	d . *
Animal food	< 200	200-300	> 300	D.F	value	Sig*
Yes	159	115	91		0.010	0.604
No	11	9	4	2	0.910	0.634

Table (14) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and animal food.

Hypothesis 15 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and animal food kind.

For testing hypothesis 15, the researcher conducted Chi Square test and the results of this analysis are shown in table (15).

 Table (15): Results of Chi Square for relation between B12 and animal food kind

Animal food kind	B12 pmol/L				Chi square	с. *
	< 200	200-300	> 300	D.F	value	Sig*
Fish	78	70	49			
Birds	78	45	40	4	6.886	0.142
Others	4	2	5			

* Statically significant at ($\alpha = 0.05$).

Table (15) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and animal food kind.

Hypothesis 16 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and food complements.

For testing hypothesis 16, the researcher conducted Chi Square test and the results of this analysis are shown in table (16).

food complements	B12 pmol/L				Chi square	G : .*
	< 200	200-300	> 300	D.F	value	Sig*
Iron tablets	2	0	3			
Vitamins	6	1	0	4	5.845	0.211
Fish oil	3	1	2			

 Table (16): Results of Chi Square for relation between B12 and food

 complements

* Statically significant at ($\alpha = 0.05$).

Table (16) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and food complements.

Hypothesis 17 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and sport practicing.

For testing hypothesis 17, the researcher conducted Chi Square test and the results of this analysis are shown in table (17).

 Table (17): Results of Chi Square for relation between B12 and sport

 practicing

	B12 pmol/	L		D.F Chi square value	Chi square	
sport practicing	< 200	200-300	> 300		Sig*	
Yes	157	113	88		0.700	0.671
No	21	14	8	2	0.799	0.671

Table (17) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and sport practicing.

Hypothesis 18 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and sport type.

For testing hypothesis 18, the researcher conducted Chi Square test and the results of this analysis are shown in table (18).

 Table (18): Results of Chi Square for relation between B12 and sport

 type

	B12 pmol/	L			Chi square	Sig*
sport type	< 200	200-300	> 300	D.F	value	
Running	35	25	29			
Fitness	28	15	11			
Swimming	10	7	11			0.045
Weight lifting	2	2	0	10	12.624	0.245
Football	60	48	24			
Others	24	18	16			

* Statically significant at ($\alpha = 0.05$).

Table (18) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and sport type.

Hypothesis 19 says : there is no significant relation at the level ($\alpha = 0.05$) between B12, and times of sport practicing.

For testing hypothesis 19, the researcher conducted Chi Square test and the results of this analysis are shown in table (19).

 Table (19): Results of Chi Square for relation between B12 and times

 of sport practicing

times of sport	B12 pmol/	L			Chi square	Sig*
practicing	< 200	200-300	> 300	D.F	value	
Daily	34	22	18			
Once a week	45	35	19		2 0 4 1	0.020
Twice a week	18	13	13	6	2.841	0.828
Undetermined	64	45	40			

* Statically significant at ($\alpha = 0.05$).

Table (19) indicates that there is no significant relation at the level ($\alpha = 0.05$) between B12, and times of sport practicing.

Appendix E

Request approval from the ministry of education to allow taking samples of blood from students

اسلط الوطنية تللسطينية الأطلاعة للمعلى الوزارة التربية والتعليم لعلى الوزارة التربية والتعليم العلى الوراية عند المعلى التاريخ : 22 / 10 /2528 الوراية : 22 / 10 /2008/بو الوراية : 23 / 10 /2008/بو الموافق : 23 / 10 /2008/بو

تحية طيبة وبعد ،..

الموضوع: التثقيف الصحى

124.

Palestinian National Authority

ry of Education & Higher Education

irectorate of Education – Tulkarm

تهديكم اطيب تحيانتا، ونعلمكم ان المعلمة (اماني ياسين) وهي طالبة ماجستير في برنامج الصحة العامة تقوم بعمل دراسة حول معدل انتشار نقص فيتامين B12 عند اليافعين في محافظات شمال الضفة الغربية، وتحتاج التي عينات دم وبعض المعلومات، يرجى مساعدة الطالبة علما بان الدراسة لأغراض البحث العلمي فقط مع ضرورة اخذ موافقة ولي أمر الطالب/ة بضصوص فصص الدم.

ملحوظة: الجدول التالي بيبن الفلة العمرية المستهدفة وأعداد الطلبة (عينة البحث) في المدارس ذات العلاقة.

أسم المدرسة	الصب	الجنس	عدد العيلات
بنك العدرية الثقوية	12.11.10	- tit	iiya 48
ينات بنصود الهبشران	9:8-7-6/5 -	انك	80
ذكري بله حسين	6.5	ذكور	32
ذكور حائظ الحداث	8.7	نگرر.	32 .
نقور خامي جنون	9	ذكرر	16
ذكور الفاضانية	12,11,10	نكرر	48
			256



83

جامعة النجاح الوطنية كلية الدر اسات العليا

تحديد المستوى الطبيعي لفيتامين B₁₂ بين المراهقين الفلسطينيين (18-10) سنة في شحديد المستوى الطبيعي لفيتامين الضفة الغربية

إعداد اماني ياسين

إشراف د .أيمن حسين

قدمت هذه الاطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الصحة العامة من كلية الدر اسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين. تحديد المستوى الطبيعي لفيتامين B₁₂ بين المراهقين الفلسطينيين (18-10) سنة في شمال الضفة

الملخص

الأهداف: تهدف هذه الدراسة إلى تحديد المستوى الطبيعي من فيتامين بـــ12 بين المراهقين الفلسطينيين (10-18) سنة في شمال الضفة الغربية وتقييم المتغيرات الاجتماعية والديمو غرافية المرتبطة به.

الطريقة (المنهجية): أجريت دراسة مسحية عن طريق جمع بيانات من مراهقين يبلغ عددهم 404 نتراوح أعمارهم بين (10-18) سنة من مناطق شمال الضفة الغربية، وأجريت اختبارات 404 وفيتامين B₁₂ جنباً إلى جنب مع تصميم استبيان للحصول على مختلف العوامل الديمو غرافية وغيرها من العوامل من المشاركين.

النتائج: أظهرت هذه الدراسة أن 43.3% (203/88) من البنين، و44.8% (201/90) من النتائج: أظهرت هذه الدراسة أن B_{12} أقل من 200 pmol/l و10 فقط من هؤلاء لديهم الفتيات لديهم مستوى فيتامين B_{12} من المصابين الذين لديهم مستوى فيتامين MCV > 92fl من المصابين الذين لديهم مستوى فيتامين $B_{12} < 200$ pmol/l لا يمكن اعتبار وجود نقص فيتامين B_{12} لديهم.

فقد أظهرت الدراسة لتلاميذ المدارس في إطار الدراسة أنه لم يكن لديهم الأعراض المرتبطة بنقص فيتامين B₁₂ ، وأشارت هذه النتائج إلى أنه يجب البحث في المستوى الطبيعي لــ B₁₂ بين المراهقين الفلسطينيين. كما أظهر التحليل للبيانات الديموغرافية والعادات الغذائية بين المشاركين أنه لا توجد علاقة وثيقة بين مستوى B₁₂ والمتغيرات التالية (P-value > 0.05): مكان الإقامة، دخل الأسرة، عدد أفراد الأسرة، العادات الغذائية.