An-Najah National University

Faculty of Graduate Studies

ASSOCIATION BETWEEN SERUM FOLATE LEVELS AND DEPRESSION AMONG ADULT FEMALE STUDENTS AT AN-NAJAH NATIONAL UNIVERSITY IN NABLUS, PALESTINE: A CROSS-SECTIONAL STUDY

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This Thesis was Defended Successfully on 29/7/2024 and approved by

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Dedication

To all who assist the science enthusiast and clear the path for him. To individuals who are self-made, diligent, and believe that success, fulfillment, and accomplishment rely on themselves rather than others. To all those who support and aid in constructing a school, or university, or purchasing school supplies for students. To each mother, father, sister, and brother who dedicate themselves to motivating their sons towards success, excellence, and innovation. To my parents and educators. To my brother and sisters who serve as the beacon of positivity in my life. To everyone who aided me in completing this task throughout my education and life.

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First and foremost, I am grateful to God for assisting me in accomplishing my mission, persevering through challenges, and successfully finishing this work.

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I want to express my gratitude and love to my wonderful family. My father is the ideal person in my life, and my mother gives me positivity and strength. I am also thankful for my brother and sisters, who are a constant source of optimism. Thank you to everyone else who supported me and may not have been named.

Finally, I sincerely hope that I have successfully achieved the intended goal of this research for the benefit of science and its people.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

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I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: Saja Esan Elewi

Signature: Saja Elewi

Date: 29 /7 /2024

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Abstract

Background: Depression is considered a major public health concern worldwide. Depression is a prevalent issue among university students compared to the general population. Folate is a B complex group water-soluble vitamin that naturally occurs in food and is essential to the brain for producing norepinephrine, dopamine, and serotonin. Previous studies have indicated that levels of folate are crucial in the development and progression of depression. However, the research findings have been inconclusive when it comes to variations in folate levels among people with depression compared to those without the condition.

Objective: This study aimed to assess the relationship between serum folate levels and depression among adult female students at An-Najah National University in Nablus, Palestine.

Method: This study was conducted with a cross-sectional observational design. A questionnaire was distributed from 15th September 2023 until 20th October 2023 to participating female students from An-Najah National University. Depressive symptoms were assessed using the DASS-21 scale. The questionnaire also collected the social and demographic characteristics of the female students and several questions related to the factors that affect folate levels in the blood. The internal reliability of the DASS-21 questionnaire was tested using Cronbach's alpha. In addition, venous blood samples were collected within the same period in the scientific research laboratory at An-Najah University after overnight fasting, and serum folate levels were measured. The number of female students participating was 180, and their ages varied from 18 to 30 years old.

Results: The mean of serum folate levels was 4.82 ng/ml (SD=3.38). The prevalence of depression among them was 16.7%, 47.2%, 14.4% 12.2%, and 9.4% for extremely severe, severe, moderate, mild, and normal depression, respectively. Levels of serum folate were significantly lower in patients with extremely severe and severe depression, the mean was (3.13 and 3.30 ng/ml) respectively. Additionally, there was a significant moderate inverse correlation between the levels of serum folate and depression severity (r=-0.537, P-value<0.001).

Conclusion: Our findings showed that there is a connection between the levels of serum folate and depression among female Palestinian students. Folate serum levels may serve as indicators to evaluate the effectiveness of depression treatment. Measuring folate levels can assist physicians in effectively managing depression. In this regard, folate appears to play a more crucial role in the improvement outcomes of mood disorders and should be assessed more attentively.

Keywords: Serum folate; depression; lifestyle; diet; clinical characteristics; An-Najah National University; Palestine.

Chapter One

Introduction

1.1 Background

Psychological distress signifies general symptoms of depression. Elevated levels of psychological distress suggest compromised mental health and could indicate typical mental disorders such as depressive disorders.[1] Depressive disorder affects around 7% of people in a year, with significant variations across different age groups. The rate is three times higher among 18- to 29-year-olds compared to those aged 60 and above. Females have a 1.5 to 3 times higher prevalence than males starting in early adolescence.[2] The global prevalence of depressive disorders in adults is 3.4%, while in adult females it is 4.1%[3]. Palestinians experience elevated levels of psychological distress due to the constant threat (the Israeli occupation) to their daily lives, resulting in a diminished quality of life and increased susceptibility to depression.[4] The World Bank and the Palestinian Central Bureau of Statistics (PCBS) announced that findings from the 2022 Psychological Conditions Survey in Palestine revealed that over half of the Palestinian population experiences depression (71% in the Gaza Strip and 50% in the West Bank). Of those affected, 58% are adults aged 18 and above, with depression rates being comparable among both genders. However, data indicated that post-traumatic stress disorder is more prevalent among individuals aged 18 and above in the Gaza Strip compared to the West Bank.[5] In a study published in 2022 researchers discovered that among 1049 students, the levels of depression were 55.8% for severe cases, 26.4% for moderate cases, and 9.8% for mild cases. The study indicated a significant rise in depression rates among Palestinian university students, with the majority experiencing moderate to severe symptoms.[6] It is well-established that diet plays a role in depression. Previous research on the link between diet and depression has primarily focused on food groups, individual foods, or specific nutrients.[7] Nutritional epidemiologists proposed using the dietary pattern approach to explore the relationships between diet and disease, taking into account the intricate connections between nutrients and foods.[8] Furthermore, this method offers a deeper understanding of the connection between diet and brain function.[9] Vitamins are essential nutrients needed for life, playing a crucial role in maintaining good health. Inadequate levels of vitamins may be associated with symptoms of psychiatric disorders.[10] The connection between

nutrition and depression is often overlooked by many. Nutrition can significantly impact both the development and intensity of depression. Many of the dietary habits that are easily recognizable before depression sets in are similar to those experienced during depression.[11] Vitamin B9 is crucial for proper neuronal functioning, and low levels have been associated with depression.[10] Studies over the last 35 years have demonstrated a correlation between folic acid deficiency and psychological symptoms, such as depression and cognitive impairment.[12]

1.2 Depression

Depression is starting at an earlier age now compared to previous decades.[13] Approximately 280 million people worldwide are living with depression,[14] which accounts for 4.4% of the global population[15], as reported by the World Health Organization (WHO).[14] Depression is a prevalent mental illness that affects individuals globally in the present day.[16] Depression is common in both developed and developing countries.[7] Many university students experience high rates of depression.[17] Depression is identified by various symptoms such as sadness, feelings of guilt or low self-esteem, loss of interest or pleasure, fatigue, changes in sleep and appetite, and difficulty concentrating.[18] A study revealed that female students at private universities have a higher prevalence of moderate to severe/extremely severe levels of depression compared to male students.[19] Epidemiological research conducted in the Middle East and North Africa regions indicates depression rates ranging from 13% to 18%.[20] Depression rates in women are up to twice as high as those in men.[21] Depression rates in Arab regions and the Gulf show significant variation across different studies.[22] A research study in Egypt found that 15.3% of female adolescents experienced depression. The rate among high school students in Oman was 17%, while in Saudi Arabia, it was 33.4%.[23] In Iran, depression ranks as the third most prevalent health issue[24], research findings reveal that 4.1% of adults experience depressive disorders, with women being nearly twice as likely as men to develop depression disorder.[7] Almost 11 million adults in the United States (U.S.) who are 18 years and older go through a major depressive episode each year.[25] Depression is projected to become the primary source of disease burden and morbidity globally by 2030.[26] Depression accounts for 6.2% of all diseases, ranking second after ischemic heart disease. The World Health Organization reports that depression is the primary cause of lost years of healthy life for women between the ages of 15 and

44.[27] Mental illness occurs when various factors interact, causing certain brain chemicals and neurotransmitters to malfunction, ultimately resulting in the onset of mental disorders. Changes in brain signal transmission at the chemical synapse level are crucial for the onset of mental disorders. Any alteration in a neurotransmitter's chemical structure or imbalance at any stage of this intricate process can impact behaviors, moods, thoughts, and emotions.[28] Depression is frequently linked to low levels of neurotransmitters like dopamine, noradrenaline, serotonin, and γ amino butyric acid (GABA).[11] Depression and other mental disorders can impact individuals of all backgrounds, and distinguishing between mental well-being and illness can be challenging. Modern advancements in technology have led to many contemporary jobs being less physically demanding, yet the mental stress associated with them has risen. Mental illness can hinder an individual's ability to manage their daily responsibilities at home and work. Feelings of shame and guilt may prevent them from seeking help for their mental health issues.[29] Depression impacts numerous individuals, but there are several treatments available that effectively manage these conditions, such as medication and psychotherapy.[30] Psychiatric drugs offer a readily available and efficient solution for this mental health issue. Nonetheless, those who use medication for depression may encounter side effects such as restlessness, increased weight, sexual problems, and emotional blunting.[31] Some studies reported that since the 1950s when antidepressants were introduced, the options for pharmacological treatments for depressive disorder have grown, yet their effectiveness has largely stayed the same. Around 50% of patients starting antidepressant therapy do not respond significantly after the first attempt. Additionally, even after trying multiple treatments, about 30% of patients still do not achieve remission.[32] The functioning of the brain relies on enzymes and coenzymes to regulate the availability and balance of macronutrients. Micronutrients play a crucial role in various coenzyme systems and can contribute to the complete activation of enzymes involved in neurotransmitter synthesis.[33] People with mood disorders may have subclinical deficiencies because of genetic differences that make some more susceptible or because of past changes in dietary patterns.[11] Numerous nutrients play a crucial role in brain function, with some, like folate, impacting various neurobiological processes linked to depression.[12] Folate supplementation could be considered for individuals who do not respond to traditional treatment or favor non-pharmacological options. Folate is typically obtained from food,

and a lack of folate is linked to depression. In the brain, folate plays a crucial role in producing neurotransmitters that influence mood.[34]

1.3 Folic acid

Folic acid (FA), is a synthetic molecule produced artificially. It is commonly used as a dietary supplement and in fortifying food products. This fully oxidized compound contains just one glutamate residue and is typically not found in the bloodstream unless consumed through food or supplements.[35] Its effectiveness in the body relies on the activity of the dihydrofolate reductase (DHFR) enzyme in the liver, which operates at a slower pace in humans compared to other functions. To carry out its biological role within the folate cycles, folic acid must undergo a process of reduction by dihydrofolate reductase to become dihydrofolate (DHF), which is then further converted to tetrahydrofolate (THF) before finally transforming into the biologically active 5-methyltetrahydrofolate (5-MTHF).[36]

1.4 Folinic acid

Folinic acid and folic acid have distinct chemical compositions, but both function similarly.[37] Folinic acid, also known as Leucovorin, is a synthetic molecule that is a derivative of THF with a 5-formyl group. It can be easily converted to 5-MTHF and 5–10 MTHF without the need for DHFR action. This substance is used to reduce the negative impacts of specific chemotherapy drugs that interfere with folate metabolism by inhibiting DHFR, like methotrexate. Additionally, it is used in conjunction with other conditions treated with anti-folate medications.[36]

1.5 Folate

Folate, a B complex water-soluble vitamin, includes natural food folate, which is found in the body as MTHF (the main form in the blood) and THF (the active form).[38] It is made up of a p-aminobenzoic acid, a pterin ring, and an γ-linked tail with one or more L-glutamic acid molecules. The structures of folates vary based on the substitution of the pterin ring and reduction and the length of the glutamate chain.[35] Naturally occurring forms are usually either fully reduced (at the 5, 6, 7, and 8-position) in THF compounds or partially reduced (at the 7, and 8-position) in DHF forms. Different folate forms vary in a one-carbon unit attached to the N10 and/or N5 positions of the pteridine ring, such as methyl (5-CH3), methenyl (5,10-CH), formyl (5- or 10-HCO), formimino

(5-CHNH), and methylene (5,10-CH2). Mammalian cells are unable to produce folate, and its deficiency has been linked to various disorders.[39]

1.5.1 Structures

Figure 1

The chemical structures of folic acid, folate, and folinic acid [40]

FOLIC ACID
$C_{19}H_{19}N_7O_6$

5-METHYLTETRAHYDROFOLATE
$C_{20}H_{25}N_7O_6$

FOLINIC ACID
$C_{20}H_{23}N_7O_7$

1.5.2 Folate vitamers (derivatives)

Folate is a general term for nine folate derivatives (FDs) present in mammalian and microbial cells, including folic acid, tetrahydrofolate, dihydrofolate, 5,10-methylenetetrahydrofolate (5,10-CH2-THF),5,10-methyltetrahydrofolate(5,10-CH+-THF),5-methyltetrahydrofolate,10-formyltetrahydrofolate (10-CHO-THF), 5-formiminotetrahydrofolate (5-NH=CH-THF), and 5-formyltetrahydrofolate (5-CHO-THF). [41]

Figure 2

The chemical structure of folate and its derivatives [39]

Folic acid (FA) Dihydrofolate (DHF)

Pterin p-Aminobenzoic acid Glutamate

R_1	R_2	Vitamers
H	H	Tetrahydrofolate (THF)
CH_3	H	5-MethylTHF
CHO	H	5-FormylTHF
HC=NH	H	5-FormiminoTHF
H	CHO	10-FormylTHF
$-CH_2-$	$-CH_2-$	5,10-MethyleneTHF
=CH-	=CH-	5,10-MethenylTHF

Note. The chemical structure of folate and its derivatives, labeled as R1 and R2, match the atom replacement shown in the accompanying table. The variable "n" indicates the number of glutamate residues linked, which can vary from 1 to 8.

1.5.3 Sources

The term folate is derived from the same origin as foliage and is a crucial micronutrient that mammalian cells cannot produce on their own.[42] Therefore, it is necessary to consume folates from natural sources[39] such as leafy green vegetables like asparagus, okra, mustard greens, brussels sprouts, spinach, peas, broccoli, bell peppers, beets, green beans, swiss chard, kale, turnip greens, green beans, collard greens, carrots, and iceberg lettuce.[10] Folate and folic acid can also be obtained from cooked dry beans such as black-eyed peas, lentils, kidney beans, pinto beans, black beans, garbanzo beans, lima beans, and navy beans, as well as bread, rice, cereals, pasta, [43], fruits like orange juice, oranges, strawberries, grapes, cantaloupe, grapefruit, raspberries, kiwi, dried fruit like almonds and walnuts, liver, cheese, eggs, sunflower seeds, peanuts, peanut butter, nuts, and seeds.[42] Synthetic folic acid is the primary way to fulfill daily requirements [39]and is produced locally by the gut microbiome, primarily as monoglutamylated folate, which is the form most readily absorbed, intestinal bacteria serve as one of the sources of this vitamin.[44]

1.5.4 Dosage

Maintaining sufficient folate levels is crucial, yet many people consume less than the recommended daily amount advised by national health authorities,[12] the current recommended daily intake of folate for non-pregnant adults who are 19 years old and older is 400 mcg.[16] Folate's total body content is estimated to be around 15 to 30 mg, with about half stored in the liver and the rest spread among different body tissues and the blood.[45] Serum folate levels are commonly utilized to evaluate folate levels. A serum folate level higher than 3 ng/mL suggests sufficient folate levels.[12]

1.5.5 Function

Evidence from both open and controlled trials indicates that folic acid and methyl folate replacement therapy may have an impact on mood, cognitive function, arousal, and social function.[46] Folates, particularly methyl folate, play a vital role in the nervous

system throughout life. They are essential for various methylation reactions within the central nervous system (CNS), including purine, thymidine, nucleotide, and deoxyribonucleic acid (DNA) synthesis. Additionally, folates are involved in nongenomic and genomic methylation processes, contributing to tissue growth, differentiation, and repair. These compounds also serve as cofactors for various biological functions.[47] Neural tube defects including spina bifida in fetuses, genetic disorders in childhood, and depression/cognitive decline in the elderly are all currently of interest.[46] There is a focus on the potential role of vitamin B9 in preventing central nervous system development disorders and mood issues.[48] Folate is essential for creating healthy red blood cells and plays a vital role in times of rapid growth, such as pregnancy and fetal development.[49] Administering folic acid inappropriately when there is a vitamin B12 deficiency can result in neurological and, subsequently, hematologic relapse.[48]

1.5.6 Fortification

Fortification is a method of enhancing staple foods with vitamins, minerals, or both, to improve the nutritional content consumed by the majority of the population. Fortified foods like rice, pasta, bread, and certain breakfast cereals in the United States may include extra vitamins, minerals, or both as specified by the regulations of the U.S. Food and Drug Administration (FDA). These rules permit both voluntary and compulsory fortification.[50]

The World Health Organization and the Food and Agricultural Organization of the United Nations (FAO) released guidelines in 2006 to assist countries in establishing the Target Fortification Level, Maximum Fortification Level, Minimum Fortification Level, and Legal Minimum Level of folic acid for fortifying flour.[51] The British Government revealed in November 2018 a proposal to seek input on requiring the fortification of flour with folic acid.[52]

1.5.7 Bioavailability

Bioavailability is the extent to which a consumed micronutrient is absorbed and utilized by the body. Factors that can affect bioavailability include the chemical form of the fortificant, the food matrix and the dietary composition, the overall dietary intake, the nutrient composition of the fortification mix, and the physiological state of the

individuals being targeted.[53] The bioavailability of folate from different foods is influenced by factors like the food's composition, the breakdown of polyglutamyl folates in the intestines, the possible degradation of delicate folates during digestion, and the presence of dietary elements that may enhance folate retention during digestion.[54] Dietary folate is less bioavailable than folic acid. Folic acid is considered to be fully bioavailable when consumed as a supplement, whereas the bioavailability of folic acid in fortified food is estimated to be around 85% compared to supplemental folic acid. This variation occurs because the natural polyglutamate form of dietary folate is hydrolyzed into the monoglutamate form in the intestinal tract prior to absorption. In most cases, folate is converted into 5-methyltetrahydrofolate in the liver, which is the standard form found in the bloodstream and utilized by body tissues.[55]

1.5.8 Folate deficiency and elevation

Folic acid deficiencies have also been associated with symptoms of depression in adults.[10] Folate deficiency can stem from various factors, including diet, lifestyle choices, as well as pathological and pharmacological processes.[56] Causes of folate deficiency include alcoholism, poor nutrition, issues with nutrient absorption, and utilization, certain medications being used simultaneously, and many times, genetic variations in folate-dependent enzymes can lead to metabolic disorders.[48] Because methylenetetrahydrofolate reductase (MTHFR) genetic variations are common in the general population and raise concerns about reduced enzyme function leading to decreased levels of biologically active L-methylfolate.[57] Inadequate dietary consumption is the primary reason for folate deficiency. Elevated folate turnover, such as during breastfeeding, pregnancy, hematological issues, and skin conditions like psoriasis, can also result in folate deficiency. Additional primary factors that contribute include issues leading to malabsorption in the gastrointestinal system. Anticonvulsants, oral contraceptives, and folate antagonists like methotrexate can also lead to folate deficiency. Severe folate deficiency is caused by inborn errors in folate metabolism.[58] Folate can be lost from foods when cooking, preparing, or storing them, especially when exposed to heat and oxidation[59], this is because these vitamins are heat, light, air, and acid-sensitive. On the other hand, grinding food can enhance the release of additional folates.[60] Adults with symptoms of depression have been associated with deficiencies in folic acid. For instance, researchers have discovered a correlation between depression symptoms and reduced plasma folate levels.[61] Studies also

suggest that folate deficiency could lead to neuropsychiatric disorders such as endogenous depression.[62] Folate deficiency is linked to a higher chance of neurological conditions and depression.[10] Adding folic acid to antidepressant medication boosts its effectiveness. The optional enrichment of food, easy access to affordable folic acid supplements, and required inclusion of folic acid in grains could result in an overabundance in one's diet, potentially causing various negative effects. The negative consequences can affect various aspects of public health, as research indicates that excessive folic acid consumption could potentially interfere with medications, increase the risk of cancer, and hinder fetal development.[56] Consider the potential risks of taking high doses of folate. Folate supplements could hide a vitamin B12 deficiency (pernicious anemia), so it is important to be cautious with at-risk individuals to prevent overlooking this condition. Furthermore, there are worries about the negative impacts of unprocessed synthetic folic acid on depression.[63] Early data indicates that using L-methyl folate instead of folic acid for supplementation may help reduce the risk [64].

1.5.9 Physiology of absorption, metabolism, and excretion

1.5.9.1 Absorption, transport, and storage

Polyglutamates are the main form found in foods rich in this vitamin, such as green vegetables, liver, kidney, yeast, and egg yolk. These compounds can dissolve in water and must undergo a conversion process into the monoglutamate form with the help of the enzyme γ-glutamylhydrolase, also referred to as folate conjugase,[65] in the intestinal mucosa before they can be absorbed [66] through a saturable pH-dependent process. Consuming pharmacological levels of folate in the monoglutamate form results in absorption through a nonsaturable process that includes passive diffusion.[67] Folate monoglutamates, such as 5-methyltetrahydrofolate, circulate in body fluids like cerebrospinal fluid (CSF) and serum.[67] The liver can absorb a significant amount of this folate, metabolizing it into polyglutamate derivatives before either keeping it or discharging it into the bile or blood. Roughly two-thirds of the folate found in plasma is attached to proteins. Different levels of plasma folate are linked to proteins with low affinity, predominantly albumin, accounting for approximately half of the bound folate. Additionally, plasma contains small amounts of high-affinity folate binders.[65] Folate levels in cerebrospinal fluid are notably two to three times higher compared to levels in

the bloodstream.[67] Confirmed that 5-MTHF is transported actively across the blood-brain barrier through the choroid plexus.[68] The relationship between CSF and serum folate is disrupted when folic acid is used in clinical treatment, as the blood-brain barrier (BBB) restricts the passage of 5-MTHF. This could be due, at least in part, to the convulsive nature of folate derivatives as shown in experiments. Mammalian cells cannot produce folates on their own, so they have developed specialized carrier-mediated transport systems for delivering and absorbing folate derivatives from food.[48] The three main types of transport systems are the reduced folate carrier (RFC), the proton-coupled folate transporter (PCFT), and a small family of folate receptors (FRs) encoded by three distinct genes: folate receptor alpha (FRα), folate receptor beta (FRβ), and folate receptor gamma (FRγ). Both FRα and RFC play roles in transporting folates across the blood-brain barrier and into neuronal cells.[69] Folate's total body content is estimated at 15 to 30 mg, with approximately half stored in the liver and the rest in body tissues and blood.[45]

1.5.9.2 Metabolism and excretion

Folic acid or folate, also called pteroylglutamic acid, consists of a pteridine ring attached to p-aminobenzoic acid, which is further connected to glutamic acid.[48] Active folates are transformed into dihydrofolate and tetrahydrofolates by adding two or four hydrogen atoms through enzymatic reduction, facilitated by the DHFR enzyme. Following this, L-methylfolate, folate's active form is generated from THF through the enzyme methylenetetrahydrofolate reductase (Figure 3).[57] Certain folate derivatives within cells contain extra glutamic acid residues, forming folylpolyglutamates.[48]

Folate monoglutamate is transformed into its polyglutamate form by the enzyme folylpolyglutamate synthetase before storage in tissue or use as a coenzyme. Upon release from tissues into circulation, folate polyglutamate is converted back to the mono-glutamate form by γ-glutamyl hydrolase. Enzymatic reduction and resynthesis into the polyglutamate form are necessary for folates to operate in single-carbon transfer reactions.[65]

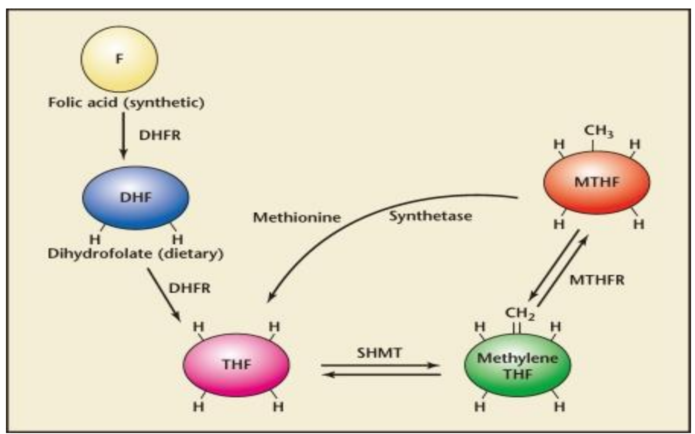
Understanding folate metabolism is crucial, with the folate cycle being a key component (Figure 4) [57]. In this cycle, serine donates a carbon unit to tetrahydrofolate (THF) and undergoes a series of reductions to form formyl (CHO), methylene (CH2), and

methenyl (CH) derivatives, leading to the production of 5-MTHF. This 5-MTHF transfers a methyl group to homocysteine, converting it into methionine with the help of an enzymatic reaction that includes vitamin B12 and methionine synthase. The cycle continues with the regenerated THF initiating a new round of methyl group synthesis. These methyl groups move from methionine to S-adenosylmethionine (SAM), which is the main provider of methyl groups in different methylation reactions in the body. These reactions take place in the nervous system and impact lipids, proteins, nucleoproteins, and monoamines. SAM also regulates methionine synthase activity. Additionally, converting various carbon-linked folate derivatives in the folate cycle provides carbon units for vital metabolic pathways, particularly the synthesis of purines and pyrimidines facilitated by 5,10-methylene-THF. This process is crucial for nucleotide, ribonucleic acid (RNA), DNA synthesis, and overall genetic function [48].

Folic acid is eliminated through feces and urine but can also be broken down through catabolism into smaller compounds. The majority of excreted substances in humans consist of these breakdown products of folate. The first stage of folate breakdown involves splitting intracellular folylpolyglutamate at the C9-N10 bond. The resulting p-aminobenzoylpolyglutamates are converted into monoglutamate, which is then N-acetylated before being excreted.[65] While the breakdown of folic acid is a well-understood process, the breakdown within cells is usually not a significant factor due to the presence of proteins, antioxidants, and specific enzymes that limit the formation of oxidizing agents. When folic acid intake is reduced, serum levels decrease rapidly within a few days. Since the body's folic acid reserves are relatively low, a lack may develop in 1 to 6 months, depending on utilization rate and nutritional status.[66]

Figure 3

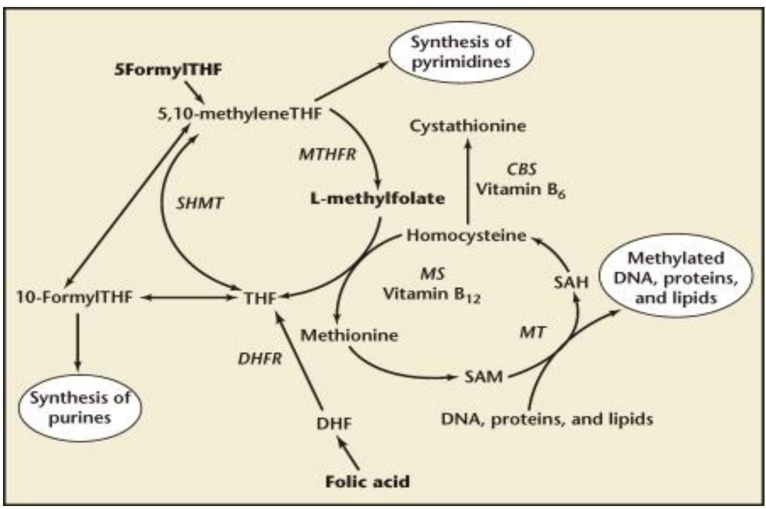
Conversion of folic acid into l-methylfolate [57]



Note. CH2 represents methylene, CH3 signifies a methyl group, DHFR denotes dihydrofolate reductase, DHF stands for dihydrofolate, H represents hydrogen, F stands for folic acid, SHMT is an abbreviation for serine hydroxy-methyl transferase, and THF refers to tetrahydrofolate.

Figure 4

Folate metabolism pathway [57]



Note. DHF stands for dihydrofolate, CBS is cystathionine beta-synthase, DHFR represents dihydrofolate reductase, MT refers to a methyltransferase, MS is shorthand for methionine synthase, MTHFR denotes methylenetetrahydrofolate reductase, SAM represents S-adenosylmethionine, SAH stands for S-adenosylhomocysteine, THF signifies tetrahydrofolate and SHMT is an abbreviation for serine hydroxymethyltransferase.

1.5.10 Mechanism of action of folate in depression

Studies on folate metabolism have offered insights into the link between low folate levels and depression. Folate is converted into S-adenosylmethionine (SAMe) in the body.[70]

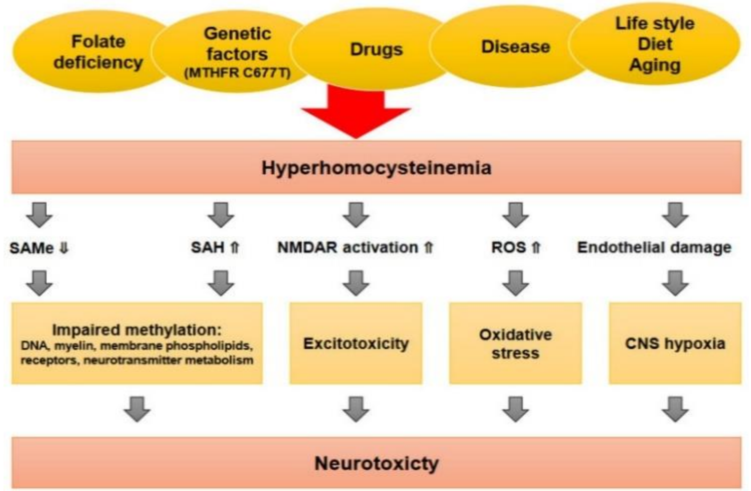
Folate forms (levomefolic acid, folic acid, folinic acid, 5-methyltetrahydrofolate or methylfolate and l-methylfolate), [71] can change into one another through the one-carbon cycle, a biochemical pathway involved in methylation processes in brains and our bodies. Numerous crucial metabolic processes are controlled by this cycle, such as the production of the neurotransmitters norepinephrine (NE), dopamine (DA), and serotonin (5HT) which are implicated in depression. Disturbances in this process could contribute to depression.[72]

Different forms of folate have varying levels of bioavailability. An active form of folate is necessary to produce serotonin, dopamine, and norepinephrine in the central nervous system. Folic acid needs to undergo a four-step conversion in the liver and intestines to become l-methylfolate, which is a biologically active form of folate capable of crossing the blood-brain barrier. This form aids in the synthesis of tetrahydrobiopterin (BH4) by assisting in the regeneration of BH4 from its oxidized state.[12] BH4 activation is crucial for the enzymes tryptophan hydroxylase and tyrosine hydroxylase, which are responsible for producing serotonin, dopamine, and norepinephrine.[25] A lack of folate can result in decreased levels of certain neurotransmitters, which may increase the likelihood of experiencing depression. Folate insufficiency also affects other biological functions related to depression.[12] Low levels of folate can cause an increase in homocysteine levels, resulting in hyperhomocysteinemia. Studies indicate a strong link between the severity of depressive symptoms and high homocysteine levels [73] because of the toxic effects on brain cells.[3] This disruption in the metabolic cycle can result in higher levels of S-adenosylhomocysteine (SAH) and lower levels of S-adenosylmethionine (Figure 5).[74] Elevated levels of homocysteine and decreased SAMe can lead to the activation of N-methyl-D-aspartate (NMDA) receptors, which are involved in excitatory neurotransmission. Overactivation of NMDA receptors may disturb the equilibrium of neurotransmitter systems, resulting in oxidative stress and neuronal excitotoxicity.[75] Higher production of reactive oxygen species (ROS) and impaired endothelial function linked to hyperhomocysteinemia can harm vascular

integrity and vascular health.[76] This endothelial dysfunction can lead to decreased blood flow and nutrient delivery to the brain, which may exacerbate mood disturbances and neuronal harm. The disruption of these chemical processes can lead to a series of effects on neurophysiology and mood control (Figure 5).[77] The dihydrofolate reductase enzyme triggers the conversion of folic acid to dihydrofolate. Individuals using lamotrigine and other DHFR inhibitors might find relief from a folate type not dependent on DHFR. Folinic acid and 5-MTHF can be converted into an active folate form without the need for DHFR, unlike folic acid.[36] Genetic studies have pinpointed a distinct genetic variation in the methyltetrahydrofolate reductase enzyme, known as the C677T TT genotype, as a contributor to depression. This specific genotype impacts the enzyme involved in folate metabolism.[78] The enzyme methylenetetrahydrofolate reductase is crucial for the one-carbon metabolism of folinic acid and folic acid; however, the C677TT polymorphism, a common genetic variation in individuals with depression, affects this enzyme and can hinder the conversion into l-methylfolate [79]. Individuals with this genetic variation may find 5-MTHF more effective than other folate forms in reducing depressive symptoms as it can cross the blood-brain barrier without needing MTHFR.[61] Consequently, biological research shows that folate has a significant impact on the development of depression.[12]

Figure 5

Mechanisms of homocysteine-induced neurotoxicity [12]



Note. The figure illustrates the different processes involved in the neurotoxic effects of high levels of homocysteine. Several factors, such as diseases, aging, folate deficiency, and MTHFR C677T polymorphism, can lead to elevated homocysteine levels. Increased homocysteine levels disturb essential biochemical processes in the central nervous system. This disruption triggers a series of events, including an increase in S-adenosylhomocysteine (SAH) levels and a decrease in S-adenosylmethionine (SAMe) levels. This imbalance results in heightened oxidative stress because of increased production of reactive oxygen species (ROS). The subsequent endothelial dysfunction and oxidative stress contribute to neurotoxicity and are associated with the development of depressive illness. The heightened oxidative stress and disrupted biochemical pathways ultimately play a role in the pathogenesis of depressive symptoms. The figure has been revised based on[77].

1.6 Problem statement

Mental disorders are one of the threats to human health that is expanding most quickly, with depression being the most common [29]. According to one study, folate levels are not inversely related to depression [80], and according to several studies, folate deficiency is consistently associated with a high risk of depression, longer depressive episodes, more severe depressive symptoms, and an increased risk of relapse and low levels of folate have been linked to a poorer response to drug treatment.[81] Nevertheless, the literature on discrepancies in folate levels between individuals either without or with depression has been incongruent [72]. Based on the studies, this study is the initial local study to determine the correlation between levels of serum folate and depression among female students based on a limited age period of 18-30 years old.

1.7 Research Questions

Is there any association between levels of serum folate and depression?

What is the association between levels of serum folate and depression?

1.8 Objectives

1.8.1 Main objective

To determine the relationship between serum folate levels and depression among adult female students at An-Najah National University in Nablus, Palestine.

1.8.2 Other objectives

To assess the prevalence of socio-demographic data.

To assess the prevalence of food types, lifestyle behaviors, and clinical characteristics.

To evaluate the concentration of levels of serum folate.

To assess the prevalence of depression, anxiety, and stress.

To compare general characteristics by serum folate levels.

To compare diet, lifestyle behaviors, and clinical characteristics by serum folate levels.

To compare general characteristics by depression severity.

To assess the correlation between levels of serum folate and depression severity.

1.9 Significance of the study

The importance of the study was to help to know the levels of serum folate among females and enable them to improve its level and fade the effects of deficiency like depression, by addressing this disorder through comprehensive management approaches and effective prevention strategies, we can alleviate the substantial burdens they impose, improve overall well-being, and improve the quality of life for individuals and communities worldwide.[12] Also to make sure that there is an actual correlation between levels of serum folate and depression and the possibility of using folic acid to relieve the symptoms resulting from it or improve the treatment of depression because, despite advancements in antidepressant treatments, their effectiveness remains limited,

with a significant percentage of patients not responding to it adequately. So combining nutraceuticals especially folate in both methyl folate forms and folic acid, may offer a unique and safer option for managing depression.[82]

1.10 Hypothesis

1.10.1 Alternative non-directional hypothesis

There is a correlation between levels of serum folate and depression.

1.10.2 Null hypothesis

There is no correlation between levels of serum folate and depression.

1.11 Literature

1.11.1 The Basis for folate deficiency and elevated levels

Various factors and conditions are linked to folate deficiency, including medications like specific anticonvulsants, antibiotics, and oral contraceptives, as well as antifolate cancer treatments. Other contributors include malabsorption syndromes, chronic illnesses such as autoimmune disorders like rheumatoid arthritis, genetic folate metabolism disorders like 5,10-methylenetetrahydrofolate reductase deficiency, inadequate dietary folate intake, alcoholism, and increased utilization during pregnancy.[48] [59] [60]

1.11.1.1 Influence of diet on folate levels

Eating a healthy diet is associated with a reduced risk of depression by raising folate levels in the blood. Conversely, an unhealthy diet is linked to an increased likelihood of experiencing depression by lowering vitamin levels in the blood.[7] Healthy diets typically include more foods rich in folate, which can help prevent depression.[11] In a case-control study, 110 individuals diagnosed with depression and 220 healthy participants were enlisted to investigate the association between dietary habits and depression with serum folate as a mediator. The results suggest that dietary patterns may affect depression by influencing folate levels in the blood.[7] In a 1999 diet intervention study, the effectiveness of natural dietary folate in enhancing folate levels was examined. A group of 66 women and 28 men volunteered for the study, which

targeted healthy individuals aged 18–45. The results suggest that eating more foods rich in folate such as citrus fruits and vegetables can enhance folate levels.[83]

1.11.1.2 Influence of body mass index on folate levels

In 2015, a study investigated the connection between metabolic factors and body mass index (BMI) with serum folate levels in 3767 adults from a nationally representative sample. The findings indicated that obesity is linked to lower serum folate levels, which align with reduced folate intake. [84] A review of past studies on the link between obesity and folate was conducted. Out of the 17 studies reviewed, 12 focused on the folate levels of the participants. The findings suggested that obese and overweight individuals typically exhibit lower serum folate levels in comparison to those of normal weight.[85] A research study investigated how folic acid affects obesity by analyzing information from the Korea National Health and Nutrition Examination Survey (KNHANES VII 2016–2018), involving 6394 adults between 19 and 80 years old. The results revealed a significant inverse correlation between obesity and folate levels.[86]

1.11.1.3 Influence of caffeine on folate levels

Caffeine consumption (in mg/day) from coffee, chocolate, cola, tea, and other soft drinks.[87] The impact of coffee on health has garnered significant interest.[88] The study shows that the consumption of caffeinated beverages can have a negative impact on serum folate levels, based on the connections found between serum folate levels, caffeine intake, and dietary folate intake.[89] A group of 486 healthy adults (250 males, 236 females) with an average age of 39 years, plus or minus 14 years, were included in the study. The research focused on analyzing the levels and factors influencing serum folate levels in a healthy adult community in Crete, Greece. The study found a negative association between levels of serum folate and coffee intake.[90] In an open-labeled randomized study without blinding, researchers examined how tea affected the absorption of folic acid (0.4 mg and 5 mg) in healthy volunteers. The study aimed to explore any potential interaction between folic acid and tea in the body. The latest findings indicate a potential in vivo interaction between tea and folic acid, where even small amounts of black and green tea extracts can reduce the body's ability to absorb folic acid.[91]

1.11.1.4 Influence of smoking and passive smoking on folate levels

The World Health Organization identifies tobacco smoking as one of the top 10 health risks [92], estimating that 1 billion men and 250 million women are current cigarette smokers. Smoking a single cigarette releases over 1015 free radicals and other oxidants, with more present in the cigarette tar. Folate levels in cigarette smokers are lower compared to non-smokers, as indicated by research showing decreased blood and tissue concentrations of serum folate in smokers.[93] Smoking exposure in non-pregnant adults is linked to lower folate levels [94], a study was conducted to investigate the impact of smoke exposure on red blood cells and serum folate levels. The results showed that individuals, whether smokers or nonsmokers, who experienced high levels of smoke exposure had lower levels of red blood cell folate than nonsmokers with minimal smoke exposure. Exposure to tobacco smoke has been associated with reduced folate levels, potentially contributing to certain health impacts of smoking, whether actively or passively.[95] Two hundred healthy Saudi men, with half being non-smokers and the other half smokers, between the ages of 19 and 50 from Riyadh, were chosen for this study. The study aimed to investigate how water-pipe smoking impacts the risk factors related to the total antioxidant capacity of healthy Saudi men. Smokers exhibited significantly lower total antioxidant capacity than non-smokers ($p<0.05$).[96]

1.11.1.5 Influence of physical activity on folate levels

Fifty animals were assigned randomly to either the regular exercise training (ET) group (n = 25) or the non-exercise training (NT) group (n = 25) for 5 weeks. The research demonstrated that muscle tissue rebuilding and repair and energy production through physical activity rely on folate as a cofactor. Engaging in regular moderate exercise training was found to lower plasma folate levels. Conversely, there were no notable alterations in levels of plasma folate when the duration of acute aerobic exercise was increased.[97]

The connection between physical activity and levels of folic acid in the plasma has not been studied. To address this gap, they looked into how prolonged endurance training and intense exercise affect total folic acid levels in the plasma of 42 highly trained male triathletes. Additionally, they found that athletes with the most training had the highest plasma folate levels.[98]

1.11.1.6 Influence of pregnancy on folate levels

Micronutrient levels fluctuate significantly during pregnancy and differ among various populations. Hemodilution linked to pregnancy leads to a reduction in the levels of several micronutrient indicators in the latter stages of pregnancy.[99] Several studies have explored the potential effects of folate levels on the likelihood of experiencing depression either during postpartum or pregnancy. A comprehensive review of these studies yielded varying outcomes. [100] One research study reviewed 709 women in Singapore and revealed that those with lower plasma folate concentrations (average 27.3 nmol/L [12.0 ng/mL]) at 26–28 weeks gestation had a notably higher likelihood of experiencing depression during pregnancy compared to women with elevated plasma folate concentrations (average 40.4 nmol/L [17.8 ng/mL]). The risk of depression did not persist after childbirth.[101] A different research involving 2,856 women from the United Kingdom showed that there were no notable connections between levels of folate intake or red blood cell folate from supplements and food during or before pregnancy and symptoms of postpartum depression.[102] A recent study involving 1,592 Chinese women revealed a decreased occurrence of postpartum depression among those who consumed folic acid supplements for over 6 months during pregnancy compared to those who took them for a shorter duration.[103]

1.11.1.7 Other factors related to folate levels

The most extensive study on serum folate levels in COVID-19 hospitalized patients is a retrospective cohort study. It indicates a high prevalence of reduced folate levels among these individuals.[104] In vitro research has examined the impact of ultraviolet (UV) on both natural folate and synthetic folic acid. Folic acid, a synthetic variant of folate, is included in supplements and foods to boost folate levels. Unlike natural folate, folic acid is resistant to UV light and heat breakdown, making it a stable choice for food preparation purposes.[56] A study suggests that feeling depressed can reduce appetite and lower folate levels, potentially hindering recovery from depression and worsening the condition.[70] A total of 4478 serum samples from healthy participants aged 2 months to 18.0 years, collected during a child population-based cohort study conducted from 2011 to 2015, underwent analysis using electrochemiluminescence immunoassay (ECLIA). The study suggests specific percentiles for serum folate based on age and sex and examines how sex and age impact folate levels in adolescents and healthy children.

Their findings indicate that folate concentrations decrease with age in both males and females.[105] A study compared 110 patients diagnosed with depression to 220 healthy individuals who filled out a semi-quantitative food frequency questionnaire. The study aimed to explore the connection between depression and dietary patterns by examining the influence of serum folate as an intermediary. Research indicates that the patients had lower levels of education compared to the individuals who were in good health. Research shows that individuals with higher levels of education are more likely to opt for nutritious food, undergo regular health screenings, and experience improved health results.[7] Adults residing in rural areas consumed notably less folate compared to their urban counterparts. This trend was also documented in other studies involving rural populations.[106] Folate deficiency rates exceeded 20% in numerous low-income countries while remaining below 5% in higher-income nations.[107]

1.11.2 Folic acid deficiency and risk of depression

Numerous studies indicate a connection between depression and a deficiency in folic acid. When the levels of folic acid are low, it can result in decreased levels of dopamine, norepinephrine, and serotonin, which can contribute to the development of depression.[72] In one study, patients with a higher rate of FA metabolism had a 1.37 times greater risk of depression.[78] Bender et al. performed a meta-analysis to investigate the disparity in folate levels among people without depression compared to those with it. The study revealed that individuals with depression had notably lower levels of folate than those without depression. Moreover, people experiencing depression tend to have reduced folate intake and lower levels of serum folate compared to individuals without depression.[72] Almeida, Ford, and Flicker found that extended folate supplementation could lead to better results in managing depressive symptoms by lessening their occurrence, intensity, and likelihood of recurrence. Interestingly, certain people may experience a folate deficiency in the central region of the body rather than the outer regions.[108] Hyland, Shoffner, and Heales examined the cerebrospinal fluid of five adolescent patients with treatment-resistant depression. They found that three patients exhibited low levels of 5-methyltetrahydrofolate in their CSF but normal folate levels in their blood, suggesting cerebral folate deficiency.[109] Studies conducted in vivo and in vitro have shown that the reduced folate carrier's functional expression at the blood-brain barrier, along with its upregulation by the vitamin D nuclear receptor (VDR), may offer a different way to transport folate to the brain. This therapeutic

approach could potentially improve brain folate levels to treat neuropathological conditions resulting from dysfunctions in the proton-coupled folate transporter or folate receptor alpha, primarily at the choroid plexus.[110] Loss of PCFT or FRα function can significantly impact brain folate absorption. Apart from the blood-brain barrier, RFC is also found in epithelial cells of the choroid plexus, which could aid in folate uptake into the central nervous system.[69] Studies on animals demonstrated that folate can reduce the permeability of the BBB and decrease oxidative nitrosative stress in the brain to avoid cognitive impairment.[111]

1.11.3 The association of folate and depression

Depression is the primary factor contributing to disability on a worldwide scale and can result in fatalities.[112] In 2008, depressive disorder was ranked as the third leading cause of the global burden of disease by the World Health Organization. The WHO has forecasted that this disorder will become the primary cause by 2030.[26]

Research involving 2,948 individuals aged 15 to 39 in the United States revealed significant decreases in erythrocyte and serum folate levels among individuals with depression when compared to those who had not previously experienced depression.[113] A study of data from the 2005-2006 NHANES revealed a connection between elevated folate levels in the blood and reduced rates of depression among 2,791 adults aged 20 and above.[114] There was a significant association among females, while no correlation among males. Nonetheless, a different analysis revealed no connections between folate consumption through dietary supplements and food and depression in 1,368 healthy Canadians aged 67–84.[115]

1.11.4 Folate in the treatment of depression

Depression has been associated with inadequate folate levels, indicating a possible use of folate in treating depressive conditions. Evidence supporting this idea shows that replacing folate has led to improved moods in patients with epilepsy or gastrointestinal issues who were deficient in folate. [116] In a randomized controlled trial that included 24 depressed patients with low red blood cell folate (RBCF) levels below 200 ng/mL, adding a dose of 15 mg/day of methylfolate to their existing treatment with standard antidepressants such as lithium, tricyclic antidepressants, or monoamine oxidase inhibitors led to improved clinical results at 3 and 6 months compared to adding a

placebo.[117] Methylfolate was chosen for its active transport into the CNS and its concentration in synaptic areas where it is essential for metabolic pathways. A retrospective survey of past cases found that psychiatric patients who were given folic acid experienced shorter hospitalizations, and showed enhanced mood and improved social functioning compared to those with low levels of folate who did not take supplemental folate.[116] A double-blind, placebo-controlled trial was conducted to examine how a daily 200-microgram folic acid supplement compared to a placebo affected the emotional well-being of 75 patients undergoing lithium therapy. The researcher discovered that including patients with bipolar and unipolar mood disorders who were taking lithium prophylaxis and giving them small daily doses of folic acid (200 pg) decreased the frequency and length of mood disorder symptoms.[118] In an open-label trial involving 20 elderly patients with depressive disorders, where only two had low folate levels, giving methylfolate (50 mg/day) by itself, instead of alongside regular antidepressants, led to a notable 81% response rate within a 6-week timeframe.[119] There is increasing evidence indicating that folate could be used alongside antidepressant medication and potentially on its own to treat depressed patients with borderline low folate levels. In a speculative scenario, folates might be beneficial in treating certain depressed patients with normal serum folate levels.[116] Research indicates that even individuals with sufficient levels of folate can gain advantages, particularly because levels of folate in the peripheral system may not accurately reflect folate levels in the central nervous system.[120]

Folate supplementation has been linked to favorable results in people with depression, including decreased length of inpatient treatment stays and enhanced effectiveness of standard therapies. [108] A recent meta-analysis showed that there was no significant variance in depressive symptoms between those who took folic acid supplements and those who took a placebo.[32] Additional studies indicate that there is insufficient support for short-term folate or folic acid supplementation compared to prolonged usage. Folate supplementation is suggested as a third-line treatment.[121] Long-term supplementation with folate may lead to better results by decreasing the occurrence, intensity, and likelihood of recurrence of depressive symptoms.[108] It is crucial to mention that taking folic acid supplements might decrease the efficacy of other psychotropic drugs.[122] Therefore, the potential interaction between folate and psychotropic medications should be taken into account before recommending

supplementation. More research is needed to fully grasp the impact of folate supplementation on psychotropic drugs. However, most individuals can safely take folate as a supplement.[123] L-methylfolate is a costly option, but it could be particularly beneficial for individuals carrying the C677T TT (vs. CC) genotype of the MTHFR enzyme. This genotype is associated with inefficient folate metabolism, and L-methylfolate bypasses the need for a further breakdown like synthetic folic acid.[124] In cases where traditional treatments do not lead to remission for people with depression, combined or dynamic treatment methods may present hopeful options. Further research on folate supplementation for depression is therefore justified.[72] Even though depression causes a significant amount of illness and death, the medications and therapies supported by the most reliable evidence do not work for almost 60% of depression patients.[125]

1.11.5 Impact of vitamin status on treatment outcome

Reynolds et al. conducted a study on 101 depressed patients who received various antidepressant medications. The study revealed that the effectiveness of the treatment was reduced in cases where serum folate levels were low. A recent study on severely depressed outpatients revealed that patients who had a positive response to desmethylimipramine treatment had notably higher folate levels compared to those who did not respond.[126] Another study found that patients with a deficiency in serum folate showed a notably higher resistance to fluoxetine therapy (which is part of a class of medications known as SSRIs.). A new study suggests that the effectiveness of the medications sertraline and nortriptyline in treating depression depends on the initial levels of RBC folate, even when all folate levels are within normal limits. Better results were seen in elderly patients with depression who had higher folate levels, particularly in those taking sertraline.[127] Antidepressant therapy is linked to an elevation in RBC folate levels. This increase is influenced by the levels of folate, with a higher increase observed in those who respond positively to the treatment compared to those who do not. SAM, the crucial methyl group provider, relies on folate for its synthesis. In certain countries, SAM is utilized in the management of depression, either on its own or alongside antidepressants. The impact has been approximated to be similar to that of tricyclic antidepressants but is better received and takes effect more quickly.[126]

1.11.6 Augmentation of antidepressants by folic acid

In 2021, a study was conducted to assess depression scores, response, and remission rates in individuals diagnosed with depression who were undergoing additional treatment with folate (L-Methylfolate or folic acid) in comparison to those receiving only serotonin-norepinephrine reuptake inhibitor or selective serotonin reuptake inhibitor therapy (SNRI or SSRI). Adjunct therapy using L-Methylfolate or folic acid was found to enhance patient response, depression scale scores, and rates of remission.[128]

In Kashan, Iran, a double-blind randomized clinical trial was carried out on 90 depression patients to investigate the impact of folic acid supplementation alongside citalopram in treating depression. The study revealed that combining folic acid with antidepressants can enhance patients' responses to treatment for major depression.[129]

The research explored how mixing low doses of venlafaxine with folic acid impacted mice. The results suggest that combining a low venlafaxine dose with folic acid may enhance its therapeutic effectiveness and allow for lower dosages.[130]

Research findings on the possible benefits of folic acid supplementation as a complementary treatment for depression alongside standard antidepressant medications have been inconclusive. During a clinical study in the United Kingdom, 127 individuals diagnosed with severe depression were randomly divided into two groups. One group was given 500 mcg of folic acid while the other group received a placebo, alongside a daily dose of 20 mg of fluoxetine for 10 weeks.[131] While there were no statistically significant effects observed in men, women who were given fluoxetine along with folic acid experienced a notably greater enhancement in depressive symptoms compared to those who were given fluoxetine along with a placebo. In the United Kingdom, another clinical study randomly assigned 475 adults with moderate to severe depression, who were already on antidepressant medications, to receive either a placebo or 5,000 mcg of folic acid daily for 12 weeks.[132] Depression measures did not show improvement in participants who were taking folic acid compared to those taking a placebo. A meta-analysis and systematic review of four trials involving folic acid (In two trials, less than 5,000 mcg/day was used, while in two other trials, 5,000 mcg/day) along with fluoxetine or other antidepressants in patients with depressive disorder, concluded that

folic acid at doses lower than 5,000 mcg/day could be helpful as a supplement to selective serotonin reuptake inhibitor (SSRI) therapy.[131] The authors stated that this conclusion was derived from poor-quality evidence. In another meta-analysis combining data from four clinical studies, it was observed that daily doses of 500–10,000 mcg of folic acid over 6–12 weeks as an additional therapy did not show a significant impact on depression indicators when compared to a placebo.[32]

Additional research has explored the impacts of 5-MTHF supplementation in conjunction with antidepressants, indicating its potential effectiveness surpassing that of folic acid.[131] A study involving 148 adults diagnosed with depressive disorder found that taking 7,500 mcg/day of 5-MTHF for 30 days, and then 15,000 mcg/day for another 30 days, alongside SSRI treatment, did not show any improvement in depression indicators when compared to SSRI treatment with a placebo.[133] However, in a subsequent study with the same design, 75 adults experienced a notable enhancement in depression symptoms when taking 15,000 mcg/day 5-MTHF alongside SSRI therapy for the entire 60-day period, as opposed to SSRI therapy combined with a placebo.[133] The researchers who conducted a meta-analysis and systematic review of three trials involving 5-MTHF (<15,000 mcg/day in one trial, and 15,000 mcg/day in two trials) alongside fluoxetine or other antidepressants, determined that 15,000 mcg/day 5-MTHF could potentially enhance SSRI therapy for individuals with depressive disorder. However, they acknowledged that limited-quality data supported this finding.[131] Furthermore, recommendations based on evidence from the British Association for Psychopharmacology (BAP) [134] and the Canadian Network for Mood and Anxiety Treatments (CANMAT) suggest that 5-MTHF could be beneficial when used alongside SSRI treatment for depressive disorders.[121]

1.11.7 Dosage of folic acid to augment antidepressants

The study by Coppen and Bailey examines if combining folic acid with fluoxetine could improve the effectiveness of the antidepressant. 127 patients were selected at random to receive either 500 micrograms of folic acid or a visually identical placebo, along with a daily dose of 20 mg of fluoxetine. All patients met the criteria for depression according to the Diagnostic and Statistical Manual of Mental Disorders (3rd ed., rev; DSM-III-R) and had a baseline depression score of 20 or higher on the Hamilton Depression Rating Scale (HDRS) (17-item version). Plasma levels of folate and homocysteine were

evaluated at the beginning and after 10 weeks to determine the significance of folic acid dosage. The study found that a 500 mg dose of folic acid did not effectively reduce plasma folate and homocysteine levels or improve the response rate in men. The study mentioned above recommends taking 2 mg of folic acid to increase plasma folate levels above 20 ng/ml in males and females.[135]

Antidepressant treatment trials require a significant amount of time to conduct, with continuation and maintenance studies extending the duration further. Integrating 2 mg of folic acid into antidepressant therapy could be seamlessly implemented in routine clinical settings. The daily supplement is affordable and safe to consume. No harm would have been done if the long-term trial results were negative. However, if they were positive, many lives and suffering would have been saved.[136]

Chapter Two

Methodology

2.1 Study design

A cross-sectional study was conducted to assess the connection between serum folate levels and depression in adult females at An-Najah National University in Nablus city, located in the northern part of the West Bank in Palestine. This university, the largest in the area, draws students from various social backgrounds and regions across the West Bank. Subjects were invited through the announcement of a research work aimed at conducting a test to measure the levels of serum folate. In addition, a questionnaire was created and used for this survey study. The questionnaire contained several variables, the questionnaire also included a commonly used scale, Arabic Depression, Anxiety, and Stress Scale-21 items (DASS-21) is a highly reliable and valid scale used to assess depression.

This study aimed to explore the common belief regarding potential connections between serum folate levels and depression.

2.2 Study population and setting

The samples were gathered from Palestinian female students of An-Najah National University at An-Najah National University Scientific Research Laboratory in Nablus, Palestine.

2.2.1 Inclusion criteria

The inclusion criteria were female students aged 18-30 and only individuals capable of providing informed consent.

2.2.2 Exclusion criteria

The exclusion criteria included: (i) Females aged <18 and 30< years old and all males, (ii) Medical conditions both physical and mental, (iii) Drug use, (iv) Pregnancy or lactation within the past year, (v) Body mass index that is equal to or greater than 40 kg/m2, (vi) B-complex injections in the form of a dietary supplement, injection, or oral form has been taken within the last three months, (vii) Inability to complete the questionnaire.

2.3 Study time

After obtaining approval from the Faculty of Graduate Studies (Appendix C) and the Institutional Review Board (IRB) of An-Najah National University on 15 March 2023 (Appendix D), sampling was conducted from 15th September to 20th October 2023. Furthermore, the data analysis, literature review, and writing of the study were ongoing until May 2024.

2.4 Sample size

The study was conducted on adult females and all males were excluded from the study. The sample size calculated for this study was 345 participants. Due to the high cost of the kit/materials, and the kit works within the limits of 180 samples, 180 samples were collected from accredited volunteers and, the sample size was smaller than the expected sample size for females (345), as per the sample size calculator. Which was calculated in two different manners:

First: An online sample size calculator is available on the website "Select Statistical Services" and is utilized for determining the precise sample size.[137]

Second: Stephen Thompson's Equation utilizes the subsequent formula to determine the

$$n = \frac{N \times p(1-p)}{\left((N-1) \times (d^2 \div z^2)\right) + p(1-p)}$$

sample size:

Where, sample size (n), population size (N), confidence level (z) set at 95% (1.96), error proportion (d) at 0.05, and population proportion (p) as a decimal of 0.65.[138]

2.5 Sampling technique

This research employed a convenience sampling method, which is classified as a type of non-probability sampling technique (non-random sampling). The research was promoted, attracting volunteers to join the study. This sample type was utilized in various socio-behavioral, psychological, and pharmacological studies. Convenience sampling was selected for its affordability and simplicity, as it allows for easy access to participants. When a sampling frame is not available, convenience sampling allows researchers to gather data that would otherwise be unattainable.[139]

2.6 Study variables

The study variables were identified and established using a comprehensive questionnaire that gathered sociodemographic data, as well as the dependent and independent variables obtained from the laboratory.

2.6.1 Dependent variable

The DASS-21 was categorized using the following scale: 0 for "Did not apply to me at all," 1 for "Applied to me to some degree or some of the time," 2 for "Applied to me to a considerable degree or a good part of the time," and 3 for "Applied to me very much or most of the time." Depression severity was classified as normal, mild, moderate, severe, and extremely severe.

2.6.2 Independent variable

Serum folate levels (continuous).

2.6.3 Covariates

This study examined sociodemographic characteristics such as age groups (18-25, 26-30) and body mass index (BMI kg/m2) was categorized as (underweight, normal weight, overweight, obese), residence area was classified as (city, village, camp), marital status was classified as (single, married, divorced), educational levels was classified as (bachelors, masters, Ph.D.), work was classified as (works, not works), family number of members was classified as (<3, 3-5, 6-8, >8), and family income was classified as (ILS/monthly) (<1500, 1501-3500, 3501-7000, >7000). Nature of food was categorized as (fruits and vegetables, sugars and starches, meats, canned and manufactured, fast food), all types of food and drinks in this questionnaire were classified as (never, 1-3, 4-6, >6), physical activity (0-1 h, 2-4 h, >4 h) smoking was classified as passive smoking (yes, no), cigarette smoking (smoker, non-smoker, ex-smoker), water-pipe smoking (yes, no), infected with covid-19 was classified as (yes, no), the environment was classified as, exposure to air pollution and sunlight (yes, no). Stress and anxiety were categorized into five levels: normal, mild, moderate, severe, and extremely severe.

2.7 Materials and methods

2.7.1 Questionnaire

2.7.1.1 Measurement of basic information

The self-report Basic Information Questionnaire (Appendix B) was implemented in this study for data collection, with guidance from the research supervisor and its authenticity was verified by many experts, including professors at An-Najah University. It was also prepared after reviewing and reading many questions and questionnaires from various relevant literature and studies. The first part of the questionnaire contained four sections, the first section was a socio-demographic questionnaire, including questions regarding age, level of education, marital status, anthropometric (weight and height), number of family members, work, monthly family income, the second section was about lifestyle habits including questions regarding nature of food, used of Folate Intake Calculation - Food Frequency Questionnaire (Fol-IC-FFQ), included questions about types of food products consumed in a usual week or month. The weekly or monthly frequency was selected according to common dietary practices in Palestine, determined by the specific food item. The daily frequency was not used to make the questionnaire simpler and avoid possible overestimation[140], physical activity, smoking, and drinks intake, a third section was about health status, including questions regarding medical conditions (physical and mental), coronavirus, pregnancy, medications, and the antioxidant intake, and the fourth section was about some environmental factors, including questions regarding pollution and radiation.

2.7.1.2 Outcome measurement

The second part of the questionnaire included the use of the Depression, Anxiety, and Stress Scale-21 items, a popular screening tool that can evaluate symptoms of depression, anxiety, and stress individually in community environments[141]. This tool includes three sub-scales: (1) the Depression sub-scale (DASS-D), which evaluates low self-esteem, hopelessness, and low positive affect; (2) the Anxiety sub-scale (DASS-A), which examines musculoskeletal symptoms, subjective experience of anxious arousal, autonomic arousal, and situational anxiety; and (3) the Stress sub-scale (DASS-S), which measures tension, negative affect, and agitation [142]. Severity ratings for the DASS sub-scales are displayed in Table 1.

2.7.1.3 Reliability

The reliability of a measurement is determined by how consistently it reflects a concept. Cronbach's alpha was used to measure internal reliability, with results reported for both the total alpha and the subscale alphas of the DASS-21 instrument. The alpha reliability coefficient can vary from 0, indicating complete independence among items, to 1, indicating a strong relationship among items. An alpha coefficient ranging from 0.70 to 0.80 was deemed satisfactory, while anything below 0.50 was unacceptable.[141] For reliability, r values below 0.5 are deemed poor, while those ranging from 0.5 to 0.75 are considered moderate, from 0.75 to 0.9 are good, and above 0.9 are excellent.[143]

The scale was translated to Arabic and validated for utilization within Arabic culture [144], as previously noted[145]. The DASS comes in two forms: a complete 42-item option and a condensed 21-item version. Both evaluate the same domains. This tool has been validated and translated in multiple other languages. Both non-English and English versions show strong internal consistency with Cronbach's alpha scores exceeding 0.7.[142] Research indicates that the DASS-21 exhibits strong internal consistency reliability, with Cronbach's alpha (α) values between 0.74 and 0.93 in various non-clinical and clinical populations.[145] Similarly, prior studies show that the DASS-21 demonstrates strong internal reliability and effectively differentiates between depression and anxiety compared to other available assessments.[146] In this study, scales of the DASS-21 had good internal consistency reliability, the overall Cronbach's alpha(α) (95% confidence interval for DASS-21 was 0.813 (95% CI = 0.771 - 0.850), α = 0.651 (95% CI = 0.567 - 0.724), for the depression scale; α = 0.666 (95% CI =0.586 - 0.736), for the anxiety scale; α = 0.576 (95% CI = 0.475 - 0.665), for the stress scale.

2.7.1.4 DASS-21 Scoring

The three sub-scales, namely Depression (DASS21-D), Anxiety (DASS21-A), and Stress (DASS21-S), each consist of seven items.[142] Items 3, 5, 10, 13, 16, 17, and 21 make up the depression subscale. The anxiety subscale is composed of items 2, 4, 7, 9, 15, 19, and 20. The stress subscale includes items 1, 6, 8, 11, 12, 14, and 18. The scale is set up in a four-point Likert scale style, where answers vary from 0 (Not applicable to me at all) to 3 (Highly applicable to me, or most of the time).[147] Each subscale's score is doubled to achieve a maximum total score of 42.[148] More elevated scores suggest increased psychological distress.[149] Levels of DAS were categorized using

predetermined limits for normal, mild, moderate to severe, or extremely severe symptom levels, as detailed in Table 1.[150]

Table 1

The severity ratings were utilized for interpreting DASS-21 [151]

Severity	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely severe	28+	20+	34+

2.7.2 Sample collection

2.7.2.1 Folate assessment

The most frequently used initial test for assessing folate levels is serum folate. This marker indicates the most recent dietary consumption and is the first indicator that hints at insufficient folate levels. Folate levels rise after consuming folic acid or folate for up to 2 hours before decreasing quickly. It has been suggested that quick elimination implies that fasting folate levels indicate diminished folates discharged by tissues.[152]

2.7.2.2 Blood sample collection

Mishaps were avoided by adhering to WHO guidelines for phlebotomy, such as wearing gloves, using alcohol wipes, disposing of needles properly, and washing hands before and after the procedure, errors were prevented.[153]

Sample collection started on 15th September 2023 until 20th October 2023 at An-Najah National University Scientific Research Laboratory in Nablus, Palestine. Participants completed a self-administered questionnaire covering their socio-demographic information, food nature, lifestyle factors, environment, health status, medication, and psychological status. Reduced folate forms are sensitive to heat, pressure, oxidation, pH, and ultraviolet light [154][155][156] and thus quickly undergo conversion or breakdown processes. Therefore, sample preparation and measurements for serum folate must be conducted in carefully controlled conditions.[157]

Serum samples were gathered to measure serum folate, which encompasses various folate forms present in the blood, commonly known as "total folate." Red blood cell folate is not tested because it is more time-consuming, costly, and complex compared to other methods. This is because polyglutamates need to be converted to monoglutamates before analysis. Red cell folate is less influenced by analytical and pre-analytical factors, making it a better reflection of folate levels over the past 36 weeks.[158] In general, serum folate is a more cost-efficient and faster method of administration compared to red cell folate. It also provides a more current indication of folate levels and intake. Additionally, the serum/plasma test can assess folate status over a 13-week timeframe[158]. Serum is the preferred choice over plasma because it may contain fibrinogen clots. Generally, both matrices provide similar results [159] from each participant following an overnight fasting period of 8 to 12 hours [160]. Folate levels in the blood can vary depending on recent dietary intake, with serum folate being influenced by acute dietary changes[161]. Participants were instructed to sit on a chair with their arms facing upward and placed on a raised table. A location in the antecubital area was chosen for the venipuncture site, and a tourniquet was applied above the elbow to limit the flow of blood from the veins. The sample was shielded from light, kept cool, and processed within a few minutes of collection. Lipemic and hemolyzed blood samples were avoided. Studies indicate that intact whole blood demonstrates better folate stability compared to hemolysate.[152] Blood samples were gathered in plain tubes without anticoagulant to prevent the rapid degradation of folate in serum at room temperature, especially when Ethylenediaminetetraacetic acid (EDTA) is present.[159] Blood samples were left to coagulate at room temperature for around 20 minutes before being spun at 3000 revolutions per minute (rpm) for 10 minutes. Once the yellow serum supernatant was removed, the white buffy layer was left undisturbed. Samples were stored at -80ºC for a few weeks until tested using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Freeze-thaw cycles were avoided because folate remains stable in serum for a maximum of three freeze-thaw cycles [162].

2.7.3 Sample analysis

Samples were analyzed by ELISA commercially available kits. The ELISA method was created in 1971,[163] a quantitative analytical techniques that display antigen-antibody reactions by detecting color changes with enzyme-linked conjugates and enzyme substrates, and are used to detect the presence and concentration of molecules in

biological fluids, are commonly referred to as enzyme immunotests, enzyme immunoassays (EIA), and enzyme-linked immunosorbent assays [164]. Molecules with very low concentrations, like vitamins, hormones, peptides/proteins, and drugs, exhibit a strong specificity towards antibodies or antigens created for them.[165] This high level of specificity arises from the difficulty for an antibody to attach to a molecule that is not its own antigen. Consequently, this technique allows for precise substance measurement at very low levels, with little possibility of interruption. In other words, if we have an antigen that is unique to a specific substance, we can identify the type and amount of the corresponding antibody present for that substance. Conversely, having the antibody allows us to pinpoint its specific antigen and the antigen's quantity using this approach. Enzyme immuno-tests are commonly known as all methods and techniques of analysis that utilize enzymes to demonstrate antigen-antibody reactions.[165] The process involves a sequence of mixing, reacting, incubating, and washing steps.[166] Enzyme-linked immunosorbent assays offer several benefits, including the prolonged durability of the reagents, the absence of radiation hazards from waste materials, the ability to analyze numerous samples quickly in diagnostic and research facilities, and cost-effectiveness.[164]

In this study, the Folic Acid ELISA Kit is a competitive enzyme immunoassay designed to quickly detect and measure folic acid in serum samples. The lab test reagent preparation included diluting the wash buffer, making a diluted solution of stabilizing and releasing agents and extracting samples in test tubes with the extraction agent. Once the extraction agent was added to the samples and the reaction was allowed to proceed, the neutralizing buffer was mixed in before being dispensed into microwells. To conduct the test, all reagents were brought to room temperature. Samples were prepared as per the extraction procedure, allowing neutralization reactions to finish. Microplate wells were set up for calibrators, controls, and specimens. Folate enzyme reagent and folate biotin reagent were introduced into the wells, gently mixed, and left to incubate. After discarding the microplate contents, wash buffer was added and removed three times. Substrate reagent was then added to the wells and incubated, followed by the addition of stop solution and gentle mixing. Absorbance was recorded at a wavelength of 450 nm 30 minutes after the stop solution was added. Finally, the product was analyzed using a microplate reader, and then calculate the results of a folate assay using a dose-response curve. It explains that a calibrator curve is made by plotting the

absorbance readings of folate standards against their concentrations. Then the absorbance of unknown samples is located on this curve to determine their folate concentration.

2.7.4 Reference values of vitamin B9 levels

No specific universal thresholds exist for evaluating folate levels. The World Health Organization suggests using metabolic indicators to establish deficiency levels. In this research, the Folic Acid ELISA kit provided the following reference values: The standard range for serum folate levels was 3.2-13.7 ng/ml.[167]

2.8 Statistical analysis

The information gathered in this research was inputted into Statistical Package for the Social Sciences (IBM's SPSS) for Windows Version 21.0. The Kolmogorov-Smirnov test was used to assess if the data were normally distributed or not. The means, standard deviations (SDs), median, and range were computed for continuous variables, while frequency and percentages were determined for categorical variables. To assess the categorical variables associated with depressive severity, the chi-squared test ($\chi 2$). Age, residence area, marital status, educational level, and work did not fulfill the assumption of the chi-squared test so we used the Fisher exact test. The Kruskal-Wallis test, a nonparametric test that relies on ranks, was utilized to examine the relationship between categorical variables, sociodemographic variables (not binary) (body mass index (BMI) group, marital status, place of residency, educational level, family income, and number of family members, nature of food, different types of food considered as sources of folate, drinks, physical activity) and continuous variable (serum folate levels), and to study the correlation between serum folate levels and depression severity (normal, mild, moderate, severe, and extremely severe), and the Mann-Whitney U test, a nonparametric test relying on ranks, was utilized to assess the relationship between categorical variables (binary) (age group, work, smoking, exposure to air pollution and to sunlight, infected by covid-19) and continuous variable (serum folate levels), The study explored correlation by utilizing Spearman's rank-order correlation coefficients, a nonparametric method that gauges the strength and direction of association between two ranked variables based on specific guidelines: .80-1.0 "very strong", 0.60-.79 "strong", 0.40-0.59 "moderate", 0.20-0.39 "weak", 0.00-0.19 "very weak".[168] A two-tailed p-value below 0.05 was deemed statistically significant.

2.9 Ethical considerations

The research was approved by the Institutional Review Board and the Scientific Research Committee of An-Najah National University of Medicine and Health Sciences. Permission to carry out the study in the scientific research laboratory. Subjects were invited through the announcement of a research work aimed at conducting a test to measure the levels of serum folate and its relation with depression for volunteers aged 18-30 years to participate in the study. Standard, written, and the same explanatory information about the study was delivered to the participants after they came to the scientific research laboratory at An-Najah University. This information contains details on the purpose, significance, confidentiality, and anonymity of the data, along with the choice of participating voluntarily. Participants who agreed to take part met the inclusion criteria as female students aged 18-30 and provided both verbal and written consent to be enrolled in the research.

Chapter Three

Results

3.1 Sample distribution according to socio-demographic data

This study is a cross-sectional study that involved 180 participants. The socio-demographic characteristics that were studied included age group, body mass index (BMI) group, marital status, place of residency, educational level, work, family income, and number of family members.

3.1.1 Distribution of the study participants according to their socio-demographic data

Table 2 illustrates that the highest age groups of study participants were 18-25 years 92.8% and 7.2% of participants aged between 26-30 years. The BMI group was classified into four groups; the highest BMI group of study participants was normal weight 50.6%, while the lowest BMI group was obese 7.2%. 49.9 % of the study participants live in the village, while 44.4% live in the city. Most of the participants in the study were single 91.1%. 90% of the study participants were at the bachelor's level. Regarding work, 89.4% do not work while 10.6% of participants work. Participants had 6-8 family members 64.4%, and 21.1% had more than eight members. 60% of the study participants have a low to middle family income, while 27.2% have a family income middle to high, but only 4.4% of the study participants have a high family income.

Table 2

Descriptive statistics of the sample characteristics (n = 180)

Variable		Frequency (*n*)	Percentage (%)
Age (Year)	18-25	167	92.8
	26-30	13	7.2
BMI (Kg/m2)	18.5)<(Underweight	24	13.3
	Normal weight (18.5-24.9)	91	50.6
	Overweight (25-29.9)	52	28.9
	30)≥(Obese	13	7.2
Residence area	City	80	44.4
	Village	89	49.4
	Camp	11	6.1
Marital status	Single	164	91.1
	Married	15	8.3
	Divorced	1	0.6
Educational level	Bachelor's	162	90.0
	Master's	16	8.9
	PhD	2	1.1
Work	Works	19	10.6
	Not works	161	89.4
Family number of members	<3	2	1.1
	3-5	24	13.3
	6-8	116	64.4
	>8	38	21.1
Family income (ILS/monthly)	<1500	15	8.3
	1500-3500	108	60.0
	3501-7000	49	27.2
	>7000	8	4.4

3.2. The prevalence of diet, lifestyle behaviors, and clinical characteristics

The frequency and proportions of food, lifestyle behaviors, and some clinical characteristics, where the highest percentage was for the nature of the food (sugars and starches, fruits and vegetables), while the meat was the lowest percentage (33.9%), (21.1%), (11.7%) respectively. Depending on the folate content of the food, the highest percentage of never intake for liver, avocado, brown bread, milk, and dark chocolate (66.7%, 42.2%, 51.7%, 43.3%, 58.3% respectively), while the highest percentage of low amount intake for the spinach, parsley, kale, and broccoli, followed legumes, zucchini, peppers, lettuce, green peas, and green beans, cucumbers, onions, eggplant, radishes, carrots, tomatoes, and cabbage, eggs, fruits, potatoes, meat, cheese (48.9%, 55.6%, 63.9%, 59.4%, 53.9%, 56.7%, 58.9%, 51.1%, 57.8%, respectively), regarding drinks the highest percentage of low amount intake, for energy drinks (48.3%) followed tea and coffee (36.7%, 36.1%, respectively), while the highest percentage of never drinks intake, for natural herbs drinks (41.1%). As for exercise, the highest percentage was for

the participants who exercise very little (60%), and for smoking the majority are not exposed to passive smoking and do not smoke cigarettes and not even a hookah (56.1%), (91.1%), (57.2%) respectively. As for exposure to the coronavirus, the majority have been infected (58.9%). As for environmental factors, there was a high percentage exposed to sunlight for a long time (56.7%) and not exposed to air pollution (63.3%). The details are shown in Table E1 in (Appendix E).

3.3 Serum levels of folate

Table 3 illustrates the mean, median, and standard deviation of the levels of serum folate was 4.82, 3.10, and 3.38 respectively. Figure 6 shows the frequency of serum folate levels, as low serum folate levels (<3.2 ng/ml) amounted to about 107, while the total frequency of normal serum folate levels was about 68, distributed as follows, the highest frequency of normal folate levels ranged between (3.2 ng/ml - 10.5 ng/ml) amounted to approximately 54, and the frequency of the upper limit of the normal level, which ranged between (10.6 - 13.7 ng /ml), was approximately 14. As for the frequency of high serum folate levels (>13.7 ng /ml), it amounted to approximately 5.

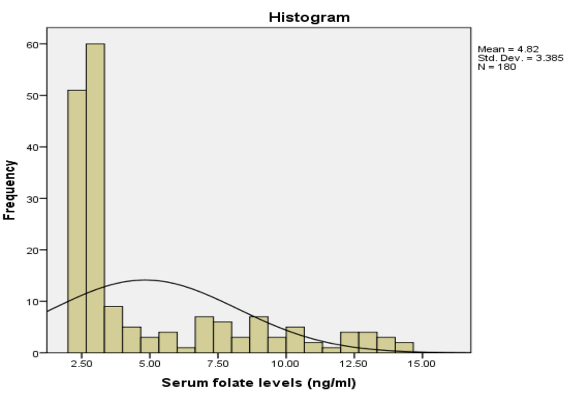
Table 3

Serum levels of folate

Mean	4.82
Median	3.10
Std. Deviation	3.38
Minimum	2.00
Maximum	14.20

Figure 6

Serum levels of folate



3.4 The prevalence of depression, anxiety, and stress is based on severity

Table 4 shows the percentage of depression as the main result of the research and the percentage of anxiety and stress as a side result of the research among the female students participating in the study. The percentage of the levels of severe and extremely severe depression among the participating students was the highest, reaching 47.2%, and 16.7% respectively, while the percentage of normal level of depression was the lowest 9.4%. As for anxiety, the percentage of extremely severe and severe anxiety was also the highest, as the percentage reached 41.7%, and 25% respectively, while the percentage of a normal level of anxiety was lowest 7.2%. Finally, with regard to stress, the highest percentage was severe and moderate levels of stress, 44.4%, and 30.6% respectively, while the lowest percentage was a normal level of stress 6.1%.

Table 4

The prevalence of depression, anxiety, and stress is based on severity

Scale	Severity	Frequency (n)	Percentage (%)
DASS-Depression	0-9 (Normal)	17	9.4
	10-13)(Mild	22	12.2
	Moderate (14-20)	26	14.4
	Severe (21-27)	85	47.2
	Extremely severe (28+)	30	16.7
DASS-Anxiety	Normal (0-7)	13	7.2
	Mild (8-9)	16	8.9
	Moderate (10-14)	31	17.2
	Severe (15-19)	45	25.0
	Extremely severe (20+)	75	41.7
DASS-Stress	Normal (0-14)	11	6.1
	Mild (15-18)	12	6.7
	Moderate (19-25)	55	30.6
	Severe (26-33)	80	44.4
	Extremely severe (34+)	22	12.2

3.5 General characteristics compared by serum folate levels (n=180)

A Kruskal-Wallis H test indicated a statistically significant variation in the levels of serum folate between the different places of residency, family number, and family income (P-value <0.05), among the residential area, family members number, and the income categories the folate concentration was the highest for the village, middle family member and high-income category. In addition, showed that there was no statistically significant variation in the levels of serum folate between the different BMI groups, marital status, and educational levels (P-value ≥ 0.05). Mann- Whitney U test indicated

that serum folate levels in the age and work were not statistically significant (P-value ≥ 0.05). A Spearman's rank-order correlation was used to establish the connection

between age, BMI group, family number, family income, and serum folate levels. There is a significant weak inverse correlation between the number of family members, and serum folate levels and a significant weak direct association between family income, and serum folate levels (r = - 0.148, P-value = 0.048, r = 0.183, P-value = 0.014 respectively), and there is no significant association between age, BMI group, and educational levels, and serum folate levels (P-value ≥ 0.05) this is shown in Table 5.

Table 5

General characteristics compared by serum folate levels (n = 180)

Characteristic		Median	χ2 (df)	*p*-value	Correlation coefficient	*p*-value
Age (Year)	18-25	3.10	_	0.474	0.053	0.476
	26-30	3.16				
BMI (Kg/m2)	Underweight (<18)	2.87	3.699 (3)	0.296	0.080	0.287
	Normal weight (18-24.9)	3.10				
	Overweight (25-29.9)	3.12				
	Obese (≥30)	5.50				
Residence area	City	2.90	17.717 (2)	<0.001	-	-
	Village	4.03				
	Camp	2.96				
Marital status	Single	3.10	_	0.464	-	-
	Married	3.10				
	Divorced	9.12				
Educational level	Bachelor's	3.10	0.144 (2)	0.931	-0.003	0.972
	Masters	3.04				
	PhD	5.14				
Work	Works	3.15	_	0.646	-	-
	Not works	3.10				
Family number	<3	5.28	16.064 (3)	0.001	- 0.148	0.048
	3-5	5.70				
	6-8	2.90				
	>8	3.12				
Family income (ILS/monthly)	<1500	3.20	8.603 (3)	0.035	0.183	0.014
	1501-3500	3.00				
	3501-7000	4.65				
	>7000	6.31				

3.6 Diet, lifestyle behaviors, and clinical characteristics compared by serum folate levels

A Kruskal-Wallis H test indicated that there was a statistically significant variation in the levels of serum folate between the nature of food, various types of food considered as sources of folate, drinks, and physical activity (P-value < 0.05). Mann-Whitney U test indicated that serum folate levels in smoking (passive, and water pipe), infected by COVID-19, and environment (exposure to air pollution and sunlight) were statistically significant (P-value < 0.05), and it also showed that serum folate levels in cigarette smoking were not statistically significant (P-value ≥ 0.05). A Spearman's rank-order correlation indicated that there is a significant moderate inverse association between drinks (caffeinated), and serum folate levels (P-value <0.05) and a significant weak direct correlation between different types of food considered as sources of folate, drinks (natural herbs), and physical activity, and serum folate levels (P-value <0.05), and there is no significant correlation between white bread consumption and the levels of serum folate (P-value = 0.831). This is shown in Table E2 in (Appendix E).

3.7 General characteristics compared by depressive severity (n = 180)

Table E3 in (Appendix E), illustrates a Chi-square or Fisher exact test that there was a significant association between place of residency, work, family income, and depression categories (P-value < 0.05), but there is no statistically significant correlation between age group, body mass index group, educational level, marital status, number of family members, and depression categories (P-value ≥ 0.05). Severe depression was positively linked with low income, living in the city, and not working. A Spearman's rank-order correlation was used to determine the relationship between age, BMI group, family number of members, family income, and depression severity. There is a significant weak inverse association between family income and depression severity (r = -0.212, P-value = 0.004), and there is no significant correlation between age, BMI group, educational levels, family number members, and depression (P-value ≥ 0.05).

3.8 The relationship and correlation coefficient between serum folate levels and depression severity (n = 180)

A Kruskal-Wallis H test indicated that there was a statistically significant difference in the levels of serum folate between the depression severity (P-value < 0.001), serum folate levels were highest for normal, followed by high in mild, moderate in moderate, and low levels for severe and the lowest for extremely severe. A Spearman's rank-order correlation was used to establish the connection between serum folate levels and depression severity. There is a significant moderate inverse correlation between serum folate levels and depression severity (r = - 0.537, P-value < 0.001), this is shown in Table 6.

Table 6

The association and correlation coefficient between serum folate levels and depression severity

	Depression severity	N	Median	χ^2 (df)	p-value	Correlation coefficient	p-value
Serum folate levels	Normal	17	12.45	64.809 (4)	<0.001	- 0.537	<0.001
	Mild	22	7.49				
	Moderate	26	6.79				
	Severe	85	2.80				
	Extremely severe	30	2.73				

Chapter Four

Discussions and Conclusions

4.1 Introduction

This research was the first research in Palestine to analyze the correlation between levels of serum folate and depression in female students at An-Najah University. This study revealed an association between levels of serum folate and depression. This aligns with findings from prior research indicating a rise in psychological issues influenced by various factors. Psychiatry is presently experiencing a crucial juncture as the dominant model, which heavily depends on medication-based treatments, has shown only minimal progress in tackling the worldwide impact of mental health challenges.[169] Acknowledging the intricate nature of factors influencing mental health, a growing body of convincing evidence emphasizes the crucial impact of nutrition on the occurrence and progression of mental disorders. This indicates that nutrition is equally important in the field of psychiatry.[170] Recognizing the significance of taking nutritional aspects into account about mental well-being emphasizes the need to broaden the range of psychiatric care to include dietary measures.[171] Insufficient nutrition is increasingly being linked to the onset and advancement of behavioral health conditions, while also potentially impeding the success of therapy and rehabilitation.[172] Suboptimal nutrition may influence the underlying pathology of depression because nutrients play an important role in the functioning of the neuroendocrine system. The International Society for Nutritional Psychiatry Research (ISNPR) supports incorporating nutritional medicine into standard psychiatric practice, highlighting the importance of policy, education, research, and health promotion to endorse this new approach,[173] including folic acid (folate) and other essential nutrients.[12] However, implementing this framework encounters challenges because of the intricate and multifaceted aspects of nutrition and mental health.[174] Depressive disorders have multiple factors contributing to their development and do not stem from a single cause. Several elements can lead to a higher likelihood of experiencing depression, such as gender, social support, socioeconomic status, stress levels, drug and alcohol consumption, epigenetic and genetic factors, the existence of medical conditions, inflammation, dietary habits, and endothelial dysfunction.[175] These elements interact and affect one another, forming an intricate network of risk factors that may lead to the onset and advancement

of depressive disorders. It is difficult to pinpoint the specific influences of separate factors on the correlation between mental well-being and diet, and analytical results may be influenced by remaining confounding variables. This study was consistent with that, as we found that there was a statistically significant relationship between residential area, work, family income (P-value = 0.017, P-value = 0.012, P-value < 0.001 respectively), and depression, as the relationship was inverse for family income, while there was no statistical relationship between age, body mass index, family members, educational level, marital status (P-value ≥ 0.05) and depression, and this was not consistent with many studies that reported the presence a relationship between these factors and depression. Studying the relationship between behavioral health disorders and diet epidemiologically can lead to reverse causation, where a poor diet can be both a consequence and a cause of these disorders, suggesting a bidirectional relationship [172]. Without healthy lifestyle habits, mental well-being can decline, making it harder to maintain healthy behaviors, resulting in a recurring cycle [176]. Imbalances in specific micronutrients can greatly affect mental well-being and mood, in addition to general dietary habits [177]. Micronutrients are essential for the metabolic processes that support the growth and healthy operation of the nervous system. Not consuming enough of these micronutrients can harm mental health and raise the likelihood of experiencing depressive disorders.[11] Not consuming enough folic acid, leading to a folate deficiency, greatly raises the risk of depression [12]. In this study, the highest frequency of low serum folate levels, and the mean and standard deviation were low, at the minimum normal range, while the median of the data was less than the normal range. This highlights various factors that impact serum folate levels, such as malnutrition and unhealthy lifestyle choices, [12] resulting in various health issues at both psychological and physical levels.

4.1.1 Serum folate levels and depression

Previous research has shown a connection between folate levels and depression, highlighting the significant role folate plays in brain function. Multiple studies have shown evidence indicating a potential link between levels of low folate and depression [178]. These results show that a lack of folate is associated with a higher risk of depression, as well as lengthier depressive episodes, more intense depressive symptoms, and a greater chance of depressive symptom recurrence [81]. The main neurobiological theory for depressive disorder focuses on the participation of monoamine

neurotransmitters like norepinephrine, dopamine, and serotonin [52]. These neurotransmitters function in distinct neural circuits in the brain, influencing the emergence of depressive symptoms [179]. Tetrahydrobiopterin (BH4) is a crucial cofactor in the synthesis of neurotransmitters like dopamine, serotonin, and nitric oxide [126]. Folate aids in BH4 synthesis by helping regenerate BH4 from its oxidized state. Insufficient folate levels may lead to decreased levels of norepinephrine, dopamine, and serotonin, increasing vulnerability to depression [52]. Individuals with depression often have low levels of folate [12]. The lower the levels of folate in the blood, the higher the percentage of depression, as the results of the current study agree with this conclusion, there was a statistically significant relationship between levels of serum folate and depression (P-value < 0.001), where the lowest level of serum folate in participants with extremely severe depression and the severe, respectively, followed by the moderate level of serum folate in participants with moderate and mild depression, and also levels of serum folate were on the high limit of normal and high, for participants without depression (normal). Also, the inverse relationship between levels of serum folate and depression ($r = -0.537$, P-value < 0.001), was consistent with the results of many of prior research. These results also align with various factors that negatively or positively affect folate levels that were studied during this study and therefore affect psychological and mental health, including depression, because folate levels are significantly associated with depression, including socio-demographic, lifestyle, and clinical characteristics. Firstly, age, in the present study the age group was young, ranging from 18 to 30 years old, there is no significant relationship between age, and serum folate levels (P-value $=0.476$), this is consistent with what was reported in some previous studies, examples of these studies are: Folic acid can affect nervous system function throughout life [55]. Some deficiencies may be linked to aging [180], with elderly individuals demonstrating a higher likelihood of low folate levels [181]. Age-related disruptions in folate transport and metabolism can also contribute to folate deficiency [182]. Folate deficiency can arise due to various factors, such as issues in the absorption process. Optimal absorption occurs within a specific pH range, while achlorhydria, common in the elderly, can significantly reduce absorption [181]. Nutritional intake declines as people get older [47]. Folate deficiency may result from connective tissue and small bowel diseases [181].

In this research, there was also a statistically significant relationship between levels of serum folate and different places of residence, number of families, and family income (P-value< 0.001, P-value = 0.01, P-value = 0.035 respectively). While there was no statistically significant correlation between levels of serum folate and the different BMI groups, and educational levels (P-value ≥ 0.05), there was a statistically significant correlation between the nature of food, and dietary folates which were present in this study, and levels of serum folate (P-value <0.05), this result was consistent with what was reported in some previous research examples of studies that agree with the results are.

Significant factors influencing daily folate intake among adults include residential area, education level, employment status, and income. Research suggests that adults residing in rural areas tend to have lower folate intake than their counterparts, with similar trends observed among unemployed, retired, and less educated individuals. Previous studies have also highlighted the impact of residing in rural areas [27], lower education levels [7], and higher BMI [84]on the risk of inadequate nutrition, including lower dietary folate intake. BMI showed a negative relationship with levels of serum folate in women who did not take folic acid supplements [183]. Additionally, individuals with lower economic status tend to have higher BMI [184], emphasizing the connection between factors contributing to poor dietary habits. This indicates that these groups are at higher risk for nutritional deficiencies, as demonstrated in Slovenia[185]. They often opt for processed foods over nutrient-dense options like fruits, vegetables, and whole grains, leading to insufficient nutrient consumption. Individuals with lower incomes are particularly impacted by the cost of food[186]. Since processed foods are typically cheaper than fresh whole foods, this presents an extra challenge for these susceptible populations[187]. Their findings reinforce the need for public health policies to address economic challenges in vulnerable populations, as the prevalence of low-cost processed foods with limited nutritional value is increasing the difficulty of maintaining healthy diets[185]. Their research revealed that across all studied population groups, the consumption of bakery and bread products significantly affects folate intake, comparable to the impact of vegetables. Certain vegetables contain high levels of folate, allowing for a greater intake of folate in smaller quantities compared to bread. Their research suggests that increasing the consumption of foods high in folate like vegetables, fruits, and whole grains can enhance folate intake among the general

population. Other studies have identified vegetables, fruits, bread, and cereal products as significant sources of dietary folate [49]. In adolescents, fruits and vegetables provided less than 30% of folate, while bakery and bread products, especially white bread, showed a higher folate contribution. Adolescents tend to consume more folate from various cereal products, such as fortified breakfast cereals, compared to other population groups[106]. These findings align with the trend of younger individuals consuming processed and refined foods more frequently. Meat, milk, and dairy products were also recognized as significant sources of folate.

In this study, there was a statistically significant inverse relationship between caffeinated drinks such as (coffee, tea, and energy drinks), smoking (water pipe), being infected by coronavirus disease (COVID-19), and levels of serum folate (P-value <0.05) and there was no statistically significant correlation between smoking (cigarette) and serum folate levels (P-value = 0.339), these results were aligned with what was reported in some previous research, drinking caffeinated beverages is linked to lower serum folate levels. Caffeine boosts the central nervous system and functions as a diuretic, leading to a faster removal of water-soluble B vitamins like folate.[89] Chemical elements in tobacco smoke react with the mentioned components, turning them into inactive substances that decrease their active levels in bodily fluids. This process could potentially change the cell's capacity to store and process folate [95]. Another discovery indicated a negative correlation between tobacco use and levels of serum folate. Smokers showed notably reduced serum folate concentrations compared to non-smokers, and the levels dropped with higher cigarette consumption [93]. Additionally, smokers were found to have lower intake of various vitamins, fruits, and vegetables, leading to reduced dietary folate consumption [188]. Low folate levels are widespread among COVID-19 patients who are hospitalized. Deficiencies and inadequacies in folate are prevalent in hospitalized COVID-19 patients and contribute to the development of severe illness. Infected with coronavirus can lead to a malfunction in the primary carrier that transports folate into intestinal cells after digestion. This carrier, known as the proton-coupled folate transporter, is a high-affinity facilitative folate carrier primarily located in the proximal jejunum and duodenum. PCFT is believed to be the main transporter of folate and folic acid into small intestine cells.[104]

In this study, there was a statistically significant direct relationship between physical activity and levels of serum folate (P-value <0.001), and there was a statistically significant inverse relationship between environment (exposure to air pollution, and sunlight) and levels of serum folate (P-value <0.05). These results were consistent with what was mentioned in some previous research. Folate serves as a co-factor in pathways utilized during physical activity, potentially raising the need for vitamins and posing a risk of low plasma concentrations.[189] Research conducted in test tubes has demonstrated that UV radiation can break down folic acid and folate in the blood of humans, a finding supported by various human studies [190]. Exposure to ambient air pollution may be greater in poor countries or communities, and the interaction between lack of nutrients and high levels of ambient air pollutants may be the main cause of various negative health effects, such as deficiencies in nutrients like folate linked to pollutant exposure.[191]

4.2 Strengths and limitations of the study

4.2.1 Strengths

This study had numerous strengths. Firstly, it is considered the first study conducted in Palestine to investigate the correlation between levels of serum folate and depression. This study provides a clearer understanding of this relationship, which can aid in diagnosing depression in students. Secondly, the participants underwent assessment using a structured research protocol along with established and standardized scales. Various covariates were taken into account, potentially impacting the results of the study. Serum folate samples were gathered instead of red cell folate samples, as the latter is susceptible to pre-analytical and analytical variables that could also have affected the study outcomes. Thirdly, in this study, the DASS-21 depression scale was chosen over the Beck Depression Inventory (BDI) scale. The key difference is that the BDI contains items like insomnia, irritability, somatic preoccupation, and weight loss, which do not effectively distinguish between depression and other emotional states. Additionally, the DASS-21 features an item assessing the devaluation of life, a factor not covered by any BDI items. Finally, the research was carried out at a single center, contributing to the increased power of the study due to the specificity of the study group.

4.2.2 Limitations

The study had multiple limitations. Firstly, it was cross-sectional, measuring baseline serum folate levels only once. As a result, causal conclusions could not be made, and the link between changes in folate levels and depression could not be evaluated. Secondly, in this study, serum folate levels were assessed, but some researchers advocate for analyzing red blood cell folate instead. Unlike serum folate, red blood cell folate offers a more consistent indication of folate status, capturing long-term stores rather than recent intake. This distinction is crucial as serum folate levels can fluctuate with immediate dietary changes, as suggested by prior studies. Consequently, incorporating red blood cell analysis in future research could yield more reliable and stable data on folate levels. Thirdly, the size of the sample was small, this is due to the lack of a sufficient budget to purchase the number of kits that cover the required sample size. Fourthly, the research was carried out at a single center, potentially restricting the applicability of the results. Finally, it relied on a limited age period.

4.3 Recommendations and Conclusion

4.3.1 Recommendations

This research aligns closely with findings from earlier studies, so it is recommended a regular intake of folate/folic acid and maintain the normal level of folate in the body, so its sources need to be consumed daily, by eating food that contains a high percentage of it, especially red meat, green vegetables, fruits, and nuts are rich sources of vitamin B9 and play a significant role in its levels. In addition, it is recommended that work be expanded on such research that is concerned with studying the content of the body of vitamins and minerals, especially folate/vitamin B9 through measuring folate levels in addition to other examinations at the level of the brain and many biochemical tests. This is because relying solely on the symptoms that the patient talks about may not be sufficient, as these can overlap with symptoms of other physical and psychological conditions. Finally, it is recommended that further studies or research should be carried out using a larger sample size.

4.3.2 Conclusion

Our findings suggest that levels of serum folate were linked to depression among Palestinian students and can be utilized as a biomarker to identify patients with depressive disorders due to folate involvement in emotional, mental, and metabolic development. It is also of great value to monitor folate intake at the recommended level is important to safeguard future generations from depression.

List of Abbreviations

Abbreviation	Meaning
BAP	British Association for Psychopharmacology
BBB	Blood-Brain Barrier
BDI	Beck Depression Inventory
BH4	Tetrahydrobiopterin
BMI	Body Mass Index
CANMAT	Canadian Network for Mood and Anxiety Treatments
CH	Methenyl
CH_2	Methylene
CH_3	Methyl
CHNH	Formimino
5,10-CH^+-THF	5,10-methyltetrahydrofolate
5,10-CH_2-THF	5,10-methylenetetrahydrofolate
5-CH_3-THF	5-methyltetrahydrofolate
CHO	Formyl
5-CHO-THF	5-formyltetrahydrofolate
10-CHO-THF	10-formyltetrahydrofolate
CI	Confidence interval
CNS	Central Nervous System
COVID-19	Coronavirus disease
CSF	Cerebro Spinal Fluid
DA	Dopamine
DASS-21	Depression, Anxiety, and Stress Scale - 21 items
DASS-A	Depression, Anxiety, and Stress Scale - Anxiety
DASS-D	Depression, Anxiety, and Stress Scale – Depression
DASS-S	Depression, Anxiety, and Stress Scale - Stress
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders (3rd ed., rev)
ECLIA	Electrochemiluminescence immunoassay
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay

ET	Exercise Training
FA	Folic acid
FAO	Food and Agricultural Organization
FDs	Folate derivatives
FDA	Food and Drug Administration
Fol-IC-FFQ	Folate Intake Calculation – Food Frequency Questionnaire
FRα	Folate Receptor Alpha
FRβ	Folate Receptor Beta
FR γ	Folate Receptor Gamma
FRs	Folate Receptors
GABA	Gamma-aminobutyric acid
HDRS	Hamilton Depression Rating Scale
5HT	Serotonin
IRB	Institutional Review Board
ISNPR	International Society for Nutritional Psychiatry Research
KNHANES	Korea National Health and Nutrition Examination Survey
mcg	microgram
5-MTHF	5-methyltetrahydrofolate
MTHFR	Methylenetetrahydrofolate reductase
N	Population size
NE	Norepinephrine
ng	Nanogram
5-NH=CH-THF	5-formiminotetrahydrofolate
NMDA	N-methyl-D-aspartate
nmol	Nanomole
NT	Non-exercise training
PCBS	Palestinian Central Bureau of Statistics
PCFT	Proton-Coupled Folate Transporter
R	Correlation coefficient
RBCF	Red blood cell Folate
RFC	Reduced Folate Carrier
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
rpm	revolutions per minute
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine

SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
SNRI	Serotonin Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
THF	Tetrahydrofolate
U.S	United State
UV	Ultraviolet
WHO	World Health Organization

References

[1] S. Viertiö *et al.*, “Factors contributing to psychological distress in the working population, with a special reference to gender difference.,” *BMC Public Health*, vol. 21, no. 1, p. 611, Mar. 2021, doi: 10.1186/s12889-021-10560-y.

[2] S. P. Chand and H. Arif, “Depression.,” Treasure Island (FL), 2022.

[3] E. H. Reynolds, “Folic acid, ageing, depression, and dementia.,” *BMJ*, vol. 324, no. 7352, pp. 1512–1515, Jun. 2002, doi: 10.1136/bmj.324.7352.1512.

[4] W. M. Sweileh, H. M. Abu-Hadeed, S. W. Al-Jabi, and S. H. Zyoud, “Prevalence of depression among people with type 2 diabetes mellitus: a cross sectional study in Palestine.,” *BMC Public Health*, vol. 14, p. 163, Feb. 2014, doi: 10.1186/1471-2458-14-163.

[5] “Palestinian Central Bureau of Statistics (PCBS),” [Online]. Available: https://www.pcbs.gov.ps/portals/_pcbs/PressRelease/Press_En_WorldHealthDay2023e.pdf.

[6] M. Ghanim, M. Rabayaa, S. Atout, N. Al-Othman, and M. Alqub, “Prevalence of anxiety and depression among Palestinian university students: a cross-sectional study during COVID-19 pandemic.,” *Middle East Current Psychiatry, Ain Shams University*, vol. 29, no. 1. 2022, doi: 10.1186/s43045-022-00238-5.

[7] M. Khosravi, G. Sotoudeh, M. Amini, F. Raisi, A. Mansoori, and M. Hosseinzadeh, “The relationship between dietary patterns and depression mediated by serum levels of Folate and vitamin B12.,” *BMC Psychiatry*, vol. 20, no. 1, p. 63, Feb. 2020, doi: 10.1186/s12888-020-2455-2.

[8] F. B. Hu, “Dietary pattern analysis: a new direction in nutritional epidemiology.,” *Curr. Opin. Lipidol.*, vol. 13, no. 1, pp. 3–9, Feb. 2002, doi: 10.1097/00041433-200202000-00002.

[9] F. N. Jacka *et al.*, “Association of Western and traditional diets with depression and anxiety in women.,” *Am. J. Psychiatry*, vol. 167, no. 3, pp. 305–311, Mar. 2010, doi: 10.1176/appi.ajp.2009.09060881.

[10] M. Bemanian *et al.*, "Folate Status as a Nutritional Indicator among People with Substance Use Disorder; A Prospective Cohort Study in Norway.," *Int. J. Environ. Res. Public Health*, vol. 19, no. 9, May 2022, doi: 10.3390/ijerph19095754.

[11] S. Demelash, "The Role of Micronutrient for Depressed Patients," *J. Neuropsychopharmacol. Ment. Heal.*, vol. 02, no. 01, pp. 2–5, 2017, doi: 10.4172/2472-095x.1000116.

[12] T. Liwinski and U. E. Lang, "Folate and Its Significance in Depressive Disorders and Suicidality: A Comprehensive Narrative Review.," *Nutrients*, vol. 15, no. 17, Sep. 2023, doi: 10.3390/nu15173859.

[13] S. Naushad, W. Farooqui, S. Sharma, M. Rani, R. Singh, and S. Verma, "Study of proportion and determinants of depression among college students in Mangalore city.," *Niger. Med. J.*, vol. 55, no. 2, pp. 156–160, Mar. 2014, doi: 10.4103/0300-1652.129657.

[14] "Depressive disorder," 2023. https://www.who.int/news-room/fact-sheets/detail/depression.

[15] R. Olum, F. N. Nakwagala, and R. Odokonyero, "Prevalence and Factors Associated with Depression among Medical Students at Makerere University, Uganda.," *Adv. Med. Educ. Pract.*, vol. 11, pp. 853–860, 2020, doi: 10.2147/AMEP.S278841.

[16] L. Zheng, X. Yu, W. Jiang, and D. Zhang, "Total folate, natural folate and synthetic folic acid intake associations with adult depressive symptoms.," *Asia Pac. J. Clin. Nutr.*, vol. 29, no. 4, pp. 846–855, 2020, doi: 10.6133/apjcn.202012_29(4).0021.

[17] J. Ndegwa, A. Mbwayo, and M. Kiarie, "Factors Related to Depression among University Students in Nairobi County, Kenya," *Int. J. Humanit. Soc. Sci.*, vol. 7, no. 2, pp. 34–41, 2020, doi: 10.14445/23942703/ijhss-v7i2p106.

[18] G. Y. Lim, W. W. Tam, Y. Lu, C. S. Ho, M. W. Zhang, and R. C. Ho, "Prevalence of Depression in the Community from 30 Countries between 1994 and 2014.," *Sci. Rep.*, vol. 8, no. 1, p. 2861, Feb. 2018, doi: 10.1038/s41598-018-21243-x.

[19] M. Kamruzzaman, A. Hossain, E. Kabir, M. A. Islam, and M. S. Ahmed, "Prevalence of depression, anxiety, stress, and their associated factors among university students in Bangladesh," *Res. Sq.*, pp. 1–20, 2022.

[20] N. Kilzieh, S. Rastam, W. Maziak, and K. D. Ward, "Comorbidity of depression with chronic diseases: a population-based study in Aleppo, Syria.," *Int. J. Psychiatry Med.*, vol. 38, no. 2, pp. 169–184, 2008, doi: 10.2190/PM.38.2.d.

[21] "Strategy for mental health and substance abuse in the Eastern Mediterranean Region 2012–2016." .

[22] Z. Al-Busaidi *et al.*, "Prevalence of Depressive Symptoms among University Students in Oman.," *Oman Med. J.*, vol. 26, no. 4, pp. 235–239, Jul. 2011, doi: 10.5001/omj.2011.58.

[23] H. A. Razzak, A. Harbi, and S. Ahli, "Depression: Prevalence and Associated Risk Factors in the United Arab Emirates.," *Oman Med. J.*, vol. 34, no. 4, pp. 274–282, Jul. 2019, doi: 10.5001/omj.2019.56.

[24] M. Khosravi, G. Sotoudeh, F. Raisi, R. Majdzadeh, and T. Foroughifar, "Comparing dietary patterns of depressed patients versus healthy people in a case control protocol.," *BMJ Open*, vol. 4, no. 2, p. e003843, Feb. 2014, doi: 10.1136/bmjopen-2013-003843.

[25] R. Altaf, I. Gonzalez, K. Rubino, and E. C. Nemec, "Folate as adjunct therapy to SSRI/SNRI for major depressive disorder: Systematic review & meta-analysis," *Complement. Ther. Med.*, vol. 61, p. 102770, 2021, doi: 10.1016/j.ctim.2021.102770.

[26] G. S. Malhi and J. J. Mann, "Depression.," *Lancet (London, England)*, vol. 392, no. 10161, pp. 2299–2312, Nov. 2018, doi: 10.1016/S0140-6736(18)31948-2.

[27] A. Siripongpan, K. Phattaramarut, N. Namvichaisirikul, S. Poochaya, and P. Horkaew, "Prevalence of depression and stress among the first year students in Suranaree University of Technology, Thailand.," *Heal. Psychol. Res.*, vol. 10, no. 3, p. 35464, 2022, doi: 10.52965/001c.35464.

[28] K. M. Davison *et al.*, "The Role of Nutrition in Mental Health Promotion and Prevention.," *Dietitians of Canada*, no. Diciembre, p. 35, 2012, [Online]. Available: www.dietitians.ca/mentalhealth.

[29] T. Ljungberg, E. Bondza, and C. Lethin, “Evidence of the Importance of Dietary Habits Regarding Depressive Symptoms and Depression.,” *Int. J. Environ. Res. Public Health*, vol. 17, no. 5, Mar. 2020, doi: 10.3390/ijerph17051616.

[30] G. Andrews *et al.*, “Royal Australian and New Zealand College of Psychiatrists clinical practice guidelines for the treatment of panic disorder, social anxiety disorder and generalised anxiety disorder,” *Aust. N. Z. J. Psychiatry*, vol. 52, no. 12, pp. 1109–1172, 2018, doi: 10.1177/0004867418799453.

[31] M. Blampied, C. Bell, C. Gilbert, and J. J. Rucklidge, “Broad spectrum micronutrient formulas for the treatment of symptoms of depression, stress, and/or anxiety: a systematic review.,” *Expert Rev. Neurother.*, vol. 20, no. 4, pp. 351–371, Apr. 2020, doi: 10.1080/14737175.2020.1740595.

[32] J. Sarris *et al.*, “Adjunctive Nutraceuticals for Depression: A Systematic Review and Meta-Analyses.,” *Am. J. Psychiatry*, vol. 173, no. 6, pp. 575–587, Jun. 2016, doi: 10.1176/appi.ajp.2016.15091228.

[33] E. A. Frazier, M. A. Fristad, and L. E. Arnold, “Multinutrient supplement as treatment: literature review and case report of a 12-year-old boy with bipolar disorder.,” *J. Child Adolesc. Psychopharmacol.*, vol. 19, no. 4, pp. 453–460, Aug. 2009, doi: 10.1089/cap.2008.0157.

[34] M. Stengler, “The Role of Folate and MTHFR Polymorphisms in the Treatment of Depression.,” *Altern. Ther. Health Med.*, vol. 27, no. 2, pp. 53–57, Mar. 2021.

[35] J. Zhang *et al.*, “The folic acid/β-casein complex: Characteristics and physicochemical implications,” *Food Res. Int.*, vol. 57, pp. 162–167, 2014, doi: 10.1016/j.foodres.2014.01.019.

[36] Y. Menezo, K. Elder, A. Clement, and P. Clement, “Folic Acid, Folinic Acid, 5 Methyl TetraHydroFolate Supplementation for Mutations That Affect Epigenesis through the Folate and One-Carbon Cycles.,” *Biomolecules*, vol. 12, no. 2, Jan. 2022, doi: 10.3390/biom12020197.

[37] B. Shea *et al.*, “Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis.,” *Cochrane database Syst. Rev.*, vol. 2013, no. 5, p. CD000951, May 2013, doi: 10.1002/14651858.CD000951.pub2.

[38] R. Iyer and S. K. Tomar, “Folate: a functional food constituent.,” *J. Food Sci.*, vol. 74, no. 9, pp. R114-22, 2009, doi: 10.1111/j.1750-3841.2009.01359.x.

[39] Wusigale and L. Liang, “Folates: Stability and interaction with biological molecules,” *J. Agric. Food Res.*, vol. 2, no. March, p. 100039, 2020, doi: 10.1016/j.jafr.2020.100039.

[40] “Folate.” https://lpi.oregonstate.edu/mic/vitamins/folate.

[41] A. Nzila, S. A. Ward, K. Marsh, P. F. G. Sims, and J. E. Hyde, “Comparative folate metabolism in humans and malaria parasites (part I): pointers for malaria treatment from cancer chemotherapy.,” *Trends Parasitol.*, vol. 21, no. 6, pp. 292–298, Jun. 2005, doi: 10.1016/j.pt.2005.04.002.

[42] “Folic Acid.,” *J. Midwifery Womens. Health*, vol. 61, no. 6, pp. 797–798, Nov. 2016, doi: 10.1111/jmwh.12584.

[43] N. Delchier, A. L. Herbig, M. Rychlik, and C. M. G. C. Renard, “Folates in Fruits and Vegetables: Contents, Processing, and Stability,” *Compr. Rev. Food Sci. Food Saf.*, vol. 15, no. 3, pp. 506–528, 2016, doi: 10.1111/1541-4337.12193.

[44] M. Rossi, A. Amaretti, and S. Raimondi, “Folate production by probiotic bacteria.,” *Nutrients*, vol. 3, no. 1, pp. 118–134, Jan. 2011, doi: 10.3390/nu3010118.

[45] “Bailey L.B., Caudill M.A. Folate. In: Erdman J.W., Macdonald I.A., Zeisel S.H., editors. Present Knowledge in Nutrition. Wiley-Blackwell; Washington, DC, USA: 2012. pp. 321–342.”

[46] E. H. Reynolds, “Benefits and risks of folic acid to the nervous system.,” *J. Neurol. Neurosurg. Psychiatry*, vol. 72, no. 5, pp. 567–571, May 2002, doi: 10.1136/jnnp.72.5.567.

[47] K. S. Crider, T. P. Yang, R. J. Berry, and L. B. Bailey, “Folate and DNA methylation: A review of molecular mechanisms and the evidence for Folate’s role,” *Adv. Nutr.*, vol. 3, no. 1, pp. 21–38, 2012, doi: 10.3945/an.111.000992.

[48] E. H. Reynolds, “The neurology of folic acid deficiency.,” *Handb. Clin. Neurol.*, vol. 120, pp. 927–943, 2014, doi: 10.1016/B978-0-7020-4087-0.00061-9.

[49] “Folate (Folic Acid) – Vitamin B9,” [Online]. Available: https://www.hsph.harvard.edu/nutritionsource/folic-acid/.

[50] “folic acid,” [Online]. Available: https://www.cdc.gov/ncbddd/folicacid/faqs/faqs-general-info.html.

[51] K. S. Crider, L. B. Bailey, and R. J. Berry, “Folic acid food fortification-its history, effect, concerns, and future directions.,” *Nutrients*, vol. 3, no. 3, pp. 370–384, Mar. 2011, doi: 10.3390/nu3030370.

[52] M. O. Abdimalik, “The kind assailant: how dementia patients view their carers,” *Br. Student Dr. J.*, vol. 3, no. 2, p. 42, 2019, doi: 10.18573/bsdj.77.

[53] D. Moretti, R. Biebinger, M. J. Bruins, B. Hoeft, and K. Kraemer, “Bioavailability of iron, zinc, folic acid, and vitamin A from fortified maize.,” *Ann. N. Y. Acad. Sci.*, vol. 1312, pp. 54–65, Apr. 2014, doi: 10.1111/nyas.12297.

[54] H. McNulty and K. Pentieva, “Folate bioavailability.,” *Proc. Nutr. Soc.*, vol. 63, no. 4, pp. 529–536, Nov. 2004, doi: 10.1079/pns2004383.

[55] J. F. 3rd Gregory, “Case study: folate bioavailability.,” *J. Nutr.*, vol. 131, no. 4 Suppl, pp. 1376S–82S, Apr. 2001, doi: 10.1093/jn/131.4.1376S.

[56] K. R. Patel and A. Sobczyńska-Malefora, “The adverse effects of an excessive folic acid intake.,” *Eur. J. Clin. Nutr.*, vol. 71, no. 2, pp. 159–163, Feb. 2017, doi: 10.1038/ejcn.2016.194.

[57] J. A. Greenberg, S. J. Bell, Y. Guan, and Y.-H. Yu, “Folic Acid supplementation and pregnancy: more than just neural tube defect prevention.,” *Rev. Obstet. Gynecol.*, vol. 4, no. 2, pp. 52–59, 2011.

[58] A. Sobczyńska-Malefora and D. J. Harrington, “Laboratory assessment of folate (vitamin B(9)) status.,” *J. Clin. Pathol.*, vol. 71, no. 11, pp. 949–956, Nov. 2018, doi: 10.1136/jclinpath-2018-205048.

[59] D. J. McKillop *et al.*, “The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet.,” *Br. J. Nutr.*, vol. 88, no. 6, pp. 681–688, Dec. 2002, doi: 10.1079/BJN2002733.

[60] J. J. Castenmiller, C. J. van de Poll, C. E. West, I. A. Brouwer, C. M. Thomas, and M. van Dusseldorp, "Bioavailability of folate from processed spinach in humans. Effect of food matrix and interaction with carotenoids.," *Ann. Nutr. Metab.*, vol. 44, no. 4, pp. 163–169, 2000, doi: 10.1159/000012840.

[61] P. S. Sachdev *et al.*, "Relationship of homocysteine, folic acid and vitamin B12 with depression in a middle-aged community sample.," *Psychol. Med.*, vol. 35, no. 4, pp. 529–538, Apr. 2005, doi: 10.1017/s0033291704003721.

[62] G. S. Kelly, "Folates: supplemental forms and therapeutic applications.," *Altern. Med. Rev.*, vol. 3, no. 3, pp. 208–220, Jun. 1998.

[63] M. S. Morris, P. F. Jacques, I. H. Rosenberg, and J. Selhub, "Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors.," *Am. J. Clin. Nutr.*, vol. 91, no. 6, pp. 1733–1744, Jun. 2010, doi: 10.3945/ajcn.2009.28671.

[64] F. R. Frankenburg, "Folate supplementation: is it safe and effective?," *The Journal of clinical psychiatry*, vol. 70, no. 5. United States, pp. 767; author reply 767-9, May 2009.

[65] *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: The National Academies Press.* Washington (DC), 1998.

[66] R. T. P. Paul, A. P. McDonnell, and C. B. Kelly, "Folic acid: neurochemistry, metabolism and relationship to depression.," *Hum. Psychopharmacol.*, vol. 19, no. 7, pp. 477–488, Oct. 2004, doi: 10.1002/hup.614.

[67] E. H. Reynolds, B. B. Gallagher, R. H. Mattson, M. Bowers, and A. L. Johnson, "Relationship between serum and cerebrospinal fluid folate.," *Nature*, vol. 240, no. 5377, pp. 155–157, Nov. 1972, doi: 10.1038/240155a0.

[68] R. Spector and A. V Lorenzo, "Folate transport in the central nervous system.," *Am. J. Physiol.*, vol. 229, no. 3, pp. 777–782, Sep. 1975, doi: 10.1152/ajplegacy.1975.229.3.777.

[69] C. Alam, M. Kondo, D. L. O'Connor, and R. Bendayan, "Clinical Implications of Folate Transport in the Central Nervous System.," *Trends Pharmacol. Sci.*, vol. 41, no. 5, pp. 349–361, May 2020, doi: 10.1016/j.tips.2020.02.004.

[70] S. N. Young, “Folate and depression--a neglected problem.,” *Journal of psychiatry & neuroscience : JPN*, vol. 32, no. 2. pp. 80–82, Mar. 2007.

[71] “Folate and folic acid for use in listed medicines,” [Online]. Available: https://www.tga.gov.au/folate-and-folic-acid-use-listed-medicines.

[72] A. Bender, K. E. Hagan, and N. Kingston, “The association of folate and depression: A meta-analysis,” *J. Psychiatr. Res.*, vol. 95, pp. 9–18, 2017, doi: 10.1016/j.jpsychires.2017.07.019.

[73] T. Tolmunen *et al.*, “Association between depressive symptoms and serum concentrations of homocysteine in men: a population study.,” *Am. J. Clin. Nutr.*, vol. 80, no. 6, pp. 1574–1578, Dec. 2004, doi: 10.1093/ajcn/80.6.1574.

[74] B. P. Kennedy, T. Bottiglieri, E. Arning, M. G. Ziegler, L. A. Hansen, and E. Masliah, “Elevated S-adenosylhomocysteine in Alzheimer brain: influence on methyltransferases and cognitive function.,” *J. Neural Transm.*, vol. 111, no. 4, pp. 547–567, Apr. 2004, doi: 10.1007/s00702-003-0096-5.

[75] S. A. Lipton *et al.*, “Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 94, no. 11, pp. 5923–5928, May 1997, doi: 10.1073/pnas.94.11.5923.

[76] S. Dayal *et al.*, “Deficiency of glutathione peroxidase-1 sensitizes hyperhomocysteinemic mice to endothelial dysfunction.,” *Arterioscler. Thromb. Vasc. Biol.*, vol. 22, no. 12, pp. 1996–2002, Dec. 2002, doi: 10.1161/01.atv.0000041629.92741.dc.

[77] T. Bottiglieri, “Homocysteine and folate metabolism in depression.,” *Prog. Neuropsychopharmacol. Biol. Psychiatry*, vol. 29, no. 7, pp. 1103–1112, Sep. 2005, doi: 10.1016/j.pnpbp.2005.06.021.

[78] S. J. Lewis *et al.*, “The thermolabile variant of MTHFR is associated with depression in the British Women’s Heart and Health Study and a meta-analysis.,” *Mol. Psychiatry*, vol. 11, no. 4, pp. 352–360, Apr. 2006, doi: 10.1038/sj.mp.4001790.

[79] C. B. Kelly *et al.*, “The MTHFR C677T polymorphism is associated with depressive episodes in patients from Northern Ireland.,” *J. Psychopharmacol.*, vol. 18, no. 4, pp. 567–571, Dec. 2004, doi: 10.1177/0269881104047285.

[80] K. A. Skarupski, C. Tangney, H. Li, B. Ouyang, D. A. Evans, and M. C. Morris, “Longitudinal association of vitamin B-6, folate, and vitamin B-12 with depressive symptoms among older adults over time.,” *Am. J. Clin. Nutr.*, vol. 92, no. 2, pp. 330–335, Aug. 2010, doi: 10.3945/ajcn.2010.29413.

[81] A. Bender, K. E. Hagan, and N. Kingston, “The association of folate and depression: A meta-analysis.,” *J. Psychiatr. Res.*, vol. 95, pp. 9–18, Dec. 2017, doi: 10.1016/j.jpsychires.2017.07.019.

[82] “Is augmentation with folate effective for major depressive disorder?,” 2018, [Online]. Available: https://www.medwave.cl/puestadia/resepis/7156.html?lang=en.

[83] I. A. Brouwer *et al.*, “Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial.,” *J. Nutr.*, vol. 129, no. 6, pp. 1135–1139, Jun. 1999, doi: 10.1093/jn/129.6.1135.

[84] J. K. Bird, A. G. Ronnenberg, S.-W. Choi, F. Du, J. B. Mason, and Z. Liu, “Obesity is associated with increased red blood cell folate despite lower dietary intakes and serum concentrations.,” *J. Nutr.*, vol. 145, no. 1, pp. 79–86, Jan. 2015, doi: 10.3945/jn.114.199117.

[85] S. Köse, S. Sözlü, H. Bölükbaşi, N. Ünsal, and M. Gezmen-Karadağ, “Obesity is associated with folate metabolism.,” *Int. J. Vitam. Nutr. Res. Int. Zeitschrift fur Vitamin- und Ernahrungsforschung. J. Int. Vitaminol. Nutr.*, vol. 90, no. 3–4, pp. 353–364, Jun. 2020, doi: 10.1024/0300-9831/a000602.

[86] M.-R. Lee and S. M. Jung, “Serum Folate Related to Five Measurements of Obesity and High-Sensitivity C-Reactive Protein in Korean Adults.,” *Nutrients*, vol. 14, no. 17, Aug. 2022, doi: 10.3390/nu14173461.

[87] S. M. Carlsen, G. Jacobsen, L. Vatten, and P. Romundstad, “In pregnant women who smoke, caffeine consumption is associated with an increased level of homocysteine.,” *Acta Obstet. Gynecol. Scand.*, vol. 84, no. 11, pp. 1049–1054, Nov. 2005, doi: 10.1111/j.0001-6349.2005.00822.x.

[88] L. Z. Hasoun, H. A. Khader, M. I. Abu-Taha, B. A. Mohammad, and M. S. Abu-Samak, “A Cross-Sectional Study on the Combined Effect of Body Weight and Coffee Consumption on Serum Levels of Leptin, Vitamin B12, and Folic Acid

in Healthy Young Adult Males.," *J. Multidiscip. Healthc.*, vol. 14, pp. 639–650, 2021, doi: 10.2147/JMDH.S290990.

[89] M. Otake, K. Sakurai, M. Watanabe, and C. Mori, "Association Between Serum Folate Levels and Caffeinated Beverage Consumption in Pregnant Women in Chiba: The Japan Environment and Children's Study.," *J. Epidemiol.*, vol. 28, no. 10, pp. 414–419, Oct. 2018, doi: 10.2188/jea.JE20170019.

[90] C. M. Hatzis, G. K. Bertsias, M. Linardakis, J. M. Scott, and A. G. Kafatos, "Dietary and other lifestyle correlates of serum folate concentrations in a healthy adult population in Crete, Greece: a cross-sectional study.," *Nutr. J.*, vol. 5, p. 5, Feb. 2006, doi: 10.1186/1475-2891-5-5.

[91] N. C. Alemdaroglu, U. Dietz, S. Wolffram, H. Spahn-Langguth, and P. Langguth, "Influence of green and black tea on folic acid pharmacokinetics in healthy volunteers: potential risk of diminished folic acid bioavailability.," *Biopharm. Drug Dispos.*, vol. 29, no. 6, pp. 335–348, Sep. 2008, doi: 10.1002/bdd.617.

[92] J. J. Guilbert, "The world health report 2002 - reducing risks, promoting healthy life.," *Education for health (Abingdon, England)*, vol. 16, no. 2. India, p. 230, Jul. 2003, doi: 10.1080/1357628031000116808.

[93] A. Tuenter *et al.*, "Folate, vitamin B12, and homocysteine in smoking-exposed pregnant women: A systematic review.," *Matern. Child Nutr.*, vol. 15, no. 1, p. e12675, Jan. 2019, doi: 10.1111/mcn.12675.

[94] T. Yamada *et al.*, "First-trimester serum folate levels and subsequent risk of abortion and preterm birth among Japanese women with singleton pregnancies.," *Arch. Gynecol. Obstet.*, vol. 287, no. 1, pp. 9–14, Jan. 2013, doi: 10.1007/s00404-012-2501-5.

[95] D. M. Mannino, J. Mulinare, E. S. Ford, and J. Schwartz, "Tobacco smoke exposure and decreased serum and red blood cell folate levels: data from the Third National Health and Nutrition Examination Survey.," *Nicotine Tob. Res. Off. J. Soc. Res. Nicotine Tob.*, vol. 5, no. 3, pp. 357–362, Jun. 2003, doi: 10.1080/1462220031000094330.

[96] K. Al-Numair, K. Barber-Heidal, A. Al-Assaf, and G. El-Desoky, "Water-pipe (shisha) smoking influences total antioxidant capacity and oxidative stress of healthy Saudi males," *J. Food, Agric. Environ.*, vol. 5, no. 3–4, pp. 17–22, 2007.

[97] Y.-N. Kim, J. H. Hwang, and Y.-O. Cho, "The effects of exercise training and acute exercise duration on plasma folate and vitamin B12.," *Nutr. Res. Pract.*, vol. 10, no. 2, pp. 161–166, Apr. 2016, doi: 10.4162/nrp.2016.10.2.161.

[98] "Exercises to Manage Symptoms of Vitamin B Deficiency," 2023, [Online]. Available: https://www.neurobion.com/en-za/nerve-health/taking-care-of-your-nerves/exercises-for-nerve-damage-symptoms.

[99] A. D. Gernand, K. J. Schulze, C. P. Stewart, K. P. J. West, and P. Christian, "Micronutrient deficiencies in pregnancy worldwide: health effects and prevention.," *Nat. Rev. Endocrinol.*, vol. 12, no. 5, pp. 274–289, May 2016, doi: 10.1038/nrendo.2016.37.

[100] J. Trujillo *et al.*, "A systematic review of the associations between maternal nutritional biomarkers and depression and/or anxiety during pregnancy and postpartum.," *J. Affect. Disord.*, vol. 232, pp. 185–203, May 2018, doi: 10.1016/j.jad.2018.02.004.

[101] M. F. F. Chong *et al.*, "Relationships of maternal folate and vitamin B12 status during pregnancy with perinatal depression: The GUSTO study.," *J. Psychiatr. Res.*, vol. 55, pp. 110–116, Aug. 2014, doi: 10.1016/j.jpsychires.2014.04.006.

[102] C. H. Blunden, H. M. Inskip, S. M. Robinson, C. Cooper, K. M. Godfrey, and T. R. Kendrick, "Postpartum depressive symptoms: the B-vitamin link.," *Ment. Health Fam. Med.*, vol. 9, no. 1, pp. 5–13, Jan. 2012.

[103] J. Yan, Y. Liu, L. Cao, Y. Zheng, W. Li, and G. Huang, "Association between Duration of Folic Acid Supplementation during Pregnancy and Risk of Postpartum Depression.," *Nutrients*, vol. 9, no. 11, Nov. 2017, doi: 10.3390/nu9111206.

[104] E. Meisel *et al.*, "Folate Levels in Patients Hospitalized with Coronavirus Disease 2019.," *Nutrients*, vol. 13, no. 3, Mar. 2021, doi: 10.3390/nu13030812.

[105] P. Kreusler *et al.*, "Folate and Cobalamin Serum Levels in Healthy Children and Adolescents and Their Association with Age, Sex, BMI and Socioeconomic Status.," *Nutrients*, vol. 13, no. 2, Feb. 2021, doi: 10.3390/nu13020546.

[106] I. Pravst *et al.*, "Dietary Intake of Folate and Assessment of the Folate Deficiency Prevalence in Slovenia Using Serum Biomarkers.," *Nutrients*, vol. 13, no. 11, Oct. 2021, doi: 10.3390/nu13113860.

[107] L. M. Rogers *et al.*, "Global folate status in women of reproductive age: a systematic review with emphasis on methodological issues," *Ann. N. Y. Acad. Sci.*, vol. 1431, no. 1, pp. 35–57, 2018, doi: 10.1111/nyas.13963.

[108] O. P. Almeida, A. H. Ford, and L. Flicker, "Systematic review and meta-analysis of randomized placebo-controlled trials of folate and vitamin B12 for depression.," *Int. psychogeriatrics*, vol. 27, no. 5, pp. 727–737, May 2015, doi: 10.1017/S1041610215000046.

[109] K. Hyland, J. Shoffner, and S. J. Heales, "Cerebral folate deficiency.," *J. Inherit. Metab. Dis.*, vol. 33, no. 5, pp. 563–570, Oct. 2010, doi: 10.1007/s10545-010-9159-6.

[110] C. Alam *et al.*, "Upregulation of reduced folate carrier by vitamin D enhances brain folate uptake in mice lacking folate receptor alpha.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 116, no. 35, pp. 17531–17540, Aug. 2019, doi: 10.1073/pnas.1907077116.

[111] N. S. K. Lam *et al.*, "The potential use of folate and its derivatives in treating psychiatric disorders: A systematic review," *Biomed. Pharmacother.*, vol. 146, p. 112541, 2022, doi: https://doi.org/10.1016/j.biopha.2021.112541.

[112] E. Beurel, M. Toups, and C. B. Nemeroff, "The Bidirectional Relationship of Depression and Inflammation: Double Trouble.," *Neuron*, vol. 107, no. 2, pp. 234–256, Jul. 2020, doi: 10.1016/j.neuron.2020.06.002.

[113] M. S. Morris, M. Fava, P. F. Jacques, J. Selhub, and I. H. Rosenberg, "Depression and folate status in the US Population.," *Psychother. Psychosom.*, vol. 72, no. 2, pp. 80–87, 2003, doi: 10.1159/000068692.

[114] X. Huang *et al.*, “Association between Serum Vitamin Levels and Depression in U.S. Adults 20 Years or Older Based on National Health and Nutrition Examination Survey 2005¯2006.,” *Int. J. Environ. Res. Public Health*, vol. 15, no. 6, Jun. 2018, doi: 10.3390/ijerph15061215.

[115] L. Gougeon, H. Payette, J. A. Morais, P. Gaudreau, B. Shatenstein, and K. Gray-Donald, “Intakes of folate, vitamin B6 and B12 and risk of depression in community-dwelling older adults: the Quebec Longitudinal Study on Nutrition and Aging.,” *Eur. J. Clin. Nutr.*, vol. 70, no. 3, pp. 380–385, Mar. 2016, doi: 10.1038/ejcn.2015.202.

[116] J. E. Alpert and M. Fava, “Nutrition and depression: The role of folate,” *Nutr. Rev.*, vol. 55, no. 5, pp. 145–149, 1997, doi: 10.1111/j.1753-4887.1997.tb06468.x.

[117] P. S. Godfrey *et al.*, “Enhancement of recovery from psychiatric illness by methylfolate.,” *Lancet (London, England)*, vol. 336, no. 8712, pp. 392–395, Aug. 1990, doi: 10.1016/0140-6736(90)91942-4.

[118] A. Coppen, S. Chaudhry, and C. Swade, “Folic acid enhances lithium prophylaxis.,” *J. Affect. Disord.*, vol. 10, no. 1, pp. 9–13, 1986, doi: 10.1016/0165-0327(86)90043-1.

[119] G. P. Guaraldi, M. Fava, F. Mazzi, and P. la Greca, “An open trial of methyltetrahydrofolate in elderly depressed patients.,” *Ann. Clin. psychiatry Off. J. Am. Acad. Clin. Psychiatr.*, vol. 5, no. 2, pp. 101–105, Jun. 1993, doi: 10.3109/10401239309148970.

[120] M. Fava and D. Mischoulon, “Folate in depression: efficacy, safety, differences in formulations, and clinical issues.,” *J. Clin. Psychiatry*, vol. 70 Suppl 5, pp. 12–17, 2009, doi: 10.4088/JCP.8157su1c.03.

[121] A. V Ravindran *et al.*, “Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical Guidelines for the Management of Adults with Major Depressive Disorder: Section 5. Complementary and Alternative Medicine Treatments.,” *Can. J. Psychiatry.*, vol. 61, no. 9, pp. 576–587, Sep. 2016, doi: 10.1177/0706743716660290.

[122] J. R. Geddes *et al.*, “Comparative evaluation of quetiapine plus lamotrigine combination versus quetiapine monotherapy (and folic acid versus placebo) in bipolar depression (CEQUEL): a 2 × 2 factorial randomised trial.,” *The lancet. Psychiatry*, vol. 3, no. 1, pp. 31–39, Jan. 2016, doi: 10.1016/S2215-0366(15)00450-2.

[123] G. I. Papakostas, C. F. Cassiello, and N. Iovieno, “Folates and S-adenosylmethionine for major depressive disorder.,” *Can. J. Psychiatry.*, vol. 57, no. 7, pp. 406–413, Jul. 2012, doi: 10.1177/070674371205700703.

[124] R. Prinz-Langenohl *et al.*, “[6S]-5-methyltetrahydrofolate increases plasma folate more effectively than folic acid in women with the homozygous or wild-type 677C-->T polymorphism of methylenetetrahydrofolate reductase.,” *Br. J. Pharmacol.*, vol. 158, no. 8, pp. 2014–2021, Dec. 2009, doi: 10.1111/j.1476-5381.2009.00492.x.

[125] G. A. 3rd Eby and K. L. Eby, “Magnesium for treatment-resistant depression: a review and hypothesis.,” *Med. Hypotheses*, vol. 74, no. 4, pp. 649–660, Apr. 2010, doi: 10.1016/j.mehy.2009.10.051.

[126] A. Coppen and C. Bolander-Gouaille, “Treatment of depression: Time to consider folic acid and vitamin B 12,” *J. Psychopharmacol.*, vol. 19, no. 1, pp. 59–65, 2005, doi: 10.1177/0269881105048899.

[127] M. Alpert, R. R. Silva, and E. R. Pouget, “Prediction of treatment response in geriatric depression from baseline folate level: interaction with an SSRI or a tricyclic antidepressant.,” *J. Clin. Psychopharmacol.*, vol. 23, no. 3, pp. 309–313, Jun. 2003, doi: 10.1097/01.jcp.0000084024.22282.cd.

[128] R. Altaf, I. Gonzalez, K. Rubino, and E. C. 2nd Nemec, “Folate as adjunct therapy to SSRI/SNRI for major depressive disorder: Systematic review & meta-analysis.,” *Complement. Ther. Med.*, vol. 61, p. 102770, Sep. 2021, doi: 10.1016/j.ctim.2021.102770.

[129] Z. Sepehrmanesh, A. Omidi, and N. Gholampoor, “Acid folic supplementation in major depressive disorder treatment: A double-blind randomized clinical trial,” *Iran. Red Crescent Med. J.*, vol. 19, no. 2, 2017, doi: 10.5812/ircmj.33243.

[130] J. Thomas, R. Khanam, and D. Vohora, “Augmentation of effect of venlafaxine by folic acid in behavioral paradigms of depression in mice: Evidence of serotonergic and pro-inflammatory cytokine pathways.,” *Pharmacol. Rep.*, vol. 68, no. 2, pp. 396–403, Apr. 2016, doi: 10.1016/j.pharep.2015.10.003.

[131] E. Roberts, B. Carter, and A. H. Young, “Caveat emptor: Folate in unipolar depressive illness, a systematic review and meta-analysis.,” *J. Psychopharmacol.*, vol. 32, no. 4, pp. 377–384, Apr. 2018, doi: 10.1177/0269881118756060.

[132] E. Bedson *et al.*, “Folate Augmentation of Treatment--Evaluation for Depression (FolATED): randomised trial and economic evaluation.,” *Health Technol. Assess.*, vol. 18, no. 48, pp. vii–viii, 1–159, Jul. 2014, doi: 10.3310/hta18480.

[133] G. I. Papakostas *et al.*, “L-methylfolate as adjunctive therapy for SSRI-resistant major depression: results of two randomized, double-blind, parallel-sequential trials.,” *Am. J. Psychiatry*, vol. 169, no. 12, pp. 1267–1274, Dec. 2012, doi: 10.1176/appi.ajp.2012.11071114.

[134] A. Cleare *et al.*, “Evidence-based guidelines for treating depressive disorders with antidepressants: A revision of the 2008 British Association for Psychopharmacology guidelines.,” *J. Psychopharmacol.*, vol. 29, no. 5, pp. 459–525, May 2015, doi: 10.1177/0269881115581093.

[135] A. Coppen and J. Bailey, “Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial.,” *J. Affect. Disord.*, vol. 60, no. 2, pp. 121–130, Nov. 2000, doi: 10.1016/s0165-0327(00)00153-1.

[136] M. T. Abou-Saleh and A. Coppen, “Folic acid and the treatment of depression.,” *Journal of psychosomatic research*, vol. 61, no. 3. England, pp. 285–287, Sep. 2006, doi: 10.1016/j.jpsychores.2006.07.007.

[137] “Select Statistical Services Limited . Population Proportion – Sample Size.,” *Retrieved from Select Statistical Services*, 2020. .

[138] “Thompson, S. K. (2012). Sampling, Third Edition. USA: John Wiley & Sons, Inc.” .

[139] "What Is Convenience Sampling? | Definition & Examples," [Online]. Available: https://www.scribbr.com/methodology/convenience-sampling/#:~:text=Advantages of convenience sampling&text=Convenience sampling is usually low,not have been possible otherwise.

[140] D. Głąbska, A. Książek, and D. Guzek, "Development and validation of the brief folate-specific food frequency questionnaire for young women's diet assessment," *Int. J. Environ. Res. Public Health*, vol. 14, no. 12, 2017, doi: 10.3390/ijerph14121574.

[141] E. Moya, L. M. Larson, R. C. Stewart, J. Fisher, M. N. Mwangi, and K. S. Phiri, "Reliability and validity of depression anxiety stress scale (DASS)-21 in screening for common mental disorders among postpartum women in Malawi," *BMC Psychiatry*, vol. 22, no. 1, pp. 1–11, 2022, doi: 10.1186/s12888-022-03994-0.

[142] T. D. Tran, T. Tran, and J. Fisher, "Validation of the depression anxiety stress scales (DASS) 21 as a screening instrument for depression and anxiety in a rural community-based cohort of northern Vietnamese women.," *BMC Psychiatry*, vol. 13, p. 24, Jan. 2013, doi: 10.1186/1471-244X-13-24.

[143] N. Yaghi, C. Boulos, R. Baddoura, M. Abifadel, and C. Yaghi, "Validity and reliability of a food frequency questionnaire for community dwelling older adults in a Mediterranean country: Lebanon," *Nutr. J.*, vol. 21, no. 1, pp. 1–13, 2022, doi: 10.1186/s12937-022-00788-8.

[144] M. Fawzy and S. A. Hamed, "Prevalence of psychological stress, depression and anxiety among medical students in Egypt.," *Psychiatry Res.*, vol. 255, pp. 186–194, Sep. 2017, doi: 10.1016/j.psychres.2017.05.027.

[145] M. T. Moussa, P. Lovibond, R. Laube, and H. A. Megahead, "Psychometric Properties of an Arabic Version of the Depression Anxiety Stress Scales (DASS)," *Res. Soc. Work Pract.*, vol. 27, no. 3, pp. 375–386, 2017, doi: 10.1177/1049731516662916.

[146] J. A. González-Rivera, O. M. Pagán-Torres, and E. M. Pérez-Torres, "Depression, Anxiety and Stress Scales (DASS-21): Construct Validity Problem in Hispanics.," *Eur. J. Investig. Heal. Psychol. Educ.*, vol. 10, no. 1, pp. 375–

389, Jan. 2020, doi: 10.3390/ejihpe10010028.

[147] O. N. Medvedev, “Depression Anxiety Stress Scales (DASS-21) in International Contexts,” *Int. Handb. Behav. Heal. Assess.*, pp. 1–15, 2023, doi: 10.1007/978-3-030-89738-3_15-1.

[148] F. Chang, X. Fan, Y. Zhang, B. Tang, and X. Jia, “Prevalence of depressive symptoms and correlated factors among pregnant women during their second and third trimesters in northwest rural China: a cross-sectional study.,” *BMC Pregnancy Childbirth*, vol. 22, no. 1, p. 38, Jan. 2022, doi: 10.1186/s12884-021-04340-0.

[149] A. M. Ali and J. Green, “Factor structure of the depression anxiety stress Scale-21 (DASS-21): Unidimensionality of the Arabic version among Egyptian drug users.,” *Subst. Abuse Treat. Prev. Policy*, vol. 14, no. 1, p. 40, Sep. 2019, doi: 10.1186/s13011-019-0226-1.

[150] E. Radwan, A. Radwan, W. Radwan, and D. Pandey, “Prevalence of depression, anxiety and stress during the COVID-19 pandemic: a cross-sectional study among Palestinian students (10-18 years).,” *BMC Psychol.*, vol. 9, no. 1, p. 187, Nov. 2021, doi: 10.1186/s40359-021-00688-2.

[151] N. E. Helwig, S. Hong, and E. T. Hsiao-wecksler, “DASS-21,” pp. 1–2.

[152] L. B. Bailey *et al.*, “Biomarkers of Nutrition for Development-Folate Review.,” *J. Nutr.*, vol. 145, no. 7, pp. 1636S-1680S, Jul. 2015, doi: 10.3945/jn.114.206599.

[153] T. Physician, M. Alomari, and O. F. Khabour, “Effect of physical activity on levels of homocysteine, folate, and vitamin B 12 in the elderly,” no. August, 2016, doi: 10.1080/00913847.2016.1135037.

[154] M. J. Akhtar, M. A. Khan, and I. Ahmad, “Photodegradation of folic acid in aqueous solution.,” *J. Pharm. Biomed. Anal.*, vol. 19, no. 3–4, pp. 269–275, Mar. 1999, doi: 10.1016/s0731-7085(98)00038-7.

[155] V. De Brouwer, G.-F. Zhang, S. Storozhenko, D. Van Der Straeten, and W. E. Lambert, “pH stability of individual folates during critical sample preparation steps in prevision of the analysis of plant folates.,” *Phytochem. Anal.*, vol. 18, no. 6, pp. 496–508, 2007, doi: 10.1002/pca.1006.

[156] I. Oey, P. Verlinde, M. Hendrickx, and A. Van Loey, "Temperature and pressure stability of l-ascorbic acid and/or [6s] 5-methyltetrahydrofolic acid: A kinetic study," *Eur. Food Res. Technol.*, vol. 223, no. 1, pp. 71–77, 2006, doi: 10.1007/s00217-005-0123-x.

[157] C.-J. L. Farrell, S. H. Kirsch, and M. Herrmann, "Red cell or serum folate: what to do in clinical practice?," *Clin. Chem. Lab. Med.*, vol. 51, no. 3, pp. 555–569, Mar. 2013, doi: 10.1515/cclm-2012-0639.

[158] M. O. L. C. S. X, "Serum Folate and RBC Folate Folate Testing," vol. 29910, no. 800, pp. 1–8, 2021.

[159] Z. Fazili, R. D. J. Whitehead, N. Paladugula, and C. M. Pfeiffer, "A high-throughput LC-MS/MS method suitable for population biomonitoring measures five serum folate vitamers and one oxidation product.," *Anal. Bioanal. Chem.*, vol. 405, no. 13, pp. 4549–4560, May 2013, doi: 10.1007/s00216-013-6854-9.

[160] B. M. H. Haynes, C. M. Pfeiffer, M. R. Sternberg, and R. L. Schleicher, "Selected physiologic variables are weakly to moderately associated with 29 biomarkers of diet and nutrition, NHANES 2003-2006.," *J. Nutr.*, vol. 143, no. 6, pp. 1001S–10S, Jun. 2013, doi: 10.3945/jn.112.172882.

[161] D. J. Harrington, "10 - Investigation of Megaloblastic Anaemia: Cobalamin, Folate and Metabolite Status," B. J. Bain, I. Bates, and M. A. B. T.-D. and L. P. H. (Twelfth E. Laffan, Eds. Elsevier, 2017, pp. 187–213.

[162] C. M. Pfeiffer, M. Zhang, and S. Jabbar, "Framework for laboratory harmonization of folate measurements in low- and middle-income countries and regions.," *Ann. N. Y. Acad. Sci.*, vol. 1414, no. 1, pp. 96–108, Feb. 2018, doi: 10.1111/nyas.13532.

[163] R. M. Lequin, "Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA).," *Clin. Chem.*, vol. 51, no. 12, pp. 2415–2418, Dec. 2005, doi: 10.1373/clinchem.2005.051532.

[164] S. Aydin, "A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA.," *Peptides*, vol. 72, pp. 4–15, Oct. 2015, doi: 10.1016/j.peptides.2015.04.012.

[165] E. Engvall, “The ELISA, enzyme-linked immunosorbent assay.,” *Clin. Chem.*, vol. 56, no. 2, pp. 319–320, Feb. 2010, doi: 10.1373/clinchem.2009.127803.

[166] F. Hou *et al.*, “Development of a microplate reader compatible microfluidic chip for ELISA.,” *Biomed. Microdevices*, vol. 14, no. 4, pp. 729–737, Aug. 2012, doi: 10.1007/s10544-012-9654-7.

[167] M. L. Hestø, K. Marie, and R. Bugge, “Graduate thesis Prevalence of folate and vitamin B12 deficiency during pregnancy among healthy , Norwegian women : association to pregnancy outcomes,” no. June, 2021.

[168] Ellis and Victoria, “Spearma n ’ s correlation,” *Environmetrics*, p. 8, 2011.

[169] A. J. Baxter, G. Patton, K. M. Scott, L. Degenhardt, and H. A. Whiteford, “Global epidemiology of mental disorders: what are we missing?,” *PLoS One*, vol. 8, no. 6, p. e65514, 2013, doi: 10.1371/journal.pone.0065514.

[170] J. Firth *et al.*, “A meta-review of ‘lifestyle psychiatry’: the role of exercise, smoking, diet and sleep in the prevention and treatment of mental disorders.,” *World Psychiatry*, vol. 19, no. 3, pp. 360–380, Oct. 2020, doi: 10.1002/wps.20773.

[171] J. Sarris *et al.*, “Nutritional medicine as mainstream in psychiatry.,” *The lancet. Psychiatry*, vol. 2, no. 3, pp. 271–274, Mar. 2015, doi: 10.1016/S2215-0366(14)00051-0.

[172] P. M. Kris-Etherton *et al.*, “Nutrition and behavioral health disorders: depression and anxiety.,” *Nutr. Rev.*, vol. 79, no. 3, pp. 247–260, Feb. 2021, doi: 10.1093/nutrit/nuaa025.

[173] J. Sarris *et al.*, “International Society for Nutritional Psychiatry Research consensus position statement: nutritional medicine in modern psychiatry.,” *World psychiatry : official journal of the World Psychiatric Association (WPA)*, vol. 14, no. 3. Italy, pp. 370–371, Oct. 2015, doi: 10.1002/wps.20223.

[174] A. L. Lopresti, S. D. Hood, and P. D. Drummond, “A review of lifestyle factors that contribute to important pathways associated with major depression: diet, sleep and exercise.,” *J. Affect. Disord.*, vol. 148, no. 1, pp. 12–27, May 2013, doi: 10.1016/j.jad.2013.01.014.

[175] C. Ménard, G. E. Hodes, and S. J. Russo, "Pathogenesis of depression: Insights from human and rodent studies.," *Neuroscience*, vol. 321, pp. 138–162, May 2016, doi: 10.1016/j.neuroscience.2015.05.053.

[176] L. Begdache, M. Chaar, N. Sabounchi, and H. Kianmehr, "Assessment of dietary factors, dietary practices and exercise on mental distress in young adults versus matured adults: A cross-sectional study.," *Nutr. Neurosci.*, vol. 22, no. 7, pp. 488–498, Jul. 2019, doi: 10.1080/1028415X.2017.1411875.

[177] C. W. Popper, "Single-micronutrient and broad-spectrum micronutrient approaches for treating mood disorders in youth and adults.," *Child Adolesc. Psychiatr. Clin. N. Am.*, vol. 23, no. 3, pp. 591–672, Jul. 2014, doi: 10.1016/j.chc.2014.04.001.

[178] D. Mischoulon and M. F. Raab, "The role of folate in depression and dementia.," *J. Clin. Psychiatry*, vol. 68 Suppl 1, pp. 28–33, 2007.

[179] C. Otte *et al.*, "Major depressive disorder.," *Nat. Rev. Dis. Prim.*, vol. 2, p. 16065, Sep. 2016, doi: 10.1038/nrdp.2016.65.

[180] E. H. Reynolds, "Folic acid, ageing, depression, and dementia.," *BMJ*, vol. 324, no. 7352, pp. 1512–1515, Jun. 2002, doi: 10.1136/bmj.324.7352.1512.

[181] J. Lökk, "News and views on folate and elderly persons," *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.*, vol. 58, no. 4, pp. 354–361, 2003, doi: 10.1093/gerona/58.4.m354.

[182] K. Björkegren and K. Svärdsudd, "Serum cobalamin, folate, methylmalonic acid and total homocysteine as vitamin B12 and folate tissue deficiency markers amongst elderly Swedes--a population-based study.," *J. Intern. Med.*, vol. 249, no. 5, pp. 423–432, May 2001, doi: 10.1046/j.1365-2796.2001.00819.x.

[183] S. C. Tinker, H. C. Hamner, R. J. Berry, L. B. Bailey, and C. M. Pfeiffer, "Does obesity modify the association of supplemental folic acid with folate status among nonpregnant women of childbearing age in the United States?," *Birth Defects Res. A. Clin. Mol. Teratol.*, vol. 94, no. 10, pp. 749–755, Oct. 2012, doi: 10.1002/bdra.23024.

[184] E. Goodman, N. E. Adler, S. R. Daniels, J. A. Morrison, G. B. Slap, and L. M. Dolan, "Impact of objective and subjective social status on obesity in a biracial cohort of adolescents.," *Obes. Res.*, vol. 11, no. 8, pp. 1018–1026, Aug. 2003, doi: 10.1038/oby.2003.140.

[185] D. Cassady, K. M. Jetter, and J. Culp, "Is price a barrier to eating more fruits and vegetables for low-income families?," *J. Am. Diet. Assoc.*, vol. 107, no. 11, pp. 1909–1915, Nov. 2007, doi: 10.1016/j.jada.2007.08.015.

[186] I. H. M. Steenhuis, W. E. Waterlander, and A. de Mul, "Consumer food choices: the role of price and pricing strategies.," *Public Health Nutr.*, vol. 14, no. 12, pp. 2220–2226, Dec. 2011, doi: 10.1017/S1368980011001637.

[187] W. E. Waterlander *et al.*, "Energy density, energy costs and income - how are they related?," *Public Health Nutr.*, vol. 13, no. 10, pp. 1599–1608, Oct. 2010, doi: 10.1017/S1368980009992989.

[188] Y. Singh *et al.*, "SARS CoV-2 aggravates cellular metabolism mediated complications in COVID-19 infection.," *Dermatol. Ther.*, vol. 33, no. 6, p. e13871, Nov. 2020, doi: 10.1111/dth.13871.

[189] A. M. Baart, M. G. J. Balvers, J. H. M. de Vries, D. S. M. Ten Haaf, M. T. E. Hopman, and J. M. T. Klein Gunnewiek, "Relationship between intake and plasma concentrations of vitamin B12 and folate in 873 adults with a physically active lifestyle: a cross-sectional study.," *J. Hum. Nutr. Diet. Off. J. Br. Diet. Assoc.*, vol. 34, no. 2, pp. 324–333, Apr. 2021, doi: 10.1111/jhn.12814.

[190] D. C. Borradale and M. G. Kimlin, "Folate degradation due to ultraviolet radiation: possible implications for human health and nutrition.," *Nutr. Rev.*, vol. 70, no. 7, pp. 414–422, Jul. 2012, doi: 10.1111/j.1753-4887.2012.00485.x.

[191] C. N. Miller and S. Rayalam, "The role of micronutrients in the response to ambient air pollutants: Potential mechanisms and suggestions for research design.," *J. Toxicol. Environ. Health. B. Crit. Rev.*, vol. 20, no. 1, pp. 38–53, 2017, doi: 10.1080/10937404.2016.1261746.

Appendices

Appendix A

Informed Consent

Palestine
An-Najah National University
Faculty of graduate study

دولــــــة فلســــــطين
جامعة النجاح الوطنية
كليـة الدراسات العليا

استمارة موافقة مسبقة للمشاركة في الدارسة البحثية

1) **الباحثة:** سجى عصام عبدالرحيم عليوي وبإشراف الدكتورة نهال الناطور.
2) **هدف الدراسة:** تهدف هذه الدراسة الى معرفة العلاقة بين مستويات حمض الفوليك(فيتامين ب9) في الدم و الاكتئاب لدى الإناث البالغات (18-30 سنة) في مركز أبحاث جامعة النجاح الوطنية في نابلس ، فلسطين.
3) **سؤال الدراسة:** هل هناك علاقة بين مستويات حمض الفوليك(فيتامين ب9) والاكتئاب، و ما هي هذه العلاقة؟
4) **نتائج البحث:** سوف تستخدم نتائج البحث للأغراض العلمية فقط وذلك للحصول على درجة الماجستير في الكيمياء الحيوية السريرية/ جامعه النجاح الوطنية.
5) **طريقة البحث:** تعبئة الاستبيانة ادناه وعمل تحليل لهذه البيانات مع نتائج فحص حمض الفوليك folic acid/ (vitamin B9).
6) **المخاطر المتوقعة:** لا يوجد أي مخاطر، حيث ان التعهد والمشاركة في تعبئة الاستبيانة اختياري. المشاركة في هذه الدراسة عبارة عن عمل تطوعي ويمكن الامتناع او الانسحاب عن المشاركة في أي وقت من دون ذكر الأسباب وبدون أي التزامات أو فقدان مزايا.
7) **الاستفادة المتوقعة للمشاركين:** يمكن للاناث المشاركات معرفة مستويات حمض الفوليك(فيتامين ب 9) في الدم وتمكينهن من تحسين مستواه وتلاشي آثار نقصه.
8) **السرية واحترام الخصوصية:** المعلومات سوف تستخدم لأغراض البحث العلمي فقط بما يضمن الحفاظ على الخصوصية والسرية التامة بحيث لا يكون هناك أي إزعاج للمشاركين. وأي استفسار او

سؤال له علاقة بهذه الدراسة يمكن للشخص المشارك مراجعة الدكتورة نهال الناطور وكما يحق لأي مشارك رفض دخول الدراسة في اي وقت من الدراسة. **كل المعلومات التي سوف يتم الحصول عليها من هذا الاستبيان هي سرية وليست للنشر**. شاكرين لكم مشاركتكم وتعاونكم البناء لما فيه من الخير.

موافقة المشارك: .... تاريخ الإجابة: ....

Appendix B

Self-administered questionnaire

ضع اشارة (✓) بجانب الاجابة

❖ أسئلة عامة				
1. الجنس:-	أنثى			
2. العمر (بالسنوات):-	☐ 25-18	☐ 30-26		
3. الوزن:-	--------------			
4. الطول:-	------------------			
5. منطقة السكن:-	☐ مدينة	☐ قرية	☐ مخيم	
6. الحالة الاجتماعية:-	☐ عزباء	☐ متزوجة	☐ مطلقة	
7. المستوى التعليمي:-	☐ بكالوريوس	☐ ماجستير	☐ دكتوراة	
8. المهنة:-	☐ تعمل	☐ لا تعمل		
9. عدد أفراد الاسرة:-	☐ أقل من 3	☐ 5-3	☐ 8-6	☐ أكثر من 8
10. معدل دخل الأسرة الشهري (بالشيقل):-	☐ أقل من 1500	☐ -1500 3500	☐ -3501 7000	☐ أكثر من 7000

❖ الطبيعة أو العادات السلوكية و الغذائيات:-					
1. طبيعة الأكل:-	☐ خضار و فواكه	☐ سكريات و نشويات	☐ لحوم	☐ معلبات و مصنعات	☐ وجبات سريعة
2. تناول الفواكه أسبوعيا ؟	☐ أبدا	☐ 3-1 حبات	☐ 6-4 حبات	☐ > 6 حبات	
3. تناول السبانخ،البقدونس،اللفت،البروكلي أسبوعيا :-	☐ أبدا	☐ 3-1 حبات	☐ 6-4حبات	☐ > 6 حبات	
4. تناول الكوسا،الفلفل،الخس،البازيلاء الخضراء، الفاصولياء الخضراء أسبوعيا:-	☐ أبدا	☐ 3-1 حبات	☐ 6-4 حبات	☐ > 6 حبات	
5. تناول الخيار ،البصل،الباذنجان،الفجل،الجزر ،الطماطم،الملفوف أسبوعيا:-	☐ أبدا	☐ 3-1 حبات	☐ 6-4 حبات	☐ > 6 حبات	
6. تناول البطاطا أسبوعيا ؟	☐ أبدا	☐ 3-1 حبات	☐ 6-4 حبات	☐ > 6 حبات	
7. تناول الأفوكادو أسبوعيا ؟	☐ أبدا	☐ 3-1 حبات	☐ 6-4 حبات	☐ > 6 حبات	
8. تناول اللحوم أسبوعيا؟	☐ أبدا	☐ 3-1 قطع	☐ 6-4 حبات	☐ > 6 قطع	
9. تناول الكبدة أسبوعيا؟	☐ أبدا	☐ 3-1 قطع	☐ 6-4 قطع	☐ > 6 قطع	
10. تناول منتجات الاسماك شهريا ؟	☐ أبدا	☐ 3-1 قطع	☐ 6-4 قطع	☐ > 6 قطع	
11. شرب الحليب أسبوعيا ؟	☐ أبدا	☐ 3-1 كوب	☐ 6-4 كوب	☐ > 6 كوب	
12. تناول الجبنة أسبوعيا ؟	☐ أبدا	☐ 3-1 قطع	☐ 6-4 قطع	☐ > 6 قطع	
13. تناول البيض أسبوعيا ؟	☐ أبدا	☐ 3-1 حبات	☐ 6-4 حبات	☐ > 6 حبات	

14. تناول البقوليات (الحمص، الذرة،الفول،العدس،قمح ، وغيرها) شهريا؟	☐ أبدا	☐ 3-1 كوب	☐ 6-4 كوب	☐ < 6 كوب
15. تناول المكسرات شهريا ؟	☐ أبدا	☐ 50 غم	☐ 100 غم	☐ < 100غم
16.تناول الخبز الأبيض أسبوعيا؟	☐ أبدا	☐ 3-1 أرغفة	☐ 6-4 أرغفة	☐ < 6 أرغفة
17. تناول الخبز الأسمرأسبوعيا ؟	☐ أبدا	☐ 3-1 أرغفة	☐ 6-4 أرغفة	☐ < 6 أرغفة
18. تناول الشوكلاتة السوداء/الداكنة أسبوعيا ؟	☐ أبدا	☐ 3-1 حبات	☐ 6-4 حبات	☐ < 6 حبات
19. تناول الشاي يوميا:-	☐ أبدا	☐ 3-1 كوب	☐ 6-4 كوب	☐ < 6 كوب
20. تناول القهوة يوميا:-	☐ أبدا	☐ 3-1 كوب	☐ 6-4 كوب	☐ < 6 كوب
21. تناول مشروبات الطاقة أسبوعيا:-	☐ أبدا	☐ 3-1 كوب	☐ 6-4 كوب	☐ < 6 كوب
22. تناول الأعشاب الطبيعية مثل: القرفة، النعنع، الزنجبيل، الميرمية و غيرها:-	☐ أبدا	☐ 3-1 كوب	☐ 6-4 كوب	☐ < 6 كوب
23. ممارسة التمارين الرياضية أسبوعيا:-	☐ 1-0 ساعة	☐ 4-2 ساعة	☐ < 4 ساعة	
24. هل تتعرضين للتدخين بشكل كبير؟	☐ لا	☐ نعم		
25. هل أنتِ مدخنة للسجائر؟	☐ مدخن	☐ غير مدخن	☐ مدخن سابق	
26.هل تدخنين الأرجيلة(الشيشة)؟	☐ لا	☐ نعم / معدل التدخين أسبوعيا		

❖ **الحالة الصحية:-**

1.هل تعانين من أمراض مزمنة؟	☐ لا	☐ السكري	☐ الضغط	☐ الغدة الدرقية	☐ فقر الدم (الأنيميا)/ الثلاسيميا	☐ غير ذلك / اسم المرض -----------
2. هل تعانين من أمراض وراثية؟	☐ لا	☐ نعم / اسم المرض -----------				
3. هل تعانين من أمراض عصبيّة؟	☐ لا	☐ نعم / اسم المرض ------------				
4. هل تعانين من الاكتئاب/القلق/التوتر/الحزن؟	☐ لا	☐ نعم				
5. هل سبق و تلقيتِ عناية نفسية؟	☐ لا	☐ نعم				
6. هل تستخدمين أدويةّ؟	☐ لا	☐ نعم / اسم الدواء -----------				
7. هل تستخدمين أدوية لها علاقة بالاكتئاب/أمراض عصبيةّ؟	☐ لا	☐ نعم / اسم الدواء ------------				
8. هل تتناولين أدوية مضادات للأكسدة مثل الفيتامينات و الاملاح المعدنية ؟	☐ لا	☐ نعم / اسم الفيتامين -----------				
9. هل تتناولين فيتامين ب 9 /الفوليك اسيد ؟	☐ لا	☐ نعم				
10. هل تناولتِ مجموعة فيتامينات ال B complex خلال الاربع شهور الماضية؟ (B1,B2,B6,B9,B12,..)	☐ لا	☐ نعم				
11. هل أصبتِ بفيروس كورونا؟	☐ لا	☐ نعم				
12. هل أنت حامل؟	☐ لا	☐ نعم				
13. هل تعتمدين على الرضاعة الطبيعيةّ؟	☐ لا	☐ نعم				

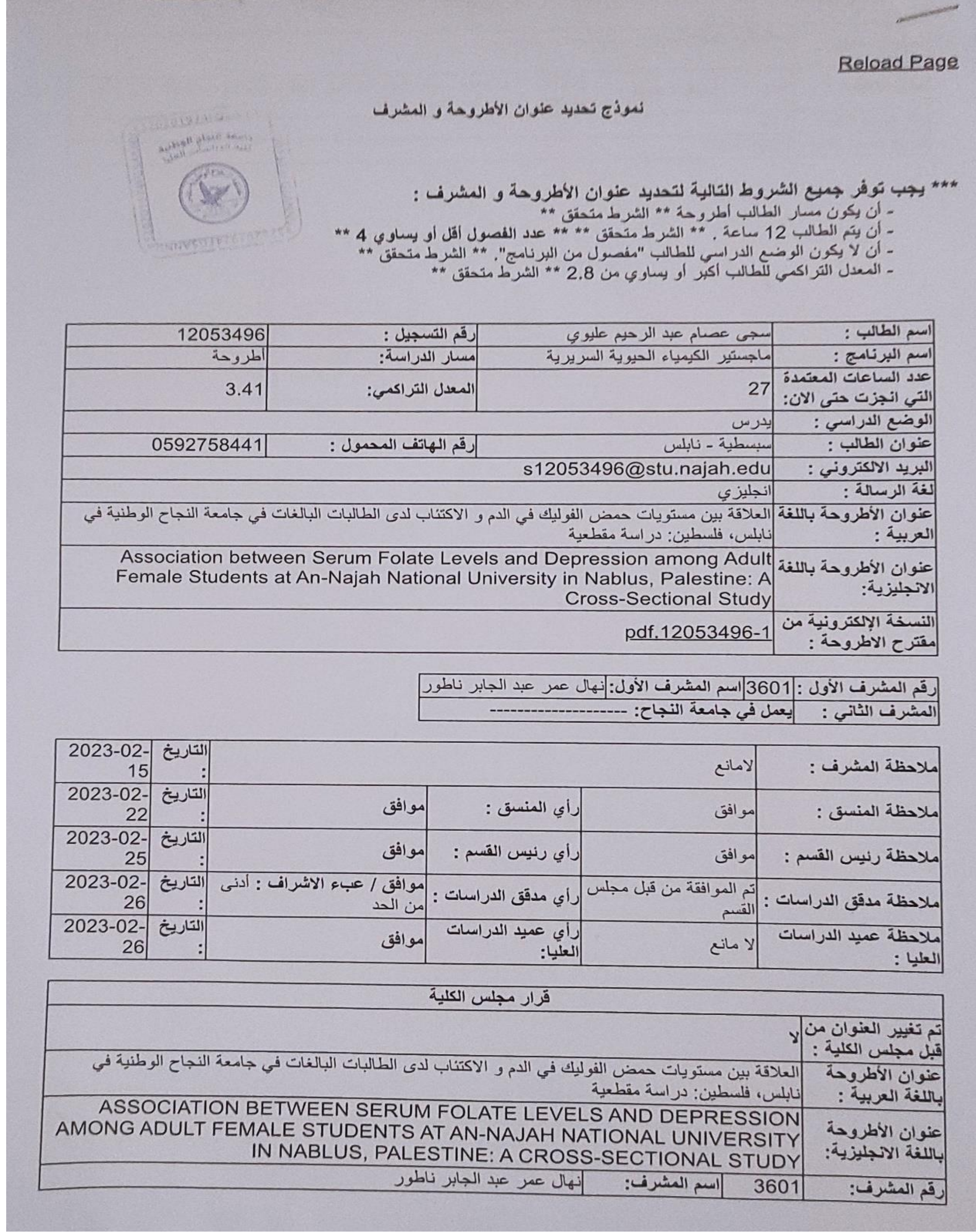
❖ **العوامل البيئية:-**		
1.هل منطقة السكن تعاني من تلوث هوائي(مثل: وجود مصانع)؟	☐ لا	☐ نعم
2. هل تتعرضين لأشعة الشمس فترة طويلة؟	☐ لا	☐ نعم

الاسم	----------------
رقم الجوال	---------------

اسم الفحص (Name of the test)	الفوليك أسيد/ فيتامين ب 9 / الفولات (Folic acid/Vitamin b9/Folate)
النتيجة (Result)	المعدل الطبيعي (Normal range)

Appendix C

Approval from the Faculty of Graduate Studies



Reload Page

نموذج تحديد عنوان الأطروحة و المشرف

*** يجب توفر جميع الشروط التالية لتحديد عنوان الأطروحة و المشرف :

- أن يكون مسار الطالب أطروحة ** الشرط متحقق **
- أن يتم الطالب 12 ساعة . ** الشرط متحقق ** ** عدد الفصول أقل أو يساوي 4 **
- أن لا يكون الوضع الدراسي للطالب "مفصول من البرنامج". ** الشرط متحقق **
- المعدل التراكمي للطالب أكبر أو يساوي من 2.8 ** الشرط متحقق **

اسم الطالب :	سجى عصام عبد الرحيم عليوي	رقم التسجيل :	12053496
اسم البرنامج :	ماجستير الكيمياء الحيوية السريرية	مسار الدراسة:	أطروحة
عدد الساعات المعتمدة التي انجزت حتى الان:	27	المعدل التراكمي:	3.41
الوضع الدراسي :	يدرس		
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عنوان الأطروحة باللغة العربية :	العلاقة بين مستويات حمض الفوليك في الدم و الاكتئاب لدى الطالبات البالغات في جامعة النجاح الوطنية في نابلس، فلسطين: دراسة مقطعية		
عنوان الأطروحة باللغة الانجليزية:	Association between Serum Folate Levels and Depression among Adult Female Students at An-Najah National University in Nablus, Palestine: A Cross-Sectional Study		
النسخة الإلكترونية من مقترح الاطروحة :	pdf.12053496-1		

رقم المشرف الأول :	3601	اسم المشرف الأول:	نهال عمر عبد الجابر ناطور
المشرف الثاني :	يعمل في جامعة النجاح:	--------------------	

ملاحظة المشرف :	لامانع			التاريخ :	2023-02-15
ملاحظة المنسق :	موافق	رأي المنسق :	موافق	التاريخ :	2023-02-22
ملاحظة رئيس القسم :	موافق	رأي رئيس القسم :	موافق	التاريخ :	2023-02-25
ملاحظة مدقق الدراسات :	تم الموافقة من قبل مجلس القسم	رأي مدقق الدراسات :	موافق / عبء الاشراف : أدنى من الحد	التاريخ :	2023-02-26
ملاحظة عميد الدراسات العليا :	لا مانع	رأي عميد الدراسات العليا:	موافق	التاريخ :	2023-02-26

قرار مجلس الكلية			
تم تغيير العنوان من قبل مجلس الكلية :	لا		
عنوان الأطروحة باللغة العربية :	العلاقة بين مستويات حمض الفوليك في الدم و الاكتئاب لدى الطالبات البالغات في جامعة النجاح الوطنية في نابلس، فلسطين: دراسة مقطعية		
عنوان الأطروحة باللغة الانجليزية:	ASSOCIATION BETWEEN SERUM FOLATE LEVELS AND DEPRESSION AMONG ADULT FEMALE STUDENTS AT AN-NAJAH NATIONAL UNIVERSITY IN NABLUS, PALESTINE: A CROSS-SECTIONAL STUDY		
رقم المشرف:	3601	اسم المشرف:	نهال عمر عبد الجابر ناطور

مشرف الثاني :	يعمل في جامعة النجاح: ---------------------		
فصل الاعتماد :	الثاني	سنة الاعتماد :	2022 ** ملاحظة : مثال العام الدراسي 2021-2022 يتم ادخاله على شكل 2021
رقم جلسة الكلية:	427		
تاريخ جلسة الكلية:	19/2/2023		

Appendix D

IRB approval

An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas.. Mar. 2023/12

IRB Approval Letter

Title of Research:

Association between Serum Folate Levels and Depression among Adult Female Students at An-Najah National University in Nablus, Palestine: A Cross-Sectional Study

Submitted by:
Saja Esam Abdel-Raheem Elewi

Supervisor:
Nihal Omar Natour

Approved:
15th. Mar. 2023

Your Study Title **"Association between Serum Folate Levels and Depression among Adult Female Students at An-Najah National University in Nablus, Palestine: A Cross-Sectional Study."** reviewed by An-Najah National University IRB committee and was approved on 15th. Mar. 2023

Hasan Fitian, MD

IRB Committee Chairman

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : IRB@najah.edu

Appendix E

Tables of Study

Table E1

The prevalence of diet, lifestyle behaviors, and clinical characteristics

Variable		Frequency (n)	Percentage (%)
Food			
Nature of food	Fruits and vegetables	38	21.1
	Sugars and starches	61	33.9
	Meats	21	11.7
	Canned and manufactures	29	16.1
	Fast food	31	17.2
Fruits intake (weekly)	Never	26	14.4
	1-3 grain	102	56.7
	4-6 grain	35	19.4
	>6 grain	17	9.4
Spinach, Parsley, Kale, and Broccoli intake (weekly)	Never	59	32.8
	1-3 grain	88	48.9
	4-6 grain	31	17.2
	>6 grain	2	1.1
Zucchini, peppers, Lettuce, Green peas, and Green beans intake (weekly)	Never	29	16.1
	1-3 grain	115	63.9
	4-6 grain	28	15.6
	>6 grain	8	4.4
Cucumbers, Onions, Eggplant, Radishes, Carrots, Tomatoes, and Cabbage intake (weekly)	Never	22	12.2
	1-3 grain	107	59.4
	4-6 grain	33	18.3
	>6 grain	18	10.0
Potatoes intake (weekly)	Never	23	12.8
	1-3 grain	106	58.9
	4-6 grain	31	17.2
	>6 grain	20	11.1
Avocado intake (weekly)	Never	76	42.2
	1-3 grain	70	38.9
	4-6 grain	29	16.1
	>6 grain	5	2.8
Meat intake (weekly)	Never	41	22.8
	1-3 piece	92	51.1
	4-6 piece	33	18.3
	>6 piece	14	7.8
Liver intake (weekly)	Never	120	66.7
	1-3 piece	47	26.1
	4-6 piece	12	6.7
	>6 piece	1	0.6
Fish products intake (monthly)	Never	71	39.4
	1-3 piece	79	43.9
	4-6 piece	29	16.1
	>6 piece	1	0.6
Milk intake (weekly)	Never	78	43.3
	1-3 cup	77	42.8
	4-6 cup	13	7.2
	>6 cup	12	6.7

Cheese intake (weekly)	Never	34	18.9
	1-3 piece	104	57.8
	4-6 piece	31	17.2
	>6 piece	11	6.1
Eggs intake (weekly)	Never	48	26.7
	1-3 grain	97	53.9
	4-6 grain	25	13.9
	>6 grain	10	5.6
Legumes (chickpeas, corn, beans, Lentils, Wheat) intake (monthly)	Never	21	11.7
	1-3 cup	100	55.6
	4-6 cup	47	26.1
	>6 cup	12	6.7
Nuts intake (monthly)	Never	20	11.1
	50 gm	93	51.7
	100 gm	40	22.2
	>100 gm	27	15.0
White bread intake (weekly)	Never	18	10.0
	1-3 loaf	39	21.7
	4-6 loaf	63	35.0
	>6 loaf	60	33.3
Brown bread intake (weekly)	Never	105	58.3
	1-3 loaf	41	22.8
	4-6 loaf	22	12.2
	>6 loaf	12	6.7
Black/dark chocolate intake (weekly)	Never	93	51.7
	1-3 piece	54	30.0
	4-6 piece	20	11.1
	>6 piece	13	7.2
Drink			
Tea intake (daily)	Never	36	20.0
	1-3 cup	66	36.7
	4-6 cup	49	27.2
	>6 cup	29	16.1
Coffee intake (daily)	Never	41	22.8
	1-3 cup	65	36.1
	4-6 cup	44	24.4
	>6 cup	30	16.7
Energy drinks intake (weekly)	Never	52	28.9
	1-3 cup	87	48.3
	4-6 cup	38	21.1
	>6 cup	3	1.7
Natural herbs intake (weekly)	Never	74	41.1
	1-3 cup	65	36.1
	4-6 cup	20	11.1
	>6 cup	21	11.7
Physical activity (weekly)	0-1 hour	108	60.0
	2-4 hour	57	31.7
	>4 hour	15	8.3
Smoking			
Passive smoking	No	101	56.1
	Yes	79	43.9
Cigarette smoking	Smoker	9	5.0
	Non-smoker	164	91.1
	Ex-smoker	7	3.9

Water-pipe smoking	No	103	57.2
	Yes	77	42.8
Infected by Covid-19	No	74	41.1
	Yes	106	58.9
Environment			
Exposure to air pollution in the residential area	No	114	63.3
	Yes	66	36.7
Exposure to sunlight for a long time	No	78	43.3
	Yes	102	56.7

Table E2

Diet, lifestyle behaviors, and clinical characteristics compared by serum folate levels

Variable		Median	χ2 (df)	*p*-value	Correlation coefficient	*p*-value
Food						
Nature of food	Fruits and vegetables	7.55	54.171 (4)	<0.001	_	_
	Sugars and starches	2.90				
	Meats	9.98				
	Canned and manufactures	2.80				
	Fast food	2.72				
Fruits intake (weekly)	Never	3.10	23.829 (3)	<0.001	0.293	<0.001
	1-3 grain	2.90				
	4-6 grain	7.13				
	>6 grain	8.80				
Spinach, Parsley, Kale, and Broccoli intake (weekly)	Never	2.98	25.163 (3)	<0.001	0.322	<0.001
	1-3 grain	3.00				
	4-6 grain	8.60				
	>6 grain	11.8				
Zucchini, peppers, Lettuce, Green peas, and Green beans intake (weekly)	Never	2.70	26.531 (3)	<0.001	0.356	<0.001
	1-3 grain	3.00				
	4-6 grain	8.29				
	>6 grain	7.46				
Cucumbers, Onions, Eggplant, Radishes, Carrots, Tomatoes, and Cabbage intake (weekly)	Never	2.64	43.365 (3)	<0.001	0.463	<0.001
	1-3 grain	2.90				
	4-6 grain	7.45				
	>6 grain	9.66				
Potatoes intake (weekly)	Never	2.90	30.581 (3)	<0.001	0.364	<0.001
	1-3 grain	2.93				
	4-6 grain	7.13				
	>6 grain	7.46				
Avocado intake (weekly)	Never	2.93	29.284 (3)	<0.001	0.383	<0.001
	1-3 grain	3.14				
	4-6 grain	8.23				
	>6 grain	9.35				
Meat intake (weekly)	Never	2.98	41.837 (3)	<0.001	0.391	<0.001
	1-3 piece	2.90				
	4-6 piece	7.80				
	>6 piece	10.55				
Liver intake	Never	3.00	17.967	<0.001	0.249	0.001

(weekly)	1-3 piece	3.19	(3)			
	4-6 piece	10.34				
	>6 piece	13.12				
Fish products	Never	3.00	25.765	<0.001	0.199	0.007
intake	1-3 piece	2.96	(3)			
(monthly)	4-6 piece	8.23				
	>6 piece	2.36				
Milk intake	Never	3.00	36.926	<0.001	0.290	<0.001
(weekly)	1-3 cup	2.90	(3)			
	4-6 cup	10.53				
	>6 cup	11.65				
Cheese	Never	2.85	36.03	<0.001	0.407	<0.001
intake	1-3 piece	2.96	(3)			
(weekly)	4-6 piece	8.90				
	>6 piece	10.53				
Eggs intake	Never	2.90	42.666	<0.001	0.358	<0.001
(weekly)	1-3 grain	2.96	(3)			
	4-6 grain	9.98				
	>6 grain	10.02				
Legumes	Never	3.00	21.319	<0.001	0.316	<0.001
(chickpeas,	1-3 cup	2.90	(3)			
corn, beans,	4-6 cup	3.79				
Lentils,	>6 cup	8.57				
Wheat)						
intake						
(monthly)						
Nuts intake	Never	3.35	36.481	<0.001	0.319	<0.001
(monthly)	50 gm	2.80	(3)			
	100 gm	3.37				
	>100 gm	9.35				
White bread	Never	4.77	13.023	0.005	- 0.016	0.831
intake	1-3 loaf	2.80	(3)			
(weekly)	4-6 loaf	3.13				
	>6 loaf	3.05				
Brown bread	Never	2.90	32.384	<0.001	0.392	<0.001
intake	1-3 loaf	3.13	(3)			
(weekly)	4-6 loaf	9.03				
	>6 loaf	10.86				
Black/dark	Never	2.97	29.512	<0.001	0.312	<0.001
chocolate	1-3 piece	3.05	(3)			
intake	4-6 piece	8.29				
(weekly)	>6 piece	10.70				
Drink						
Tea intake	Never	8.93	46.127	<0.001	- 0.449	<0.001
(daily)	1-3 cup	3.00	(3)			
	4-6 cup	2.97				
	>6 cup	2.60				
Coffee intake	Never	8.35	54.350	<0.001	- 0.501	<0.001
(daily)	1-3 cup	2.98	(3)			
	4-6 cup	2.98				
	>6 cup	2.50				
Energy	Never	8.47	57.734	<0.001	- 0.521	<0.001
drinks intake	1-3 cup	2.90	(3)			
(weekly)	4-6 cup	2.73				

	>6 cup	2.39				
Natural herbs intake (weekly)	Never	2.97	37.197 (3)	<0.001	0.364	<0.001
	1-3 cup	2.90				
	4-6 cup	6.96				
	>6 cup	10.70				
Physical activity (weekly)	0-1 hour	2.96	16.727 (2)	<0.001	0.297	0.001
	2-4 hour	4.40				
	>4 hour	10.70				
Smoking						
Passive smoking	No	3.75	_	<0.001	_	_
	Yes	2.90				
Cigarette smoking	Smoker	2.80	_	0.339	_	_
	Non-smoker	3.10				
	Ex-smoker	3.16				
Water-pipe smoking	No	4.53	_	<0.001	_	_
	Yes	2.80				
Infected by Covid-19	No	3.53	_	0.001	_	_
	Yes	2.96				
Environment						
Exposure to air pollution in the residential area	No	3.62	_	<0.001	_	_
	Yes	2.80				
Exposure to sunlight for a long time	No	3.24	_	0.002	_	_
	Yes	2.96				

Table E3

General characteristics are compared by depressive severity

Variable		Depression severity					χ^2 or Fisher exact test	p-value	Correlation coefficient	P-value
		Normal N (%)	Mild N (%)	Moderate N (%)	Severe N (%)	Extremely severe N (%)				
Age (year)	18-25	15 (88.2)	19 (86.4)	26 (100.0)	77 (90.6)	30 (100.0)	6.954	0.085	- 0.092	0.220
	26-30	2 (11.8)	3 (13.6)	0 (0.0)	8 (9.4)	0 (0.0)				
BMI (Kg/m2)	Underweight (<18)	2 (11.8)	5 (22.7)	2 (7.7)	15 (17.6)	0 (0.0)	17.864	0.120	0.118	0.114
	Normal weight(18-24.9)	9 (52.9)	11 (50.0)	18 (69.2)	36 (42.4)	17 (56.7)				
	Overweight (25-29.9)	4 (23.5)	3 (13.6)	6 (23.1)	29 (34.1)	10 (33.3)				
	Obese (≥30)	2 (11.8)	3 (13.6)	0 (0.0)	5 (5.9)	3 (10.0)				
Residence area	City	3 (17.6)	6 (27.3)	9 (34.6)	47 (55.3)	15 (50.0)	16.939	0.017	-	-
	Village	14 (82.4)	15 (68.2)	14 (53.8)	32 (37.6)	14 (46.7)				
	Camp	0 (0.0)	1 (4.5)	3 (11.5)	6 (7.1)	1 (3.3)				
Marital status	Single	15 (88.2)	21 (95.5)	25 (96.2)	75 (88.2)	28 (93.3)	9.499	0.269	-	-
	Married	2 (11.8)	1 (4.5)	0 (0.0)	10 (11.8)	2 (6.7)				
	Divorced	0 (0.0)	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)				
Educational level	Bachelors	14 (82.4)	20 (90.9)	26 (100.0)	73 (85.9)	29 (96.7)	10.083	0.154	-0.033	0.66
	Masters	3 (17.6)	1 (4.5)	0 (0.0)	11 (12.9)	1 (3.3)				

	PhD	0 (0.0)	1 (4.5)	0 (0.0)	1 (1.2)	0 (0.0)				
Work	Works	3 (17.6)	2 (9.1)	0 (0.0)	14 (16.5)	0 (0.0)	11.263	0.012	-	-
	Not works	14 (82.4)	20 (90.9)	26 (100.0)	71 (83.5)	30 (100.0)				
Family number members	<3	0 (0.0)	1 (4.5)	0 (0.0)	1 (1.2)	0 (0.0)	20.761	0.054	- 0.009	0.901
	3-5	3 (17.6)	3 (13.6)	4 (15.4)	14 (16.5)	0 (0.0)				
	6-8	9 (52.9)	12 (54.5)	12 (46.2)	61 (71.8)	22 (73.3)				
	>8	5 (29.4)	6 (27.3)	10 (38.5)	9 (10.6)	8 (26.7)				
Family income (ILS/monthly)	<1500	2 (11.8)	1 (4.5)	2 (7.7)	8 (9.4)	2 (6.7)	39.149	<0.001	- 0.212	0.004
	1500-3500	7 (41.2)	5 (22.7)	15 (57.7)	62 (72.9)	19 (63.3)				
	3501-7000	8 (47.1)	15 (68.2)	5 (19.2)	13 (15.3)	8 (26.7)				
	>7000	0 (0.0)	1 (4.5)	4 (15.4)	2 (2.4)	1 (3.3)				

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

العلاقة بين مستويات حمض الفوليك في الدم والاكتئاب لدى الطالبات البالغات في جامعة النجاح الوطنية في نابلس، فلسطين: دراسة مقطعية

إعداد

سجى عليوي

إشراف

د. نهال الناطور

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين

2024

العلاقة بين مستويات حمض الفوليك في الدم والاكتئاب لدى الطالبات البالغات في جامعة النجاح الوطنية في نابلس، فلسطين: دراسة مقطعية

إعداد

سجى عليوي

إشراف

د. نهال الناطور

الملخص

الخلفية: يعتبر الاكتئاب من أهم المشاكل الصحية العامة في جميع أنحاء العالم. يعد الاكتئاب مشكلة منتشرة بين طلاب الجامعات مقارنة بعامة السكان. حمض الفوليك، هو فيتامين ب المركب القابل للذوبان في الماء، والذي يتواجد بشكل طبيعي في الطعام وهو ضروري في الدماغ لإنتاج النورإبينفرين والدوبامين والسيروتونين. أشارت الدراسات السابقة إلى أن مستويات حمض الفوليك حاسمة في تطور الاكتئاب. ومع ذلك، فإن نتائج البحث لم تكن حاسمة عندما يتعلق الأمر بالاختلافات في مستويات حمض الفوليك بين الأشخاص المصابين بالاكتئاب مقارنة بأولئك الذين لا يعانون من هذه الحالة.

الهدف: هدفت هذه الدراسة إلى تقييم العلاقة بين مستويات حمض الفوليك في الدم والاكتئاب لدى الطالبات البالغات في جامعة النجاح الوطنية في فلسطين.

المنهجية: أجريت هذه الدراسة بتصميم وصفي مقطعي. تم توزيع استبيان من 15 أيلول 2023 الى 20 تشرين الأول 2023 على الطالبات المشاركات من جامعة النجاح الوطنية. تم تقييم أعراض الاكتئاب باستخدام مقياس DASS-21. كما جمع الاستبيان الخصائص الاجتماعية والديموغرافية للطالبات والعديد من الأسئلة المتعلقة بالعوامل التي تؤثر على مستويات حمض الفوليك في الدم. تم اختبار الموثوقية الداخلية لاستبيان DASS-21 باستخدام ألفا كرونباخ. بالإضافة إلى ذلك، تم جمع عينات الدم الوريدي

خلال نفس الفترة في مختبر الأبحاث العلمية بعد صيام ليلة كاملة، وتم قياس مستويات حمض الفوليك في الدم. وبلغ عدد الطالبات المشاركات 180، وتراوحت أعمارهن بين 18 و30 عاماً.

النتائج: كان متوسط مستويات حمض الفوليك في الدم 4.82 نانوغرام/مل (الانحراف المعياري=3.38). وبلغت نسبة انتشار الاكتئاب بينهم 16.7%، 47.2%، 14.4%، 12.2%، و9.4% للاكتئاب الشديد للغاية، الشديد، المتوسط، الخفيف، والطبيعي على التوالي. وكانت مستويات حمض الفوليك في الدم أقل بشكل ملحوظ في المرضى الذين يعانون من الاكتئاب الشديد للغاية والشديد، وكان المتوسط (3.13 و 3.30 نانوجرام/ مل) على التوالي. بالإضافة إلى ذلك، كان هناك ارتباط عكسي متوسط بين مستويات حمض الفوليك في الدم وشدة الاكتئاب ($r = -0.537$، قيمة $p<0.001$).

الخلاصة: أظهرت النتائج التي توصلنا إليها إلى وجود علاقة بين مستويات حمض الفوليك في الدم والاكتئاب بين الطالبات الفلسطينيات. قد تكون مستويات حمض الفوليك في الدم بمثابة مؤشرات لتقييم فعالية علاج الاكتئاب. يمكن أن يساعد قياس مستويات حمض الفوليك الأطباء في إدارة الاكتئاب بشكل فعال. في هذا الصدد، يبدو أن حمض الفوليك يلعب دورًا أكثر أهمية في تحسين نتائج اضطرابات المزاج ويجب تقييمه بعناية أكبر.

الكلمات المفتاحية: حمض الفوليك في الدم، الاكتئاب، نمط الحياة، النظام الغذائي، الخصائص السريرية، جامعة النجاح الوطنية، فلسطين.