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# FACTORS AFFECTING COENZYME COBALAMIN (VITAMIN $B_{12}$) LEVELS IN HEALTHY AND PATIENT INDIVIDUALS FROM PETRA REGION (JORDAN)

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## ABSTRACT

The aim of this study was to investigate levels of vitamin $B_{12}$ deficiency in healthy and patient individuals in Petra region in Jordan. The study was designed to include different subject groups based on different factors including gender, age, body mass index (BMI), diet, smoking, and disease (diabetes, hypertension, anemia, and gastrointestinal tract (GIT) problems). 400 participants were enrolled, indeed; (126) healthy (normal), (119) diabetics and (155) hypertension patients. Participants were classified based on age into three groups; (20-39), (40-59), and (60-80) years. Vitamin $B_{12}$ levels for hypertensive were significantly

higher than those of diabetics for the same group. It was found that vitamin $B_{12}$ levels for individuals with good diet, non-smokers, non-anemic and without GIT problems were significantly higher than those of individuals with poor diet, smokers, anemic and GIT problems. In conclusion Vitamin $B_{12}$ level is adversely affected by smoking, GIT problems, anemia and poor diet quality.

**Keywords: Vitamin $B_{12}$; Cobalamin; Diabetes; Hypertension; Coenzyme**

## 1. INTRODUCTION

The vast majority of all known enzymes are globular proteins [1, 2]. Some of these enzymes are made of polypeptide chains only whereas other enzymes contain both polypeptide chains (protein portions) called *apoenzyme*s and nonprotein portions called *cofactors*. The cofactors can be metallic ions, such as $Mg^{2+}$ and $Zn^{2+}$, or organic compounds called coenzymes. B vitamins are an important group of coenzymes, which are essential to the activity of many enzymes [3, 4].

Vitamin $B_{12}$ is a water-soluble vitamin that is naturally occurring in some foods, added to others, and a prescription medication and available as a dietary supplement. It plays a role in DNA synthesis and in neurological development.

Vitamin $B_{12}$ or cobalamin, is an important coenzyme required for a range of cellular metabolism processes (e.g., neurological development, DNA synthesis and methylation, mitochondrial metabolism, and etc.) [5]. Transporting ingested $B_{12}$ to the blood is a multi-step process [6]. In stomach $B_{12}$ binds to a glycoprotein (intrinsic factor, IF) forming a complex facilitating receptor-mediated endocytosis in ileum [7], a deficiency in the IF leads to autoimmune disease pernicious anaemia [6].Vitamin $B_{12}$ deficiency, also known as hypocobalaminemia, refers to low blood levels of Vitamin $B_{12}$ [8].

The higher risk groups for $B_{12}$ deficiency are children, pregnant women, the elderly, vegetarians and vegans, and individuals with gastrointestinal disorders and surgery. The main causes of vitamin $B_{12}$ deficiency include postsurgical malabsorption, dietary deficiency, vitamin $B_{12}$ malabsorption, and pernicious anemia. Also, agents like neomycin, biguanides and nitric oxide anesthetics that block or inhibit vitamin $B_{12}$ absorption, could also cause deficiency of this vitamin. The extensive use of acid blocking agents, such as proton pump inhibitors [9] and histamine $H_2$ receptor antagonists [10], is a common cause of the deficiency especially in the elderly population [11].

Additionally, Metformin, a hypoglycemic agent for diabetes treatment, may reduce vitamin $B_{12}$absorption [12] by altering the

intestinal mobility, alterations in the calcium-dependent uptake by ileal cells of the vitamin $B_{12}$-intrinsic factor complex, or increased bacterial over growth [13].

However, the exact cause of Vitamin $B_{12}$ deficiency is still unknown in many cases. The aim of the present study was to investigate the levels of Vitamin $B_{12}$ deficiency in Diabetics and hypertension patients, to assess the Vitamin $B_{12}$ status of the people in Petra region at national level. And also study the factors that affect Vitamin $B_{12}$ deficiency like gender, age, body mass index (BMI), diet, anemia, and smoking.

## 2. METHODS

**Instrument**

Cobas e411analyzer, (Electro-chemiluminescence (ECL) technology, second generation instrument, Roche Elecsys), was used to analyze the samples and determine vitamin $B_{12}$ levels. Sample volume required in standard Hitachi cup 150 Ul and reduced volume is 60 ul in primary sample tube.

**Study population**

The case report form (CRF) was self-completed by the study participants before study. The study was conducted on 109 males and 291 females' Jordanian subjects in Petra region to measure vitamin $B_{12}$serum levels. Subjects were divided into 3 groups; group one includes 126 normal subjects, group two includes 119 diabetics and group three includes 155 hypertension patients. Variables including gender, age, BMI, diet, smoking, gastrointestinal tract (GIT) problems and anemia were examined to investigate the correlation with vitamin$B_{12}$ levels. The subjects chosen in this study were selected from Petra countryside (Wadi Musa), south Jordan.

**Sample collection and Vitamin $B_{12}$ analysis**

Experimental protocols were approved by the appropriate institutional review committee and meet the guidelines of responsible governmental agency. Participating subjects in this study were verbally informed about the study and provided with an information sheet and were asked to sign a consent form. The implemented variables included age, gender, height, weight, BMI, medical history, vitamin $B_{12}$ supplements intake, diet, symptom such as: poor memory, depression, fatigue and cold hand. Blood samples of 3 – 5 ml were drawn by using syringes then transferred to tubes followed by centrifugation and the serum was collected and kept in labeled tubes. Serum samples were stored at -20$^{o}$C until the analysis.250 µl of the obtained serum was transferred to the cuvette of Cobas e411 analyzer. Vitamin $B_{12}$ concentration in human serum was determined using

vitamin $B_{12}$ kit. Samples were analyzed according to the procedures recommended by the manufacturer. The kit is based on the principal of competitive binding.

## 3. ANALYSIS

Data analyses were carried out using SPSS 17. Data were expressed as mean ± SD. Factors including gender, BMI, age, smoking, GIT problems, anemia and diet, were tested. Comparisons of variables were performed using both, descriptive statistics and analytical procedure including correlation analysis to analyze demographic and clinical factors associated with vitamin $B_{12}$ levels after logarithmic transformation. Vitamin $B_{12}$ normal range was defined as a serum level (145 pmol/l - 569 pmol/l), these values should only be used as guidelines. The statistical analysis, performed with t-test and Fisher test, was to analyze the differences among group mean and their associated. A P-value< 0.05 was considered significant.

## 4. RESULTS AND DISCUSSION

Vitamin $B_{12}$ levels were measured for 400 Jordanian subjects recruited in this study; the majority of the study participants were female (about 73 %). The study aimed to find a correlation between vitamin $B_{12}$ levels and a number of factors including; gender, age, BMI, diet, diseases (diabetes and hypertension), smoking, anemia, and GIT problems.

**Gender factor**

As shown in table 1, no significant differences ($p>0.05$) were detected between levels of vitamin $B_{12}$on the basis of gender. In males the concentration was 291 pmol/l whereas 284 pmol/l for females. This result indicates that gender is not a risk factor for vitamin $B_{12}$ deficiency. According to dietary allowances (RDAS) for Vitamin$B_{12}$ the requirements of vitamin $B_{12}$ at the same age of both gender is equal.

**Age factor** The 400 subjects are classified into 3 groups in terms of age: first: (20-39) years; second: (40-59) years; third: (60-80) years. According to Fisher test, no significant differences ($p>0.05$) were found in the vitamin $B_{12}$ levels between the three groups of age (Table 2). This means that age may not be a risk factor for Vitamin $B_{12}$ deficiency, while it is related to another factor. **Body Mass Index (BMI) factor:** Subjects were classified according to BMI into 3 groups; first: (18-24, Normal); second: (25-30, Over weight); third (>30, Obese). Fisher test indicated no significant differences ($p>0.05$) were found in the vitamin $B_{12}$ levels between the three groups (Table 3).

**Diet factor**

The 400 subjects were divided into good and poor diet groups. Good diet means that the volunteer food sources are rich with Vitamin $B_{12}$ and they have enough

requirements. Poor diet means that the volunteer food sources don't have enough Vitamin $B_{12}$. Table (4) shows all results in details. Both t-test and Fisher test confirmed the significant differences between poor and good diet ($p<0.05$). The average concentration of for good diet group was 347 pmol/l, while for poor diet group was 180 pmol/l. The % def (<145)pmol/l was 4.75% and 21.37% for good and poor diet groups, respectively. The %Over (>569) pmol/l was 7.45% and 0% for good and poor diet groups, respectively. It can be revealed from these results that poor diet could be considered as a Risk factor for vitamin $B_{12}$ deficiency and this is in agree with the federal government's 2015-2020 dietary guidelines for Americans which stated that "Nutritional needs should be met primarily from foods. Foods in nutrient-dense forms contain essential vitamins and minerals and also dietary fiber and other naturally occurring substances that may have positive health effects. In some cases, fortified foods and dietary supplements may be useful in providing one or more nutrients that otherwise may be consumed in less-than-recommended amounts".

**Disease factor**

Results of this study showed significant differences ($p<0.05$) in vitamin $B_{12}$levels between normal subjects (265 pmol/l), and hypertension patients (316 pmol/l), whereas there was no significant difference in vitamin $B_{12}$ level between normal subjects and diabetics ($p>0.05$) (Table 5).

The % deficiency values were 6.45%, 6.80% and 9.67% for normal, diabetics and hypertensive subjects, respectively. % Over values were 8.0%, 8.40% and 8.38% for normal, diabetics and hypertensive subjects, respectively. These results indicates that the low levels of vitamin$B_{12}$ for diabetic patients compared to those with hypertension might be attributed to metformin drug, which might reduce the absorption of vitamin $B_{12}$[12] or possibly through alterations in intestinal mobility, increased bacterial overgrowth, or alterations in the calcium-dependent uptake by ileal cells of the Vitamin $B_{12}$-intrinsic factor complex. A number of studies and case reports suggest that 10%–30% of patients who take metformin have reduced vitamin $B_{12}$ absorption [13, 14]. In a randomized trial in patients with type 2 diabetes, metformin treatment for more than four years raised the risk of vitamin $B_{12}$ deficiency by 7.2% and significantly decreased vitamin $B_{12}$ levels by 19% compared with placebo [11]. Some studies suggest that vitamin $B_{12}$ malabsorption caused by metformin might be controlled by supplemental calcium [13, 14].

Individuals, who experience, even slightly, vitamin $B_{12}$deficiency also tend to have raised homocysteine levels. Raised homocysteine levels are associated with heart diseases and strokes [15, 16]. According to the American heart association, plasma homocysteine has been associated with high blood pressure in large community studies. It was found that individuals with hypertension have raised homocysteine levels and reducing the potential for increased homocysteine levels by taking enough vitamin $B_{12}$[17]. It can be concluded that the deficiency in vitamin $B_{12}$ might increase homocysteine levels that are associated with high blood pressure.

Also, when studying the effect of diet in normal, diabetics and hypertension patients results showed a significant effect on vitamin $B_{12}$levels ($p<0.05$) between normal subjects and diabetes subject with good diet (337 pmol/l, 294 pmol/l for good diet of normal and diabetes, respectively). Also, there is a significant effect found on vitamin $B_{12}$levels ($p<0.05$) between normal subjects with poor diet and diabetes subjects with poor diet. The average was 190pmol/l and 163 pmol/l, respectively. Diabetes with poor dietary is risky for vitamin $B_{12}$ deficiency than normal with poor diet. Experimental studies found that metformin induces serotonin release by human duodenal mucosa through neural and no neuronal mechanisms [18]. Experimentally it has been shown that administration of these 5-HT3 agonists causes vomiting and diarrhea in animal models [19, 20].Bile salts malabsorption can also be responsible for the metformin induced diarrhea [21].

Additionally, a significant effect found on vitamin $B_{12}$levels ($p<0.05$) between good diet in normal and hypertensive patients. The averages were 337 pmol/l and 408 pmol/l for normal and hypertensive patients, respectively.

**Smoking factor**

The 400 subjects participated in this study were divided into smokers and nonsmokers. Next, smoking effect on vitamin $B_{12}$ levels has been studied (Table 6).

Both t-test and Fisher test showed a significant effect on vitamin $B_{12}$ levels ($p<0.05$) between smoking and nonsmoking subjects, the averages were 302 pmol/l and 208 pmol/l for smoking and nonsmoking subjects, respectively (Table 6). For nonsmoking and smoking subjects the (% def) values were 7.22%, 27.94%, respectively. That means smoking might be a risk factor for vitamin $B_{12}$ deficiency.

Smoke contains HCN which is a poisonous colorless gas. Linnell and Mathews [22] investigated the influence of tobacco smoke on vitamin $B_{12}$excretion and

metabolism. They found that smokers have higher urine excretion levels and lower serum levels of vitamin $B_{12}$than nonsmokers. Consequently, they concluded that high cyanide intake due to smoking can produce abnormal vitamin $B_{12}$ metabolism. Therefore, smokers may require vitamin $B_{12}$ supplementation. Smoking may interfere with absorption of vital vitamins and minerals causing deficiencies. Smoking may be a risk factor of vitamin $B_{12}$ deficiency, especially if associated with other factors such as disease.

**Anemia**

To study the effect of anemia on vitamin $B_{12}$ level, we classified the 400 subjects into anemic subjects and non-anemic subjects.

Both t-test and Fisher test confirmed that there is a significant difference in vitamin $B_{12}$ level between anemic and non-anemic subjects ($p<0.05$). The averages were 162 pmol/l, and 320 pmol/l for anemic and non-anemic subjects, respectively. The (% deficiency) values were 33.72% and 5.09%, respectively (Table 7). Results reveal that anemia may be a risk factor for vitamin $B_{12}$ deficiency.

**Gastro intestinal tract (GIT) medications factor**

The 400 subjects were divided into 2 groups according to the usage of GIT medication. There is a significant difference in vitamin $B_{12}$ level ($p<0.05$) between subjects taking GIT medications and the other who do not. The average concentrations of vitamin $B_{12}$were 197pmol/l and 332pmol/l for subjects taking GIT medications and those who do not, respectively (Table 8). The (% deficiency) values were 3.78% and 2.42%. That means that taking GIT medications can be considered as a risk factor for vitamin $B_{12}$ deficiency. In addition, we investigated the correlation between diseases (diabetes and hypertension) and taking GIT medications in the context of their influence on vitamin $B_{12}$ levels. In this investigation, the 136 subjects that used GIT medications were divided into three groups; (1) normal who are neither diabetics nor hypertensive subjects, (2) hypertensive subjects, and (3) diabetics. Table 9 shows that there is a significant difference ($p<0.05$) in vitamin $B_{12}$ levels among subjects who use GIT medication from normal group and disease (diabetes and hypertension). The average vitamin $B_{12}$ levels were 228 pmol/l, 169 pmol/l and 171 pmol/l for normal, hypertensive and diabetics, respectively. One can consider GIT medications as a risk factor of vitamin $B_{12}$ deficiency, especially when associated with other factors such as disease.

Table (1): Concentration of vitamin $B_{12}$: Gender of all the subjects

| Gender | Total% | Average (pmol/l) | %def (<145)pmol/l | %Over(>569)pmol/l | P |
|---|---|---|---|---|---|
| Female | 72.75 | 284 | 9.3% | 9.6% | 0.68 |
| Male | 27.25 | 291 | 18.3% | 9.17% | |

Table (2): Concentration of vitamin $B_{12}$: Age of all the subjects

| Age | Total% | Average (pmol/l) | %def(<145)pmol/l | %Over(>569)pmol/l | P |
|---|---|---|---|---|---|
| 1 | 25.75% | 266 | 9.70% | 5.8% | (1,3)=0.59 |
| 2 | 48.75% | 292 | 12.30% | 10.77 | (1,2)=0.24 |
| 3 | 25.50% | 295 | 12.75% | 10.78 | (2,3)=0.62 |

Table (3): Concentration of vitamin $B_{12}$: BMI of all the subjects

| BMI | %Total | Average (pmol/l) | %def(<145)pmol/l | %Over(>569)pmol/l | P |
|---|---|---|---|---|---|
| 1 | 17.50% | 301 | 8.10% | 9.45% | (1,2)=0.22 |
| 2 | 44.25% | 286 | 12.5% | 9.65% | (3,2)=0.86 |
| 3 | 38.25% | 278 | 11.25% | 9.27% | (1,3)=.167 |

Table (4): Concentration of vitamin $B_{12}$: Diet of all the subjects

| Diet | %Total | Average (pmol/l) | %def(<145)pmol/l | %Over(>569)pmol/l | P |
|---|---|---|---|---|---|
| Good | 63.75 | 347 | 4.75% | 7.45% | 0.00 |
| Poor | 36.25 | 180 | 21.37% | 0.00% | |

Table (5): Concentration of vitamin $B_{12}$: Disease of all the subjects

| Disease | %total | Average (pmol/l) | %def (<145pmol/l) | %Over (>569)pmol/l | P |
|---|---|---|---|---|---|
| Normal | 31.50% | 265 | 6.45% | 8.0% | |
| Diabetes | 29.75% | 267 | 6.80% | 8.40 | 0.63 |
| Hypertension | 38.75% | 316 | 9.67% | 8.38 | 0.03 |

Table (6): Concentration of vitamin $B_{12}$: Smoking of all the subjects

| Smoking | %Total | Average (pmol/l) | %def (<145pmol/l) | %over(569)pmol/l | P |
|---|---|---|---|---|---|
| No | 83% | 302 | 7.22 | 5.72 | 0.00 |
| Yes | 17% | 208 | 27.94 | 0.00 | |

Table (7): Concentration of vitamin $B_{12}$: Anemia of all the subjects

| Anemia | %Total | Average (pmol/l) | %def(<145) pmol/L | %over(>569) pmol/L | P |
|---|---|---|---|---|---|
| Yes | 21.50% | 162 | 33.72% | 0.00 | 0.00 |
| No | 78.50% | 320 | 5.09% | 12.10 | |

Table (8): Concentration of vitamin $B_{12}$: usage of GIT medications of all subjects

| GIT | %Total | Average (pmol/l) | %def(<145)pmol/l | %over(>569)pmol/l | P |
|---|---|---|---|---|---|
| No | 66.0% | 332 | 3.78% | 7.20 | 0.00 |
| Yes | 34.0% | 197 | 2.42% | 0.00 | |

Table (9): Concentration of vitamin $B_{12}$: usage of GIT medicationsassociated with disease factor

| GIT problems | %Total | Average(pmol/l) | %def(<145)pmol/l | %over(>569)pmol/l | P |
|---|---|---|---|---|---|
| Normal | 15.75 | 228 | 11 | 3.20 | |
| Hypertensive | 8.50 | 169 | 38 | 0 | 0.0001 |
| Diabetes | 9.75 | 171 | 36 | 0 | 0.0002 |

## 5. CONCLUSION

Vitamin $B_{12}$ levels have been determined for 400 Jordanian subjects from Petra region. The 400 subjects were classified into several groups. Factors tested included gender, BMI, age, disease (diabetes, hypertension), smoking, GIT medications usage, anemia and diet. After using both descriptive statistics and analytical procedure including correlation analysis we determined the demographic and clinical factors associated with vitamin $B_{12}$, we found that there are significant effects on vitamin $B_{12}$ levels by the following factors; disease (including hypertension, diabetes type 2), poor diet, smoking, anemia, and GIT problems. These factors decrease vitamin $B_{12}$ levels and increase its deficiency. On the contrary, there is no significant effect of age, gender, and BMI factors on vitamin $B_{12}$ levels. It is recommended to make regular checks for vitamin $B_{12}$ levels for hypertension patients and diabetics (type 2),to follow good and healthy diet, and to avoid smoking to avoid the deficiency in vitamin $B_{12}$. Our future plan is to study the relation between vitamin $B_{12}$ levels and other common and important diseases like cardiac diseases, cancer, dementia, attention deficit hyperactivity disorder (ADHD) and infertility.

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