Genetic Bases of VitaminB12 Deficiency: Impact of MTHFR, TCN-II and GIF Polymorphisms on Vitamin B12 Level

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Abstract— Vitamin B12 deficiency is associated with serious health problems such as neurological disorders. In Jordan, few studies have evaluated the level of vitamin B21 in the Jordanian population with different prevalence. Genetic predisposition, lifestyle, environment, socioeconomic status, and geographic have been linked to vitamin B12 deficiency. Polymorphisms in the GIF, MTHFR, and Transcobalamins, have been proposed to be associated with the level of vitamin B12. The aim of the current study was to evaluate the impact of certain polymorphisms in MTHFR, TCN-II and GIF genes on the level of vitamin B12 in the Jordanian population. Polymorphic sites of the MTHFR (c.677 C>T, rs1801133 and c.1286A>C, rs1801131), TCN2-776C>G (Arg259Pro) (rs1801198) and GIF-68 A>G (Q5R) genes were analyzed by RFLP and DNA sequencing in a group of vitamin B12 deficient individuals (n = 100). The control group included 100 matching individuals with a normal level of vitamin B12 (>200 ng/mL). Our results showed a significant association between the homologous variant of the TCN2 gene (G776G) and MTHFR c.677C>T genes and vitamin B12 deficiency. On the other hand, The MTHFR c.1286A>C variant and GIF variants did not show significant association with vitamin B12 deficiency. This study expounds the association of TCN2 and MTHFR polymorphisms with cobalamin levels in a Jordanian population and highlights the necessity of further studies to elucidate the molecular basis and impact of TCN2, GIF, and MTHFR gene polymorphisms on vitamin B12 deficiency and associated disorders.

Keywords— MTHFR, TCN-2, GIF, Polymorphisms, Vitamin B12 Deficiency.

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1. Introduction

Cobalamin (Vitamin B12) is an essential vitamin for the synthesis of methionine, DNA, red blood cells and the myelin of the nerve cells [1-3]. Therefore, it is crucial to be obtained from the natural sources such as meat, milk, and eggs [4]. Naturally, vitamin B12 deficiency is caused by malnutrition, malabsorption or genetic predisposition and it takes up to 5 years to develop [5-7]. Vitamin B12 deficiency can lead to serious pathology including megaloblastic anemia and certain neurological disorders [8-12]. In Jordan, vitamin B12 deficiency is a serious health concern. However, few studies have evaluated the level of vitamin B12 in the Jordanian population with different prevalence [13-17].

Many factors have been proposed to be associated with the deficiency of vitamin B12 including genetic predisposition, lifestyle, environment, socioeconomic status and geography [17-19]. For instance, certain polymorphisms in the gastric intrinsic factor, MTHFR, and Transcobalamins have been found to be related to the level of vitamin B12 [20-22]. Furthermore, in an interesting Chilean study, Cabrera et al. reported that the deficiency of vitamin B12 is associated with the UV light exposure [19].

Geographically, Jordan is a Middle East country, located in Southwest Asia and northern of Tropic of Cancer. Despite the small size of Jordan, the geography in Jordan is very diverse and includes the lowest point on the earth (Dead Sea).

In this study, we aimed to evaluate the prevalence of vitamin B12 deficiency, and genetic polymorphisms of TCN-2, MTHFR and GIF genes in Jordanian population in different geographical locations.

2. Materials and Methods

The study population included 2,880 Jordanian individuals (890 men and 1990 women) from four governorates covering three geographical locations in Jordan (Irbid, Karak, Maan, and Tafilah). Blood samples were collected in EDTA tubes after signing the informed consent form which was approved by the research ethics committee at Yarmouk University. 200 samples (100 with vitamin B12 deficiency and 100 as a control group) were selected for genetic study of the polymorphic variation in three genes (TCN-2, MTHFR and GIF) including four SNPs.

Genomic DNA extraction was performed by using commercially availble kit from Qiagen (QIAamp® DNA Blood Mini Kit (QIAGEN, Germany) According to the manufactuer's instructyions. The last wash was removed and the pellet mixed with 100 uL of TE buffer and stored at – 20° C.

PCR, RFLP and DNA sequencing were performed as described before [23, 24].

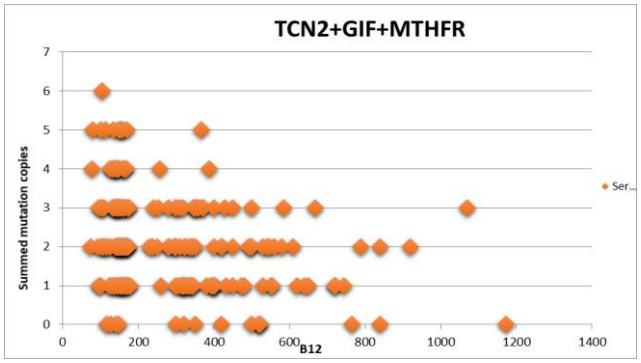
3. Results

Univariate OR showed an increase in the risk of low vitamin B12 with increasing age in men and women. Both univariate OR and age-adjusted OR in men showed a significant decrease in low vitamin B12 risk in the region of Tafela when compared to Irbid. The similar significant lower risk was observed in

women from Maan and Tafela. No significant change in risk was found in women reporting pregnancy or newborns.

Our results showed a significant association between homologous G776G genotype of the TCN2 gene and the low level of vitamin B12 ($p < 0.05$). On the other hand, the genotype distribution; A68G genotypes of the GIF gene did not show significant association with the deficiency of vitamin B12 ($p = 0.2$)

DNA sequencing analysis for the C677T polymorphisms of the MTHFR gene showed that the frequency of homozygous CC genotype is lower in the B12 deficient individualscompared to the control group. Hence, the C677T genotypes frequencies distribution revealed a significant difference in individuals with vitamin B12 compared to controls. B12 deficient individuals did not show any significant difference in the genotypes frequencies distribution for the A1298C polymorphisms in comparison with the control group.



4. Discussion

In the current study, we found a significant difference in B12 level between different geographical groups. The northern population showed the lowest level of vitamin B 12 and the highest percentage of deficient individuals in comparison with the other groups. On the other hand, the southern populations showed a higher level of vitamin B12 and lowest percentage of vitamin B 12 deficiency. Our findings are consistent with previous reports that underscored the health problem of vitamin B12 deficiency in Jordan [17, 25-27]. The southern population showed a higher level of vitamin B12 and a lower percentage of deficiency. Therefore, we are suggesting the role of other genetic, lifestyle, dietary and environmental factors that could be associated with vitamin B12 level in Jordan. Genetically, the results suggest that the homologous G776G genotype of the TCN2 gene and MTHFR T677T genotype have significant association with vitamin B12 level in a Jordanian population. On the other hand, the A68G genotype of the GIF gene and the 1298A>C polymorphisms of the MTHFR gene did not show an association with the deficiency of B12 showed no significant association with B12 deficiency.

Combination of the four geneotypes showed a higher associoation between the tested polymorphisms and the presence of vitamin B12 deficiency in the Joprdanian population. Further studies are required to elucidate the impact of TCN2, GIF and MTHFR genes polymorphisms on B12 indices and B12 associated disorders.

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