Hindawi
Biochemistry Research International
Volume 2024, Article ID 8827402, 7 pages
https://doi.org/10.1155/2024/8827402

Research Article

Exploring the Impact of Cigarette Smoke Extracts on Vitamin B_{12}: Insights into the Transformation of Methylcobalamin and Hydroxycobalamin to Cyanocobalamin through *In Vitro* Evaluation

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Received 18 November 2023; Revised 10 March 2024; Accepted 28 March 2024; Published 18 April 2024

Academic Editor: Zubeyir Huyut

Vitamin B_{12} (cobalamin) is a water-soluble molecule required for the proper functioning of metabolism, blood and DNA synthesis, and neurological development. Vitamin B_{12} exists in several forms: methylcobalamin (MeCbl), adenosylcobalamin (AdoCbl), hydroxycobalamin (OHCbl), and cyanocobalamin (CNCbl). This study aimed to evaluate the effect of cigarette smoke on the chemical structure of methylcobalamin and hydroxycobalamin forms of vitamin B_{12}. MeCbl and OHCbl were markedly affected by exposure to cigarette smoke. The resemblance of the Rt between MeCbl and OHCbl and CNCbl indicates that exposure to cigarette smoke extracts chemically alters MeCbl and OHCbl to CNCbl, warranting *in vivo* research investigations.

1. Introduction

Vitamin B_{12} (cobalamin) is a water-soluble vitamin that plays an important role in certain metabolic processes [1, 2]. The main supply of cobalamin comes from animal resources such as liver, kidney, meat, egg, and milk derivatives. The highest level of cobalamin comes from fish, and there is a small level that is produced by some kind of intestinal macrobiotic [3]. In the last few decades, there was a lot of debate about cobalamin affectivity and deficiency. It was found that nearly 6% of Western people at the age of 60 had a low level of cobalamin serum and around 20% have shown marginal cobalamin status [4]. This subtle deficiency may be responsible for cognitive function and may cause dementia in older people due to poor food habits and poor absorption which may lead to a low cobalamin concentration [5, 6]. Vitamin B_{12} has many vital roles such as the synthesis of DNA, some neurological functions, and the metabolism of proteins and carbohydrates [7]. Furthermore, it has a role in myelin synthesis and erythropoiesis [8, 9]. The most significant symptoms related to vitamin B_{12} deficiency are fatigue, memory impairment, skin pallor, glossarist, and severe hematological and neurological disorders [10–14].

Vitamin B_{12} has a complex structure that contains a corrin ring that includes four pyrrole rings and a central cobalt ion that is attached to four nitrogen atoms as illustrated in Figure 1. In addition, a dimethyl benzimidazole

group and variable *R* group are located below and above the plane of the corrin ring, respectively [9, 15–18].

Vitamin B_{12} exists in several forms; one of them being methylcobalamin (MeCbl), it is an active form that is necessary to maintain the nervous system and the most efficient form that is consumed by neurons and cells. MeCbl form, considered a cofactor for methionine synthase in the methionine synthesis process is used in the treatment of vitamin B_{12} deficiency and Alzheimer's disease [19, 20]. Another active analog of vitamin B_{12} is adenosylcobalamin (AdoCbl), a very sensitive derivative to light, which is known as a coenzyme for methyl malonyl-CoA mutase [21–23]. Cyanocobalamin (CNCbl), an inactive form of vitamin B_{12}, is described as an element of antipernicious anemia, exists in trace amounts in food and is eliminated quickly in the urine [24]. CNCbl is not a coenzyme and is mostly used as a supplement. It is partially transformed into active forms upon being absorbed in the intestine [21–23, 25, 26]. Hydroxycobalamin (OHCbl) is a unique form that is converted into its active form and has a relatively long shelf life with minimal side effects. Notably, OHCbl is one of the effective therapies used in cases of cyanide poisoning [21, 25].

The absorption process of vitamin B_{12} involves the ingestion of vitamin B_{12} (cobalamin); it will bind first to haptocorrin within the salivary glands and then will undergo a proteolytic cleavage via the stomach to the duodenum where pancreatic proteases break down haptocorrin proteins and release vitamins. The intrinsic factor attaches to cobalamin to form a complex that is actively absorbed through cubilin receptors at the ileum level. In the enterocytes, the cobalamin is released from the intrinsic factor and exported to the circulation, where it binds with transcobalamin. Enterohepatic circulation releases cobalamin into the bile associated with haptocorrin. This cobalamin may be reabsorbed into the ileum if there is an intrinsic factor [27]. The two distinct metabolic cascades have been established in the two active forms of vitamin B_{12} (methylcobalamin and adenosylcobalamin (AdoCbl)) [28–30]. AdoCbl form is stored in the cellular tissues, particularly in the mitochondria. Other forms of vitamin B_{12} are found in the cytosol, blood and some body fluids [31].

Various factors have been suggested to affect the structural integrity and conformation of vitamin B_{12} such as heat, light, and microwaves, and to lead to vitamin B_{12} deficiency [32–36]. Likewise, cigarette smoking has been proposed to be a causative agent of vitamin B_{12} deficiency by possibly converting the active forms of vitamin B_{12} into cyanocobalamin. This inactive form is, in turn, excreted with urine [24]. The mechanism of this conversion and inactivation of vitamin B_{12} is enigmatic; however, certain ingredients of the smoke such as organic nitrates, nitrous oxides, cyanate, and isocyanates could be responsible, warranting critical research assessments [37]. Therefore, in the current study, we aimed to evaluate the *in vitro* effect of cigarette smoke on the structure and integrity of methylcobalamin and hydroxycobalamin forms of vitamin B_{12}.

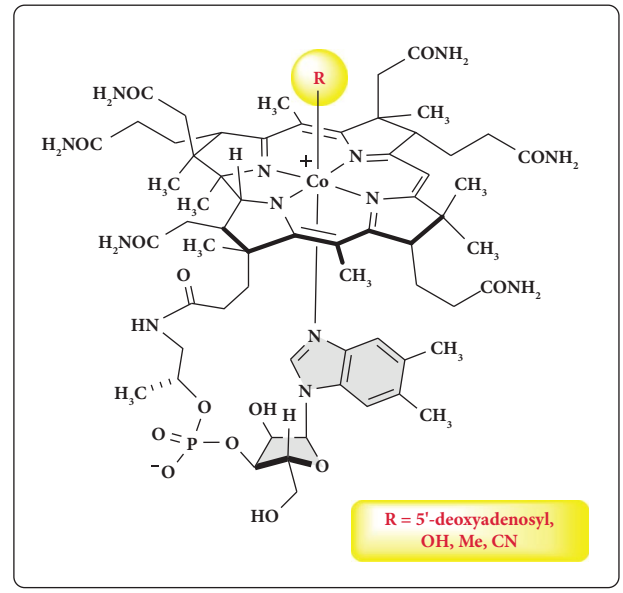

FIGURE 1: Vitamin B_{12} chemical structure and forms. *R* is bound to cobalt from the β-face.

2. Materials and Methods

Three different forms of cobalamin were used in this experiment (MeCbl, OHCbl, and CNCbl) where the concentrations were 1000 μg/mL. Vitamin B_{12} forms were obtained from the local pharmaceutical market (Jordan pharmaceutical manufacturing, Rotexmedica, and Panbiotic laboratories). Cigarette smoke was extracted by a homemade device (Figure 2) and mixed with the methylcobalamin and hydroxycobalamin solutions at different concentrations (i.e., 5 cigarettes, 10 cigarettes, and 20 cigarettes) in triplicates. The mixing process was performed under dark conditions due to the sensitivity of vitamin B_{12} to light. Samples were examined on auto-sampler high-performance liquid chromatography using Thermo Scientific, Dionex UltiMate-3000 Series. C8-column Phenosphere 5 μm with 250 $*$ 4.6 mm internal diameter from (Phenomenex, USA) and variable wavelength detectors (VWD-3100 and VWD-3400RS) were used. Two mobile phases were used, and gradient elution was performed with 0.025% (w/v) trifluoroacetic acid (TFA) in water (mobile phase A) and pure acetonitrile (mobile phase B) at a flow rate of 1.0 mL/min.

2.1. Treatment by Cigarette Smoke Extracts. Each form of cobalamin (OHCbl, MeCbl, and CNCbl) was divided into three groups (5.0 mL each (1000 μg/mL)) and mixed with cigarette smoke extracts (5 cigarettes, 10 cigarettes, and 20 cigarettes).

2.2. Treatment by Potassium Cyanide (KCN). An additional experiment was performed to confirm the role of cyanide (CN) in cigarette contents by the conversion of vitamin B_{12} forms to CNCbl. Briefly, 0.1 mM of KCN was prepared and mixed with OHCbl and measured by High-Performance Liquid Chromatography (HPLC).

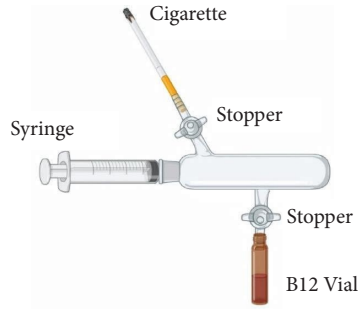

Figure 2: Cigarette smoke extraction apparatus which consists of a syringe with equal volume collection champer with two valved exits for CSE collection and cobalamin vial connection.

2.3. High-Performance Liquid Chromatography (HPLC). The samples were analyzed by using the HPLC auto-sampler (Thermo Scientific) with variable wavelength detectors (VWD-3100 and VWD-3400RS) (Germany). Gradient elution was performed with 0.025% (w/v) trifluoroacetic acid (TFA) in water (mobile phase A) and pure acetonitrile (mobile phase B) at a flow rate of 1.0 mL/min. The total run time was 18.0 minutes for each sample. C8-column Phenosphere 5 μm with 250 $*$ 4.6 mm internal diameter from (Phenomenex, USA) was used in the study.

3. Results

3.1. HPLC Analysis. The HPLC results showed no impact of cigarette smoke treatment on cyanocobalamin, giving the same peak and retention time (Rt) (6 min) as shown in Figure 3(a). On the other hand, the HPLC results showed a drastic change in the Rt time of both methylcobalamin and hydroxycobalamin after exposure to cigarette smoke extracts as shown in Figures 3(b) and 3(c), respectively. The chromatogram clearly showed a change in the Rt of methylcobalamin peak from 11.5 mins en route to 6.0 mins for both control and treated samples, respectively. For hydroxycobalamin, the Rt also shifted from 13.0 mins to 6.0 mins for both control and treated samples, respectively, as shown in Figure 3(c).

The inherent overlapping in Rt of both treated OHCbl and MeCbl with the untreated CNCbl implies the chemical conversion of OHCbl and MeCbl to CNCbl after treatment with cigarette smoke extracts (Figure 3(d)).

To confirm the chemical change in both hydroxycobalamin and methylcobalamin-treated samples and also to investigate further the role of cyanide (CN) extracted from the cigarette smoke in the conversion of OHCbl and MeCbl to CNCbl, we performed a direct mix between hydroxycobalamin and KCN solution at a concentration of 0.1 mM. It provided a new peak, which is matched perfectly in Rt with the peak of treated OHCbl with cigarette smoke extracts as shown in Figure 3(e). Interestingly, the results showed that the different doses of cigarette smoke extracts (5, 10, and 20 cigarettes) have the same effect on vitamin B_{12} forms.

4. Discussion

Vitamin B_{12} deficiency is a worldwide health concern and plays a vital role in many metabolic pathways in the human body [38–42]. Many pieces of evidence in the literature suggest that the structure of vitamin B_{12} is affected by several factors such as heat and UV light [32, 43]. Other studies reported an association between vitamin B_{12} deficiency and smoking. Accordingly, we assumed in this study that the exposure effect of vitamin B_{12} forms to cigarette smoke extracts might alter the chemical structure and therefore, lose their benefits as a vitamin in our body. According to the structure of vitamin B_{12}, we believe that the exposure of vitamin B_{12} forms to cigarette smoke extracts will be able to exchange the perpendicular *R* group, giving one of the existing forms or a new and inactive form. The three most common forms (OHCbl, MeCbl, and CNCbl) were used in this study. The experiments were conducted by treating OHCbl, MeCbl, and CNCbl with cigarette smoke extracts. Only one of these three forms of vitamin B_{12} is known to be inactive, the CNCbl form, which is known to be excreted by urine from the body.

The HPLC chromatogram of the cigarette-smoke-treated MeCbl showed a significant shift in the Rt from 12 mins to 6 mins resembling the Rt of the CNCbl. No difference in Rt for CNCbl (control) and cigarette-smoke-treated CNCbl (treated). The varying doses of cigarette smoke extracts have an identical impact on the forms of vitamin B_{12}. These results are in line with a previous study that demonstrated that urine B_{12} excretion was raised in smokers and a relatively low serum B_{12} concentration [44]. It is worth noting, as numerous other published manuscripts have indicated, that it is challenging to ensure that upon burning the same quantity of cigarettes, even of the same brand, consistently contain identical quantities and compositions of cigarette extracts.

In several studies, smoking is related to the reduced vitamin B_{12} concentration in serum and the increased vitamin B_{12} secretion through the urine. For instance, a study reported a significant reduction in B_{12} concentration in the serum in the smokers' cohort compared to the nonsmokers' control sample. The B_{12} concentration in serum was 444 μg/ml and 472 μg/ml for both smokers and nonsmokers patients, respectively [43]. Additionally, the authors reported a significant increase in the concentration of B12 in urine samples (81.2 m μg/24 hours) for the smokers' cohort compared to 60.3 m μg/24 hours for nonsmokers' control. Overall, cigarette smoking will possibly decrease vitamin B12 concentration in serum by increasing its excretion in urine [44]. Singh reported a study that enrolled 300 males, 150 among them, were chronic cigarette smokers who have been smoking for more than 20 years and the other 150 were nonsmokers recruited as a control group. Their results showed that the concentration of vitamin B12 in the smokers' group was 346 pg/ml, whereas the concentration of B12 in nonsmokers was 481 pg/ml. The findings of these studies are steady with our results that hydroxycobalamin and methylcobalamin can be converted to cyanocobalamin by altering the *R* group in cobalamin to be CN and then excreted B12 out of the body leading to a decline in B12 concentration in the serum [45]. In addition, another study recruited 33 pregnant women between 16 and 22 weeks of gestation, among them, 19 patients were smokers and 14

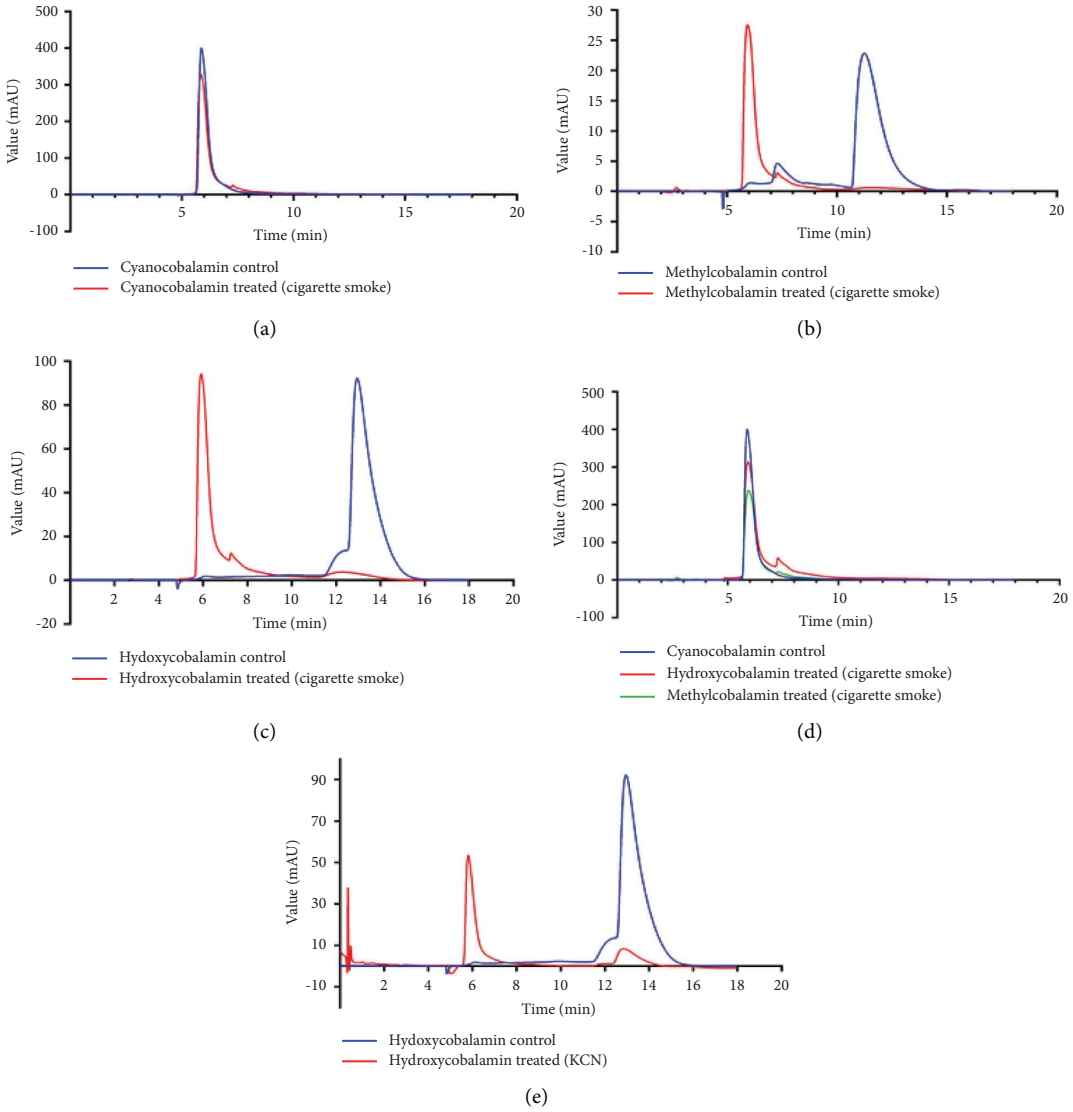

Figure 3: HPLC chromatogram of both control and CSE-treated samples (5 cigarettes); blue: control, red: treated with cigarette smoke extracts: (a) control and treated samples of cyanocobalamin; (b) control and treated samples of methylcobalamin; (c) control and treated samples of hydroxycobalamin; (d) control cyanocobalamin with treated samples of hydroxycobalamin and methylcobalamin; (e) KCN-treated hydroxycobalamin.

were nonsmokers. Their findings reported a lower concentration of vitamin B12 in the smokers' group compared to the nonsmokers' control group [46]. Pagán et al. published a study on the effect of smoking on vitamin B12, 285 women in mid-pregnancy were enrolled and showed a significant difference in vitamin B12 concentration between the smokers' group and the nonsmokers' group [47]. A systematic review included 13 studies with a total of 8661 patients concluded that low levels of vitamin B12 in smokers compared to nonsmokers were found in 8 out of 13 studies [48]. Collectively, the previously published reports and their findings support our results of smoking's effect on vitamin B12 structure and conversion of vitamin B12 to CNCbl and therefore, led to a lower vitamin B12 concentration in blood. Moreover, CNCbl was not recommended for smokers due to a possible alteration in the metabolism of CNCbl and an increase in excretion [44]. Many studies and research have shown the superior tissue retention rates of Cbl after the OHCbl supplement was taken instead of the cyano-Cbl (CNCbl) which led to a rise in the urinary secretion of CNCbl [49–57].

The results of the current study demonstrated the possible impact of cigarette smoke extracts on the structure of cobalamin formulas *in vitro*, however, this proof principle approach requires further in vivo experiments to provide us with the exact effect of smoking on vitamin B12 status in humans. Despite the previous reports about the association between smoking and vitamin B12 deficiency, more updated and well-designed experiments are required to elucidate this correlation since some of these reports were conducted more than 40 years ago. For instance, the case-control study with follow-up measures will be a good starting-up approach to

finding a correlation between cigarette smoking and vitamin B12 levels. To the best of our knowledge, this is the first study that reports an alteration in vitamin B12 structure post-cigarette smoke treatment. Further investigation into the effect of thiocyanate in vitamin B12 forms is needed.

5. Conclusion

The shifting in the Rt time of treated MeCbl and OHCbl forms of vitamin B_{12} indicates that exposure to cigarette smoke induces a chemical conversion to CNCbl. This is likely correlated with decreased levels of vitamin B_{12} in smokers and its loss after supplement administration. Thus, smokers diagnosed with vitamin B_{12} deficiency require primary healthcare and medical consultation. Further, *in vivo* studies are recommended to verify our results.

Data Availability

The data that supports the findings in this study are available from the corresponding authors upon reasonable request.

Additional Points

Highlights. (i) MeCbl and OHCbl were markedly affected by exposure to cigarette smoke extracts. (ii) Exposure to cigarette smoke extracts chemically altered MeCbl and OHCbl to CNCbl. (iii) This study suggests that cigarette smoking may cause a deficiency in vitamin B12 due to the formation of urine excretable form of vitamin B12 (CNCbl).

Conflicts of Interest

All authors declare that there are no conflicts of interest.

Authors' Contributions

MSA conceptualized the study. FAY, MIA, AA, BA, MA, and SAA designed the study, contributed to data collection, and performed data analysis. MAA wrote the original draft. RMZ, KA, and MSA reviewed and edited the final manuscript. All authors have reviewed, contributed, and approved the final manuscript version.

Acknowledgments

The APC of this article was generously funded by the Qatar National Library.

References

[1] R. Malouf and A. A. Sastre, "Vitamin B12 for cognition," *Cochrane Database of Systematic Reviews*, vol. 3, 2003.

[2] A. Ankar and A. Kumar, "Vitamin B12 deficiency," in *Treasure Island (FL)*, StatPearls Publishing, FL, USA, 2023.

[3] J. C. Fyfe, U. Giger, C. A. Hall et al., "Inherited selective intestinal cobalamin malabsorption and cobalamin deficiency in dogs," *Pediatric Research*, vol. 29, no. 1, pp. 24–31, 1991.

[4] L. H. Allen, "How common is vitamin B-12 deficiency?" *The American Journal of Clinical Nutrition*, vol. 89, no. 2, pp. 693S–696S, 2009.

[5] J. Lindenbaum, I. H. Rosenberg, P. Wilson, S. P. Stabler, and R. H. Allen, "Prevalence of cobalamin deficiency in the Framingham elderly population," *The American Journal of Clinical Nutrition*, vol. 60, no. 1, pp. 2–11, 1994.

[6] A. D. Smith and H. Refsum, "Vitamin B-12 and cognition in the elderly," *The American Journal of Clinical Nutrition*, vol. 89, no. 2, pp. 707S–711S, 2009.

[7] A. Pupić-Bakrač, A. Pervan, J. Pupić-Bakrač, and J. Končurat, "Extremely severe vitamin B12 deficiency–case presentation and review of literature," *Medicina Fluminensis*, vol. 55, no. 3, pp. 301–310, 2019.

[8] U. Gröber, "Micronutrients: metabolic tuning-prevention-therapy," *Drug Metabolism and Drug Interactions*, vol. 24, no. 2-4, p. 331, 2009.

[9] U. Gröber, K. Kisters, and J. Schmidt, "Neuroenhancement with vitamin B12—underestimated neurological significance," *Nutrients*, vol. 5, no. 12, pp. 5031–5045, 2013.

[10] R. Clarke, H. Refsum, J. Birks et al., "Screening for vitamin B-12 and folate deficiency in older persons," *The American Journal of Clinical Nutrition*, vol. 77, no. 5, pp. 1241–1247, 2003.

[11] S. P. Stabler, "Vitamin B12 deficiency," *New England Journal of Medicine*, vol. 368, no. 2, pp. 149–160, 2013.

[12] A. Hunt, D. Harrington, and S. Robinson, "Vitamin B12 deficiency," *BMJ*, vol. 349, no. sep04 1, p. g5226, 2014.

[13] S. Lee, D. O'Dell, J. Hohenstein, S. Colt, S. Mehta, and D. Erickson, "NutriPhone: a mobile platform for low-cost point-of-care quantification of vitamin B 12 concentrations," *Scientific Reports*, vol. 6, no. 1, pp. 28237-28238, 2016.

[14] R. Moll and B. Davis, "Iron, vitamin B12 and folate," *Medicine*, vol. 45, no. 4, pp. 198–203, 2017.

[15] J. H. Martens H Barg M Warren D Jah, H. Barg, M. Warren, and D. Jahn, "Microbial production of vitamin B 12," *Applied Microbiology and Biotechnology*, vol. 58, no. 3, pp. 275–285, 2002.

[16] M. J. Nielsen, M. R. Rasmussen, C. B. Andersen, E. Nexø, and S. K. Moestrup, "Vitamin B 12 transport from food to the body's cells—a sophisticated, multistep pathway," *Nature Reviews Gastroenterology and Hepatology*, vol. 9, no. 6, pp. 345–354, 2012.

[17] L. Mahmood, "The metabolic processes of folic acid and Vitamin B12 deficiency," *Journal of Health Research and Reviews*, vol. 1, no. 1, p. 5, 2014.

[18] T. Brody, *Clinical Trials: Study Design, Endpoints and Biomarkers, Drug Safety, and FDA and ICH Guidelines*, Academic Press, MA, USA, 2016.

[19] K. Okada, H. Tanaka, K. Temporin et al., "Methylcobalamin increases Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model," *Experimental Neurology*, vol. 222, no. 2, pp. 191–203, 2010.

[20] M. Zhang, W. Han, S. Hu, and H. Xu, "Methylcobalamin: a potential vitamin of pain killer," *Neural Plasticity*, vol. 2013, Article ID 424651, pp. 1–6, 2013.

[21] F. O'Leary and S. Samman, "Vitamin B12 in health and disease," *Nutrients*, vol. 2, no. 3, pp. 299–316, 2010.

[22] R. Kozyraki and O. Cases, "Vitamin B12 absorption: mammalian physiology and acquired and inherited disorders," *Biochimie*, vol. 95, no. 5, pp. 1002–1007, 2013.

[23] S. M. Chemaly, "New light on vitamin B12: the adenosylcobalamin-dependent photoreceptor protein CarH," *South African Journal of Science*, vol. 112, no. 9/10, p. 9, 2016.

[24] T. Fukuwatari, E. Sugimoto, T. Tsuji, J. Hirose, T. Fukui, and K. Shibata, "Urinary excretion of vitamin B12 depends on

urine volume in Japanese female university students and elderly," *Nutrition Research*, vol. 29, no. 12, pp. 839–845, 2009.

[25] C. J. Carlsson, H. E. Hansen, L. Hilsted, J. Malm, L. Ødum, and P. B. Szecsi, "An evaluation of the interference of hydroxycobalamin with chemistry and co-oximetry tests on nine commonly used instruments," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 71, no. 5, pp. 378–386, 2011.

[26] R. Obeid, S. N. Fedosov, and E. Nexo, "Cobalamin coenzyme forms are not likely to be superior to cyano-and hydroxyl-cobalamin in prevention or treatment of cobalamin deficiency," *Molecular Nutrition and Food Research*, vol. 59, no. 7, pp. 1364–1372, 2015.

[27] J. M. Scott and A. M. Molloy, "The discovery of vitamin B12," *Annals of Nutrition and Metabolism*, vol. 61, no. 3, pp. 239–245, 2012.

[28] E. V. Quadros, "Advances in the understanding of cobalamin assimilation and metabolism," *British Journal of Haematology*, vol. 148, no. 2, pp. 195–204, 2010.

[29] L. Randaccio, S. Geremia, N. Demitri, and J. Wuerges, "Vitamin B12: unique metalorganic compounds and the most complex vitamins," *Molecules*, vol. 15, no. 5, pp. 3228–3259, 2010.

[30] C. Gherasim, M. Lofgren, and R. Banerjee, "Navigating the B12 road: assimilation, delivery, and disorders of cobalamin," *Journal of Biological Chemistry*, vol. 288, no. 19, pp. 13186–13193, 2013.

[31] B. A. Cooper and D. S. Rosenblatt, "Inherited defects of vitamin B metabolism," *Annual Review of Nutrition*, vol. 7, no. 1, pp. 291–320, 1987.

[32] F. Watanabe, K. Abe, T. Fujita, M. Goto, M. Hiemori, and Y. Nakano, "Effects of microwave heating on the loss of vitamin B12 in foods," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 1, pp. 206–210, 1998.

[33] J. Romani, A. Caixas, J. Carrascosa, M. Ribera, M. Rigla, and J. Luelmo, "Effect of narrowband ultraviolet B therapy on inflammatory markers and body fat composition in moderate to severe psoriasis," *British Journal of Dermatology*, vol. 166, no. 6, pp. 1237–1244, 2012.

[34] S. Cabrera, D. Benavente, M. Alvo, P. De Pablo, and C. J. Ferro, "Vitamin B12 deficiency is associated with geographical latitude and solar radiation in the older population," *Journal of Photochemistry and Photobiology B: Biology*, vol. 140, pp. 8–13, 2014.

[35] A. Juzeniene, Z. Baturaite, Z. Lagunova et al., "Influence of multiple UV exposures on serum cobalamin and vitamin D levels in healthy females," *Scandinavian Journal of Public Health*, vol. 43, no. 3, pp. 324–330, 2015.

[36] A. H. Lie, M. V. Chandra-Hioe, and J. Arcot, "Sorbitol enhances the physicochemical stability of B12 vitamers," *International Journal for Vitamin and Nutrition Research*, vol. 90, no. 5-6, pp. 439–447, 2019.

[37] M. A. Khaled, C. L. Watkins, and C. L. Krumdieck, "Inactivation of B12 and folate coenzymes by butyl nitrite as observed by NMR: implications on one-carbon transfer mechanism," *Biochemical and Biophysical Research Communications*, vol. 135, no. 1, pp. 201–207, 1986.

[38] R. Carmel, "Current concepts in cobalamin deficiency," *Annual Review of Medicine*, vol. 51, no. 1, pp. 357–375, 2000.

[39] K. M. Al-Batayneh, M. S. A. Zoubi, M. Shehab et al., "Association between MTHFR 677C> T polymorphism and vitamin B12 deficiency: a case-control study," *Journal of Medical Biochemistry*, vol. 37, no. 2, pp. 141–147, 2018.

[40] K. M. Al-Batayneh, M. Salim Al Zoubi, B. Al-Trad et al., "Homologous G776G variant of transcobalamin-II gene is linked to vitamin B12 deficiency," *International Journal for Vitamin and Nutrition Research*, vol. 90, no. 1-2, pp. 151–155, 2020.

[41] M. S. Al Zoubi, K. M. Al-Batayneh, B. Al-Trad et al., "Evaluation of vitamin B12, folate and ferritin serum levels in Jordanian population," *Journal of Nutritional Science and Vitaminology*, vol. 65, no. 4, pp. 309–317, 2019.

[42] J. Qar, M. Al Zoubi, L. Azzam et al., *Vitamin B12 Deficiency in the South of Jordan: A Possible Geographical Correlation*, Authorea Preprints, Hoboken, NJ, USA, 2020.

[43] D. Singh, "Effect of cigarette smoking on serum homocysteine and vitamin B12 level in male population of Udaipur," *Biochemistry and Analytical Biochemistry*, vol. 5, no. 2, p. 282, 2016.

[44] J. Linnell, A. Smith, C. Smith, J. Wilson, and D. Matthews, "Effects of smoking on metabolism and excretion of vitamin B12," *British Medical Journal*, vol. 2, no. 5599, pp. 215-216, 1968.

[45] D. Singh, "Effect of cigarette smoking on serum lipid profile in male population of Udaipur (Rajasthan)," *International Journal of Clinical Biochemistry and Research*, vol. 3, no. 4, pp. 368–370, 2016.

[46] E. Özerol, I. Özerol, R. Gökdeniz, I. Temel, and O. Akyol, "Effect of smoking on serum concentrations of total homocysteine, folate, vitamin B12, and nitric oxide in pregnancy: a preliminary study," *Fetal Diagnosis and Therapy*, vol. 19, no. 2, pp. 145–148, 2004.

[47] K. Pagán, J. Hou, R. L. Goldenberg, S. P. Cliver, and T. Tamura, "Effect of smoking on serum concentrations of total homocysteine and B vitamins in mid-pregnancy," *Clinica Chimica Acta*, vol. 306, no. 1-2, pp. 103–109, 2001.

[48] A. Tuenter, P. K. Bautista Nino, A. Vitezova et al., "Folate, vitamin B12, and homocysteine in smoking-exposed pregnant women: a systematic review," *Maternal and Child Nutrition*, vol. 15, no. 1, 2019.

[49] H. R. Skeggs, E. J. Hanus, A. B. McCauley, and V. J. Rizzo, "Hydroxocobalamin: physiological retention in the dog," *Experimental Biology and Medicine*, vol. 105, no. 3, pp. 518–521, 1960.

[50] V. Herbert, R. Zalusky, and H. R. Skeggs, "Retention of injected hydroxocobalamin versus cyanocobalamin versus liver extract-bound cobalamin," *The American Journal of Clinical Nutrition*, vol. 12, no. 2, pp. 145–149, 1963.

[51] H. Hertz, H. P. Ø Kristensen, and E. Hoff-Jørgensen, "Studies on vitamin B12 retention comparison of retention following intramuscular injection of cyanocobalamin and hydroxocobalamin," *Scandinavian Journal of Haematology*, vol. 1, no. 1, pp. 5–15, 1964.

[52] J. Withey and G. Kilpatrick, "Hydroxocobalamin and cyanocobalamin in Addisonian anaemia," *The Lancet*, vol. 283, no. 7323, pp. 16–18, 1964.

[53] J. Marshall Chalmers and N. Shinton, "Comparison of hydroxocobalamin and cyanocobalamin in the treatment of pernicious anaemia," *The Lancet*, vol. 286, no. 7426, pp. 1305–1308, 1965.

[54] G. B. J. Glass, H. R. Skeggs, and D. H. Lee, "Hydroxocobalamin: V. Prolonged maintenance of high vitamin B12

blood levels following a short course of hydroxocobalamin injections," *Blood*, vol. 27, no. 2, pp. 234–241, 1966.

[55] T. Zwickler, M. Lindner, H. I. Aydin et al., "Diagnostic work-up and management of patients with isolated methylmalonic acidurias in European metabolic centres," *Journal of Inherited Metabolic Disease*, vol. 31, no. 3, pp. 361–367, 2008.

[56] R. Gräsbeck, "Correspondence on "Involuntary movements during vitamin B12 treatment": was cyanocobalamin perhaps responsible?" *Journal of Child Neurology*, vol. 25, no. 6, pp. 794-795, 2010.

[57] C. Paul and D. M. Brady, "Comparative bioavailability and utilization of particular forms of B12 supplements with potential to mitigate B12-related genetic polymorphisms," *Integrative Medicine*, vol. 16, no. 1, pp. 42–49, 2017.