INT. J. BIOL. BIOTECH., 19 (4): 429-432, 2022.

EFFECTS OF VITAMIN B12 SUPPLEMENTATION FOR MANAGEMENT OF DYSLIPIDEMIA IN PATIENTS WITH DIABETES MELLITUS

Junaid Mahmood Alam[1*], Ishrat Sultana[1], Afsheen Sardar[1], Syed Riaz Mahmood[2] and Maqsood Ali Ansari[3]

[1]*Department of Clinical Biochemistry laboratory services and Chemical Pathology, Liaquat National Hospital and Medical College, Karachi-74800. Pakistan*
[2]*Department of Pathology, Lyari General Hospital, Karachi, Pakistan*
[3]*Department of Genetics, University of Karachi, Karachi-75270, Pakistan*

ABSTRACT

Etiology and basis of secondary dyslipidemia and related abnormalities in lipid metabolism are known conditions such as diabetes mellitus, hyper and hypothyroidism, cirrhosis, alcoholism and deficiency of some micro nutrients such as Vitamins. It has been reported that Vitamin B12 supplementation in metabolically compromised individuals helps in reduction of insulin resistance, obesity and normalcy of dyslipidemic state. In this study, we investigated protective role of Vitamin B12 supplementation in individuals with Diabetes mellitus co-morbid with hypertryglycerdemia, hypercholstreima and lipoprotein abnormalities. Thirty four (n = 34) individuals which were confirmed cases of Diabetes mellitus, suffering various forms of dyslipidemia, were selected via clinical and laboratory investigation scrutiny. Subjects were advised Vitamin B12 supplementation, one (2.4 mg) Oral Dose/day for 3 months or more by attending physicians. Blood analysis of patients was performed before start of Vitamin B12 supplementation, and at the end of 90th day of treatment for HbA1c, Cholesterol, triglycerides, HDL, LDL and Vitamin B12 by standardized methods. Data exhibited marked significant ($P < 0.00001$) changes between biochemical parameters of zero day and after 90 day in all parameters suggesting protective and facilitating efficacy of Vitamin B12 supplementation.

Key words: dyslipidemia, hypertryglycerdemia, hypercholstreima, Vitamin B12 supplementation.

ABBREVIATIONS: DM: disbetes mellitus; HDL: high density lipoprotein; LDL: low density lipoprotein; A1AT: alpha-1-antitrypsin; HbAlc: glycated Hemoglobin .

INTRODUCTION

Lipids metabolism disorders, inclusive of lipoproteins, commonly described as Dyslipidemias, both primary and secondary (Sezgin and Becel, 2019). While etiology of primary dyslipidemia has been noted as of genetic disposition, basis of secondary dyslipidemia and related abnormalities in lipid metabolism are diabetes mellitus, hyper and hypothyroidism, cirrhosis, alcoholism and deficiency of micro and macro nutrients (Oh and Brown, 2003; Sezgin and Ozcakar, 2011,). Treatments of dyslipidemia includes controlling diabetes, changes in life style, diet, and multiple level medications to eliminate or control secondary causes (Oh and Brown, 2003; Sezgin and Ozcakar, 2011). It is a well known fact that Vitamin B12 involves in many metabolic pathways, inclusive of fatty acid catabolism, and persisting deficiency of it known to increase risk of obesity and myocardial infarction (Pinhas-Hamiel *et al*., 2006; Sezgin and Becel , 2018, Saraswathy *et al*., 2018). It is also reported that Vitamin B12 plays decisive role in maintaining normal levels of triglycerides and High density lipoprotein (HDL) and also suggestively involved in synthesis of antiprotease enzyme, alpha-1-antitrypsin (A1AT), associated with evading obesity (Sezgin *et al*., 2018).

Vitamin B12 is a water soluble vitamin that serves as significant cofactor in DNA methylation, and maintaining normal blood levels of lipids and amino acids via regulation of one carbon atom metabolism (Lyon *et al*., 2020; Ashok *et al*., 2021). It has been reported that Vitamin B12 supplementation in metabolically compromised individuals helps in reduction of insulin resistance, obesity and decline in cognitive skills associated with metformin usage by Diabetes Mellitus (DM) patients (Xie *et al*., 2016; Akbari *et al*., 2018; Asbhagi *et al*., 2021). In this study, we investigated protective role of Vitamin B12 supplementation in individuals with Diabetes co-morbid with hypertryglycerdemia, hypercholstreima and lipoprotein abnormalities.

MATERIALS AND METHODS

Patients selection and study protocols: Thirty four (n = 34) individuals, confirmed cases of Diabetes mellitus, mostly obese (n = 25) and suffering various forms of dyslipidemia, inclusive of elevated blood levels of HbA1c,

*Corresponding author: Dr. Junaid Mahmood Alam, Department of Clinical Biochemistry lab services and Chemical Pathology, Liaquat National Hospital and Medical College, Karachi-74800. Pakistan

triglycerides, cholesterol, LDL (low density lipioproteins) and low level of HDL were selected for current study. Selection was made from evaluating initial blood chemistry reports of around 98 patients and their history from Internal medicine and endocrine specialties. However, only 54 were according to criteria, and after completion of 90 days (or 3 months), only 34 were found to comply with medication and physicians advises. We, therefore, ensured that only those patients (final number = 34) were enrolled for data analyses who were advised Vitamin B12 supplementation, one (2.4 mg) Oral Dose/day, for 3 months or more by attending physicians and complied with physicians advises. Blood analysis of patients was performed before start of Vitamin B12 supplementation, and at the end of 90^{th} day of treatment.

Biochemical analysis: Blood was collected in designated vaccuttes for analysis of HbA1c, Cholesterol, triglyceride, HDL, LDL and Vitamin B12 by standardized methods (Fiedewald *et al*., 1972; Fossati and Prencipe, 1982; Goldstein *et al*., 1986; NIH Publication No 90-2964, 1990; Ng 2004, Allen, 2012). Each sample was analyzed on automated chemistry analyzer Cobas c501 (Roche Diagnostics, Basil, Sweden) with precipath and percinorm controls (Roche Diagnostics, Basil, Sweden). Each test was run in duplicate for quality assurance purpose.

Statistical analysis: Data was analyzed using SPSS ver 22 (SPSS, USA). Data presented as mean ± SD and considered significant when $P < 0.05$.

RESULTS

Results are summarized in Table 1. Ninety eight patients were selected from internal medicine and endocrine out patients departments, after evaluating their initial blood chemistry reports showing abnormal HbA1c, Cholesterol, triglyceride, HDL, LDL and Vitamin B12 levels. However, as time passed, only 54 were found to fall into our inclusion criteria, and after completion of 90 days (or 3 months), only 34 were found to comply with medication and physicians advises. Since it was initially decided that only those patients will finally be enrolled for data analyses who were advised Vitamin B12 supplementation for 3 months or more by attending physicians and complied with it, final tally was thirty four (n = 34). Blood analysis of patients was performed again before start of Vitamin B12 supplementation, and at the end of 90^{th} day of treatments. Data exhibited marked significant ($P < 0.00001$) changes between biochemical parameters of zero day (before start of supplementation) and after 90 day, suggesting protective and facilitating efficacy of Vitamin B12 supplementation. HbA1c, Cholesterol, triglyceride, HDL, LDL and Vitamin B12 all showed normalizing of its concentration after 90 days of B12 one (2.4 mg) Oral Dose/day. Patients felt less lethargic, normalization of blood pressure, heart rate, probably due to stabilizing of blood chemistry parameters.

Table 1. Comparison of Biochemical parameters before and after 90 days of Vitamin B12 treatments in selected individuals (N = 34).

Parameters	Pre-treatment	Post-Treatment	P < 0.05	t-Values
Cholesterol	274.97 ± 28.86	218.97 ± 19.57	<0.00001	9.36143
Triglyceride	218.97 ± 19.57	152.76 ± 14.93	<0.00001	15.70165
LDL	152.76 ± 14.93	125.64 ± 11.01	<0.00001	8.52014
HDL	35.82 ± 6.00	54.29 ± 5.93	<0.00001	-12.76272
HbA1c	6.90 ± 0.39	5.61 ± 0.38	<0.00001	13.63807
Vitamin B12	334.82 ± 54.57	803.44 ± 97.56	<0.00001	-24.44333.

Results are expressed as mean ± SD, Data considered significant when $P < 0.05$

DISCUSSION

It is reported that malabsorption, genetic disposition, or insufficiency in dietary intakes can be some of the reasons of Vitamin B12 deficiency (Palacios *et al*., 2013; Al-Mushraf *et al*., 2020). Several studies reported during last two decades indicated an inverse relationship between vitamin B12 levels and metabolic disorders abnormal lipid profile, body mass index, insulin resistance, and cardiovascular abnormalities (Khrisnaveni *et al*., 2009; Knight *et al*., 2015; Sarawathy *et al*., 2018; Sun *et al*.,2019 ; Boachei *et al*., 2020). Present study revealed abnormal lipid profile, glycated hemoglobin levels in diabetic patients with low Vitamin B12 levels. However, when they all went through 90 days (3 months) of Vitamin B12 supplement treatments, not only lipid profile components such as

cholesterol, triglycerides, HDL, LDL levels normalized but HbA1c levels also declined from higher percentage to under normal values. Several studies suggested vitamin B12 supplementation as remedy not only to normalize vitamin levels but also initiating normalcy in altered lipid profile levels, which mostly observed in diabetic and obese individuals (Pinhas-Hamiel *et al*., 2006; Saraswathy *et al*., 2018; Sezgin and Becel , 2018,). Several studies done earlier reported facilitating efficacy of Vitamin B12 supplementation in patients with hypertriglyceridemia, hypercholestermia, lipoproteinemia, diabetes, related metabolic conditions and even pregnancy (Al-Mushraf *et al*., 2020; Ashok *et al*., 2021)

Vitamin B12 deficiency, either due to improper diets or elevated plasma homocysteine levels, affects phospholipid metabolism and cause hyper secretions of very low density lipoproteins resulting in abnormal lipid profile (Al-Mushraf *et al*., 2020). Since Vitamin B12 acts as a cofactor in the conversion of enzyme methylmalonyl CoA to succinyl-CoA, its deficiency may hinders this particular step, consequentially causing methylmalonyl CoA accretion leading to lipigensis (Brindle eta l., 1985; Strain *et al*., 2004,). It has also been reported that subdued Gene expression involving lipogenesis due to Vitamin B12 deficiency might be one of the mechanism of abnormal lipid profiles, which then results inflammatory responses and damaged metabolic pathways (Al-Musharraf *et al*., 2020). In our study, normalization of elevated levels of triglyceride, cholesterol, LDL and HbA1c was observed. Normalization of HbA1c was probably due to indirect effects of decline in triglyceride, which possibly causing excessive glycerol for Gluconeogenesis, further aggravating glycemic state. Nonetheless, due to effective fatty acid oxidative pathways, now maintained by supplemented vitamin B12, TG undergoes potential catabolism, subsequently resulting in near normal or normal glycemic state, leading to maintained HbA1c. Known reason for Vitamin B12 deficiency in diabetics was already reported as due to metformin activity, the medicine which all our selected patients were taken daily Oral Dose, 500mg to 750mg XR.

REFERENCES

Allen, L.H. (2012). Vitamin B-12. *Adv Nutr*., 3(1):54-55. doi: 10.3945/an.111.001370. Epub 2012 .

Akbari, M., R. Tabrizi, K.B. Lankarani, S.T. Heydari, M. Karamali, F. Keneshlou, K. Niknam, F. Kolahdooz and Z. Asemi (2018). The effects of folate supplementation on diabetes biomarkers among patients with metabolic diseases: a systematic review and meta-analysis of randomized controlled trials. *Horm Metab Res.*, 50:93-105. 10.1055/s-0043-125148

Al-Musharaf, S., G.S. Aljuraiban, S.D. Hussain, A.M. Alnaami, P. Saravanan and N. Al-Daghri (2020). Low Serum Vitamin B12 Levels Are Associated with Adverse Lipid Profiles in Apparently Healthy Young Saudi Women. *Nutrients*, 12: 2395; doi:10.3390/nu12082395

Asbaghi, O., D. Ashtary-Larky, R. Bagheri, S.P. Moosavian , H.P. Olyaei, B. Nazarian, M. Rezaei Kelishadi, A. Wong, D.G. Candow, F. Dutheil, K. Suzuki and A. Alavi Naeini (2021). Folic acid supplementation improves glycemic control for diabetes prevention and management: a systematic review and dose-response meta-analysis of randomized controlled trials. *Nutrients*, 13:2355. 10.3390/nu13072355

Ashok, T., H. Puttam, V.C.A. Tarnate, S. Jhaveri, C. Avanthika, A. G. T. Treviño, S.L. Sandeep and N. T. Ahmed (2021). Role of Vitamin B12 and Folate in Metabolic Syndrome. *Cureus*, 13(10): e18521. DOI 10.7759/cureus.18521

Boachie, J. A. Adaikalakoteswari, J. Samavat and P. Saravanan (2020). Low Vitamin B12 and Lipid Metabolism: Evidence from Pre-Clinical and Clinical Studies. *Nutrients*, 12(7):1925. doi: 10.3390/nu1207192

Brindle, N.P., V.A. Zammit and C.I. Pogson (1985). Regulation of Carnitine Palmitoyltransferase Activity by malonyl-CoA in Mitochondria from Sheep Liver, a Tissue With a Low Capacity for Fatty Acid Synthesis. *Biochem. J.*, 232: 177–182.

Fiedewald, W.T., R.I. Levy and D.S. Fredrickson (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*., 18:499-502.

Fossati, P. and L. Prencipe (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem*., 28:2077–80.

Goldstein, D.E., R.R. Little, H.M. Wiedmeyer, J.D. England and E.M. McKenzie (1986). Glycated hemoglobin: methodologies and clinical applications. *Clin Chem*., 32: B64-B70;

Knight, B.A. B.M. Shields, A. Brook, A. Hill, D.S. Bhat, A.T. Hattersley and C.S. Yajnik (2015). Lower Circulating B12 is Associated with Higher Obesity and Insulin Resistance During Pregnancy in a Non-Diabetic White British Population. *PLoS ONE*, 10: e0135268.

Krishnaveni, G.V. J.C. Hill, S.R. Veena, D.S. Bhat, A.K. Wills, C.L. Karat, C.S. Yajnik and C.H. Fall (2009). Low Plasma Vitamin B12 in Pregnancy is Associated with Gestational 'Diabesity' and Later Diabetes. *Diabetologia*, 52: 2350–2358.

Lyon, P., V. Strippoli, B. Fang and L. Cimmino (2020). B vitamins and one-carbon metabolism: implications in human health and disease. *Nutrients*, 12:2867. 10.3390/nu12092867

Ng, D.S. (2004). Treating Low HDL-From bench to bedside. *Clinical Biochemistry*, 37:649-659;

Oh, R.C. and D.L. Brown (2003). Vitamin B12 deficiency. *Am Fam Physician*, 67: 993-4.

Palacios, G., R. Sola, L. Barrios, K. Pietrzik, M.J. Castillo and M. González-Gross (2013). Algorithm for the Early Diagnosis of Vitamin B12 Deficiency in Elderly People. *Nutr. Hosp.*, 28, 1447–1452

Pinhas-Hamiel, O., N. Doron-Panush, B. Reichman, D. Nitzan-Kaluski, S. Shalitin and L. Geva-Lerner (2006). Obese children and adolescents, a risk group for low vitamin B12 concentration. *Arch Pediatr Adolesc Med*. 160: 933-6.

NIH Publication (1990). Recommendations for Improving Cholesterol Measurement: A Report from the Laboratory Standardization Panel of the National Cholesterol Education Program. NIH Publication No 90-2964. February 1990;

Saraswathy, K.N., S. Joshi, S. Yadav and P.R. Garg (2018). Metabolic distress in lipid & one carbon metabolic pathway through low vitamin B-12: a population based study from North India. *Lipids Health Dis*., 17: 96.

Sezgin, Y., M. Kartal and A.D. Güldal (2018). A comparison of serum alpha-1-antitrypsin and vitamin B12 levels in patients with vitamin B12 deficiency. *J Clin Anal Med*., 9: 192-4.

Sezgin, Y. and S. Becel (2018). Comparison of serum lipid parameters and serum vitamin B12 levels. *J Clin Anal Med*., 10 (2): 198-201.

Sezgin, Y. and S. Becel (2019). Evaluation of Lipid Parameters in Patients Receiving Vitamin B12 Therapy. *İstanbul Med J*., 20(3): 214-7.

Sezgin, Y. and N. Özçakar (2011). Management of dyslipidemia. *Turkish Family Physician*, 2: 30-5.

Strain, J.J., L. Dowey, M. Ward, K. Pentieva and H. McNulty (2004). B-Vitamins, Homocysteine Metabolism and CVD. *Proc. Nutr. Soc.*, 63, 597–603.

Sun, Y., M. Sun, B. Liu, Y. Du, S. Rong, G. Xu, L.G. Snetselaar and W. Bao (2019). Inverse Association Between Serum Vitamin B12 Concentration and Obesity Among Adults in the United States. *Front. Endocrinol*., 10: 414.

Xie, R.H., Y.J. Liu, R. Retnakaran, A.J. MacFarlane, J. Hamilton, G. Smith, M.C. Walker and S.W. Wen (2016). Maternal folate status and obesity/insulin resistance in the offspring: a systematic review. *Int J Obes*. (Lond). 40:1-9. 10.1038/ijo.2015.189

(*Accepted for publication in September 2022*)