

**Original Research Article****Correlation between Visual Evoked Potentials and Vitamin B12 Levels in Patients with Controlled Diabetes Mellitus**

Authors

Vivek Kumar Verma¹, Dheeraj Kela², Premshanker Singh³, Durga Prasad Singh⁴, Geeta Singh⁵, Ganga Prasad Vaishya⁶, Jay Prakash Pal⁷^{1,4}Senior Resident, Dept of Medicine, UP University of Medical Sciences (UPUMS), Saifai, Etawah (UP)^{2,6,7}Junior Resident-2, Dept of Medicine, UP University of Medical Sciences (UPUMS), Saifai, Etawah (UP)³Professor & Head, Dept of Medicine, UP University of Medical Sciences (UPUMS), Saifai, Etawah (UP)⁵SMO, UP University of Medical Sciences (UPUMS), Saifai, Etawah (UP)

Corresponding Author

Dr Vivek Kumar Verma

Senior Resident, Dept of Medicine, UPUMS, Saifai, Etawah (UP) - 206130

Email: vk.rims@gmail.com, Mobile: +919450865362**ABSTRACT**

Background: Diabetes is the epidemic of the modern era. In this disease, the morbidity is more than mortality and results from various micro- and macro-vascular complications. The goal of therapy in diabetes is to maintain the blood glucose levels within the normal range to prevent complications. The nervous system frequently gets involved with the complications of diabetes mellitus as the duration of disease increases. So far most of the studies on diabetic neuropathy have been concerned with peripheral and autonomic nerve but recently with refinement of evoked potential technique, detailed exploration of sensory pathway in central nervous system has been possible.

Aim: To study the correlation between visual evoked potentials and vitamin B12 levels in patients with controlled diabetes mellitus.

Methods: This was a cross sectional study. Total 60 cases of diabetes mellitus were examined and investigated. Simple random sampling was done among controlled diabetes mellitus patients. VEP of all diabetic patients was done and two groups were created. Group 1 comprises diabetic patients with abnormal VEP. Group 2 comprises diabetic patients with normal VEP. Serum vitamin B12 levels of patients of both groups was estimated. All patients selected in the study had clinical symptoms and signs of peripheral neuropathy but only those patients who had normal fundus examination were included in the study. Statistical analysis of data was done by using chi-square tests for desired parameters. Data compared to obtain 'p' value for knowing the significance and correlation between the different variables of the study.

Results: Deficiency of vitamin B12 was found in 48.33% total cases in this study. VEP latency was prolonged in 65% cases and low VEP amplitude found in 56.67% cases. There was significant correlation between duration of diabetes and VEP abnormalities in this study. VEP abnormalities are more frequent in controlled diabetes mellitus patients having vitamin B12 deficiency and infrequent in controlled diabetes patients having normal serum vitamin B12 levels.

Conclusion: Visual evoked potentials latencies are more common in diabetic patients having vitamin B12 deficiency.

Keywords: Diabetes mellitus, Visual evoked potentials, Vitamin B12

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.¹

Autonomic and peripheral neuropathy, nephropathy, retinopathy and hearing impairment are some of the late micro-vascular complications of diabetes mellitus which are related to the type of diabetes, duration of disease and instability in blood sugar levels. Type II diabetes mellitus has a long asymptomatic period of hyperglycemia, and many of the individuals have complications at the time of diagnosis due to the asymptomatic period². The nervous system frequently gets involved with the complications of diabetes mellitus as the duration of disease increases. The peripheral neuropathy is far more common having prevalence varies from 55% if based on signs, 62% based on subjective symptoms and 100% if based on motor conduction velocities³. Cranial mono-neuropathies are also commonly observed in diabetes. The 3rd, 4th and 6th nerves are commonly involved. Optic nerve affection manifests as optic atrophy, which as a result of diabetes alone is estimated to occur in about 0.6% cases⁴. So far most of the studies on diabetic neuropathy have been concerned with peripheral and autonomic nervous system but recently with refinement of evoked potential techniques, detailed exploration of sensory pathway in central nervous system has been possible.

The terms visual evoked potential (VEP), visual evoked response (VER) and visual evoked cortical potential (VECP) are equivalent. They refer to electrical potentials initiated by brief visual stimuli, which are recorded from the scalp overlying visual cortex. VEP wave forms are extracted from the electro-encephalogram (EEG) by signal averaging. VEPs are used primarily to measure the functional integrity of the visual pathways from retina via the optic nerves to the visual cortex of the brain⁵. The VEP abnormalities may be:- 1) Latency

prolongation 2)Amplitude reduction 3)Combined latency & amplitude abnormalities.

Vitamin B12 is a water-soluble vitamin that is required for proper red blood cell formation, neurological function, and DNA synthesis⁶. Vitamin B12 deficiency is characterized by megaloblastic anaemia, fatigue, weakness, constipation, loss of appetite, and weight loss⁷. Neurological changes, such as numbness and tingling in the hands and feet can also occur⁷. Additional symptoms of vitamin B12 deficiency include difficulty in maintaining balance, depression, confusion, dementia, poor memory, and soreness of the mouth or tongue⁸. The neurological symptoms of vitamin B12 deficiency can occur without anaemia, so early diagnosis and intervention is important to avoid irreversible damage²³.

Vitamin B12 deficiency can occur in diabetic patients due to use of metformin⁹, PPIs¹⁰ and become a cause of neuropathy in patients with diabetes mellitus.

Vitamin B12 deficiency and accompanying hyperhomocysteinemia and elevated MMA levels have been documented to cause a distinct sensory polyneuropathy that closely mimics diabetic neuropathy. Worsening of diabetic neuropathy is also noted in patients with co-existing vitamin B12 deficiency¹¹.

Materials and Methods

This study was carried out in the UPUMS, Saifai from Sept 2016 to Feb 2017 on diabetic patients attending indoor and outdoor.

This was a cross sectional study. Total 60 cases of diabetes mellitus were examined and investigated. Simple random sampling was done among controlled diabetes mellitus patients.

Inclusion criteria

A known case of controlled diabetes mellitus of age group 30-60 years.

Exclusion criteria

- Patients of diabetes mellitus with altered sensorium, disturbed mental state, pregnant and lactating females.

- Patients of diabetes mellitus having any other diseases known to cause peripheral neuropathy like chronic renal failure, liver failure, hypothyroidism, leprosy, porphyria etc.
- Patients with diabetes mellitus on drugs known to cause peripheral neuropathy like isoniazid, linezolid, metronidazole and phenytoin.
- Patient with raised serum creatinine level (males-1.5 mg/dl, females-1.2 mg/dl).
- Patient having high risk category like HIV or Alcohol.

Step 1: VEP of all diabetic patients was done and two groups were created.

Group 1 comprises diabetic patients with abnormal VEP.

Group 2 comprises diabetic patients with normal VEP

All patients was subjected to a detailed history and thorough clinical examination specially focussing on neurological examination after obtaining his/her informed consent.

Step 2: Serum vitamin B12 levels of patients of both groups was estimated.

Investigations

- 1) VEP of patients with DM
- 2) Vitamin B12 levels in all patients
- 3) Other additional investigations- chest x-ray, electro-cardiogram (ECG), Haemogram, Fasting and postprandial blood sugar, kidney function test, liver function test, refraction etc.

All patients selected in this study had clinical symptoms and signs of peripheral neuropathy.

Visual acuity with Snellen’s chart and ophthalmoscopy were done to rule out any visual disorder.

Fundus examination of all patients included in this study was normal. Fundus Fluorescein Angiography (FFA) was done to rule out diabetic retinopathy in doubtful cases and patients having normal FFA was included in study.

Determination of serum vitamin B12 levels was done: Normal Range is 211-911pg/mL (Method: Chemiluminescence-CLIA)

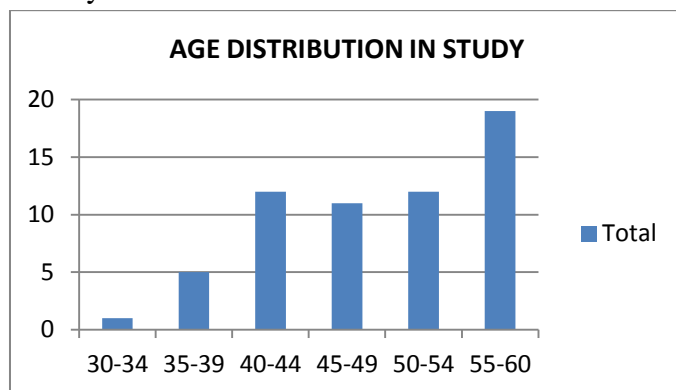
Visual evoked potential was recorded. VEP represent mass response of cortical and sub-cortical areas. Amplitude and latency of P100 was taken. Normal values as per Shahrokhi et al (1978), Mishra and Kalita are P100 latency (ms) 96.9 + 3.6 and amplitude (mV) 7.8 + 1.9.

Parameters	Shahrokhi et al (1978)	Misra and Kalita
P100 Latency (ms)	102.3±5.1	96.9±3.6
Amplitude (µV)	10.1±4.2	7.8±1.9

Statistical analysis of data was done by using chi-square tests for desired parameters. Data compared to obtain ‘p’ value for knowing the significance and correlation between the different variables of the study.

Results

In this study total 60 patients was taken which satisfies the inclusion and exclusion criteria. Minimum age in this study was 32 years and maximum 60 years with mean age of study was 49.25 years.



Out of 60 patients 57 patients (95%) had type 2 diabetes mellitus and 3 patients (5%) have type 1 diabetes mellitus.

Out of total 60 patients 25 patients (41.67%) were female and 35 patients (58.33%) were male patients. Sex ratio is 1.4:1.

Out of 60 patients, 13 patients (21.67%) had positive family history of diabetes, 47 patients (78.33%) had no family history of diabetes.

There was no significant difference in VEP latency (mean right eye P100 latency was 114 ms and mean

left eye P100 latency was 113.92 ms) and VEP amplitude of right and left eye in this study. VEP latency (P100) in ms and VEP Amplitude (N75-P100) in μ V was taken for all stastical purposes.

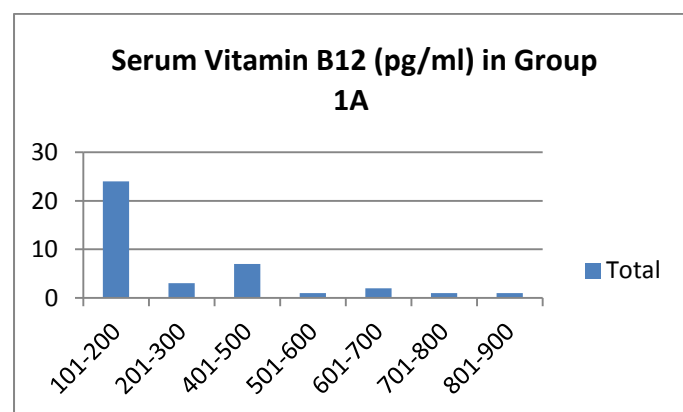
Out of 39 patients (of group 1A) 14 patients (35.90%) had normal vitamin b12 levels and 25 patients (64.10%) had low vitamin b12 levels. 24 patients (61.54%) were male and 15 patients (38.46%) were female (M:F=1.6:1). 7 patients (17.95%) had positive family history and 32 patients (82.05%) had no family history of diabetes. 4 patients (10.26%) were on insulin alone for glycemic control, 5 patients (12.82%) on insulin and metformin based oral hypoglycemic agents and 30 patients (76.92%) on metformin based oral hypoglycemic agents.

Table: Correlation between VEP latency and vitamin B12 levels

VEP latency	ABNORMAL	NORMAL	TOTAL	P Value=0.001 Continuity correlation=0.002 Linear by linear association=0.001
Vitamin B12				
ABNORMAL	25	4	29	
NORMAL	14	17	31	
TOTAL	39	21	60	

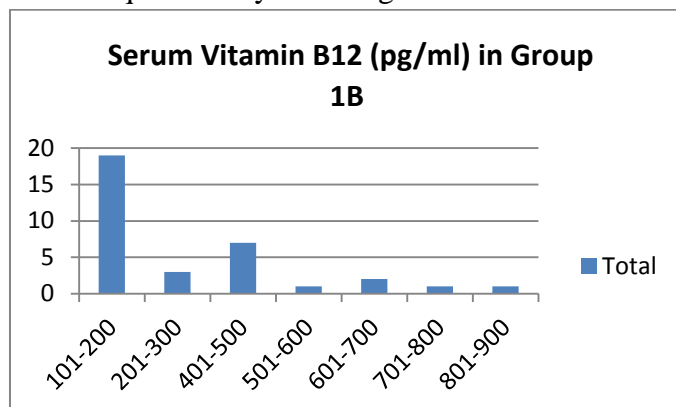
Table: Correlation between VEP amplitude and vitamin B12 levels

VEP Amplitude	ABNORMAL	NORMAL	TOTAL	P value=0.32 Continuity correlation=0.60 Linear by linear association=0.31
Vitamin B12				
ABNORMAL	20	9	29	
NORMAL	14	17	31	
TOTAL	34	26	60	

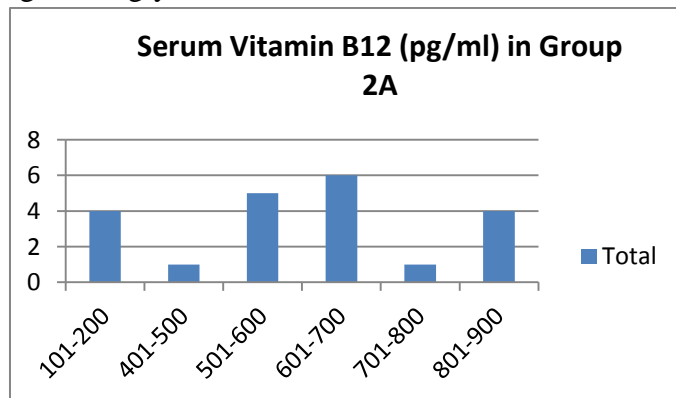


Out of 34 patients (of group 1B) 12 patients were female (35.29%) and 22 patients (64.71%) were male, 6 patients (17.65%) had positive family history and 28 patients (82.35%) had no family history of diabetes, 3 patients (8.82%) were using

insulin alone for glycemic control, 5 patients (14.71%) were using insulin and metformin based OHA for glycemic control, 26 patients (76.47%) were using metformin based OHA for glycemic control. All 34 patients (100%) had VEP latency. 14 patients (41.18%) had normal vitamin B12 levels and 20 patients (58.82%) had low serum vitamin B12 levels. 8 patients (23.53%) were below 45 years of age and 26 patients (76.47%) were more than or equal to 45 years of age.



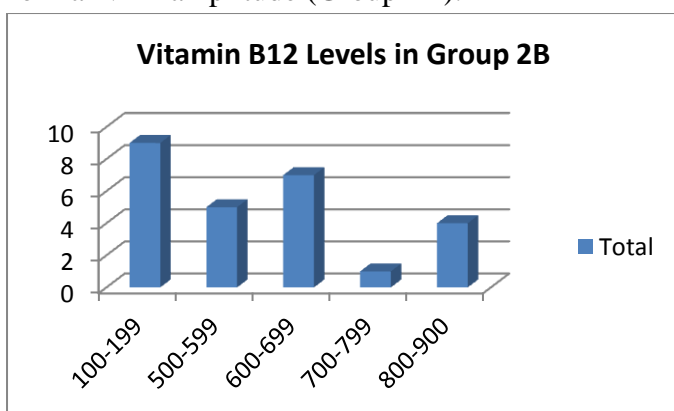
Out of 21 patients (of group 2A) 17 patients (80.95%) had normal vitamin b12 levels and 4 patients (19.05%) had abnormal vitamin b12 levels. 11 patients (52.38%) were male and 10 patients (47.62%) were female (M:F=1.1:1). 6 patients (28.57%) had positive family history of diabetes and 15 patients (71.43%) had no family history of diabetes. 7 patients (33.33%) were on insulin alone for glycemic control, 2 patients (9.52%) were taking insulin and metformin based oral hypoglycemic agent for glycemic control, and 12 patients (57.14%) were taking metformin based oral hypoglycemic agent for glycemic control.



Out of 26 patients (of group 2B), 13 patients (50%) were male and 13 patients (50%) were female, 7 patients (26.92%) had positive family history of

diabetes and 19 patients (73.08%) did not have family history of diabetes. Duration of diabetes was less than 10 years in 23 patients (88.46%) and more than 10 years in 3 patients (11.54%), 8 patients (30.77%) were taking insulin alone for glycemic control, 16 patients (61.54%) were taking metformin based OHA for glycemic control and 2 patients (7.69%) were taking insulin and metformin based OHA for glycemic control, low serum vitamin B12 levels found in 9 patients (34.62%) and normal serum vitamin B12 levels found in 17 patients (65.38%).

Out of total 60 patients, 22 patients (36.67%) were known diabetic for less than or equal to 5 years, 18 patients (30%) were known diabetic for 6 to 9 years and 20 patients (33.33%) were known diabetic for 10 years or more. Out of 22 patients with duration of diabetes less than or equal to 5 years, 6 patients (27.27%) had prolonged VEP latency (Group 1A) and 16 patients (72.73%) had normal VEP latency (Group 2A) and 5 patients (22.73%) had low VEP amplitude (Group 1B) and 17 patients (77.27%) had normal VEP amplitude (Group 2B).



Normal vitamin B12 levels were found in 14 patients (63.64%) and low vitamin B12 levels were found in 8 patients (36.36%). Out of 6 patients of group 1A 5 patients (83.33%) had low serum vitamin B12 levels and 1 patient (16.67%) had normal serum vitamin B12 level. Out of 5 patients of group 1B 4 patients (80%) had low serum vitamin B12 levels and 1 patient (20%) had normal serum B12 level. Out of 16 patients of Group 2A, 3 patients (18.75%) had low serum vitamin B12 levels and 13 patients (81.25%) had normal serum vitamin B12 levels. Out of 17 patients of group 2B, 4 patients (22.53%) had low serum vitamin B12 levels

and 13 patients (76.47%) had normal serum vitamin B12 levels.

Out of 20 patients with duration of diabetes more than or equal to 10 years, 15 patients (75%) were male and 5 patients (25%) were female, 15 patients (75%) with no family history of diabetes and 5 patients (25%) with positive family history, 4 patients (20%) were using insulin alone, 3 patients (15%) were taking insulin and metformin based oral hypoglycemic agents and 13 patients (65%) were using oral hypoglycemic agents for glycemic control. 8 patients (40%) had normal vitamin B12 levels, 12 patients (60%) had low serum vitamin B12 levels, all patients (100%) had VEP latency (group 1A) and 17 patients (85%) had low VEP amplitudes (group 1B) and 3 patients (15%) had normal VEP amplitudes (Group 2B).

Discussion

In this study, family history of diabetes was present in 21.67% of cases. Ramchandran A. et al¹² (2001) found prevalence (16.9%) of diabetes in families of diabetic patients in Indian population.

VEP latency was prolonged in 65% cases and low VEP amplitude found in 56.67% cases. Bortec et al¹³ (1989) found PVEP abnormalities in 77% of diabetic patients. Jawad H et al¹⁴ found abnormal latencies in 60% of diabetic patients.

Deficiency of vitamin B12 among diabetic patients has multi-factorial mechanism. Type 1 Diabetes Mellitus is an autoimmune condition. It is invariably associated with other organ and non organ specific autoimmune and endocrine conditions leading to development of autoimmune poly-glandular syndromes¹⁵.

Several cross sectional studies⁽¹⁶⁻¹⁸⁾ and have documented an increased frequency of vitamin B12 deficiency among type 2 Diabetes Mellitus patients. Metformin use has been unequivocally demonstrated as the prime factor associated with vitamin B12 deficiency among patients with Type 2 Diabetes Mellitus¹⁹. Studies assessing Type 2 Diabetes Mellitus on Metformin have reported the prevalence of vitamin B12 deficiency ranges from 5.8% to 33%²⁰.

Deficiency of vitamin B12 was found in 48.33% total cases in this study. Qureshi et al. (2011) found vitamin B12 deficiency of 33% among adult patients with T2DM, they used serum vitamin B12 concentrations <150 pg/ml as deficiency²¹.

In this study vitamin B12 deficiency in patients on metformin based oral hypoglycaemic drugs were found in 53.06%. Iftikhar R et al²² (2013) found prevalence of vitamin B12 deficiency in 31% in Pakistan.

Omer et al²³ (1996) who found significant correlation between the duration of diabetes and VEP abnormalities. There was significant correlation between duration of diabetes and VEP abnormalities in this study.

In this study, among patients with duration of diabetes less than or equal to 5 years (n=22), 27.27% patients (n=6) had prolonged VEP latency and 72.73% patients (n=16) had normal VEP latency and 22.73% patients (n=5) had low VEP amplitude and 77.27% patients (n=17) had normal VEP amplitude. Normal vitamin B12 levels in 63.64% patients (n=14) and low serum vitamin B12 levels in 36.36% patients (n=8).

In this study among patients with duration of diabetes more than or equal to 10 years (n=20), 100% patients (n=20) had VEP latency, 85% patients (n=17) had low VEP amplitude and only 15% patients (n=3) had normal VEP amplitude. 40% patients (n=8) had normal vitamin B12 levels and 60% patients (n=12) had low vitamin B12 levels.

Conclusion

By this study, it can be concluded that the abnormalities in the VEP response occur in diabetic patients much before the development of overt retinopathy and these changes are positively correlated with the duration of the disease. VEP abnormalities are more frequent in controlled diabetes mellitus patients having vitamin B12 deficiency and infrequent in controlled diabetes patients having normal serum vitamin B12 levels. So there is a positive correlation between VEP abnormalities and serum vitamin B12 levels in controlled diabetes mellitus patients. Hence

measurement of VEP and serum vitamin B12 levels is recommended in all diabetes mellitus patients. VEP is a highly sensitive, reliable, non-invasive and reproducible method for detecting the early alterations in the central optic pathways in diabetes mellitus patients, should be recommended in patients of diabetes mellitus, whenever possible and VEP must be added to the list of screening tools for assessment of central neuropathy for a more complete and early assessment of the neurological involvement in diabetic patients, to advise them for an early and proper management of the disease. However further research work with many more subjects is required for establishment of role of vitamin B12 supplementation in diabetic patients and to make suitable and acceptable recommendations for proper management of disease.

Conflicts of interest: None

Source of Funding: None

Ethical Issue: None

References

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2009 Jan; 32(Suppl 1): S62–S67.
2. Andrew J. M. Boulton, Rayaz A. Malik, Joseph C. Arezzo, Jay M. Sosenko. Diabetic somatic neuropathies. Diabetes Care June 2004 vol. 27 no. 6 1458-1486
3. Melton JL, Dyck PJ. Epidemiology. In Dyck PJ, Thomas PK, Asbury AK et al (Eds). Diabetic Neuropathy: Philadelphia WB Saunders, 1987; 27-35.
4. Simeon Locke. Nervous System in Diabetes. In Joslin's Diabetes. Lea and Febiger, Philadelphia. 1971; 562-4
5. Creel, D.J. (2012) Visually Evoked Potentials, <http://webvision.med.utah.edu/book/electrophysiology/the-electroretinogram-clinical-applications>
6. Creel, D.J. (2011) Electroretinograms and Electro-oculograms: Clinical Electrophysiology. <http://>

- webvision.med.utah.edu/book/electrophysiology/the-electroretinogram-clinical-applications
7. Combs G. Vitamin B12 in The Vitamins. New York: Academic Press, Inc., 1992. <http://www.fda.gov/ScienceResearch/FieldScience/ucm172553.htm>
 8. Bottiglieri T. Folate, vitamin B12, and neuropsychiatric disorders. *Nutr Rev* 1996; 54:382-90.
 9. Liu KW, Dai LK, Jean W. Metformin-related vitamin B12 deficiency. *Age Ageing* 2006; 35:200-1.
 10. Natural Medicines Comprehensive Database. Vitamin B12. <http://naturaldatabase.therapeuticresearch.com/nd/Search.aspx?pt=100&id=926&AspxAutoDetectCookieSupport=1>
 11. Wile D, Toth C: Association of metformin, elevated homocysteine, and methylmalonic acid levels and clinically worsened diabetic peripheral neuropathy. *Diabetes Care* 2010, 33:156-61.
 12. Ramchandran A, Snehlatha C, Kapur A, et al. Diabetes Epidemiology Study Group in India (DESI). High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia*. 2001;44:1094-1101
 13. Bártek L, Gat'ková A, Rybka J, Kalita Z, Smecka Z. Visual evoked potentials in diabetics. *Cesk Oftalmol*. 1989 May; 45(3):192-6.
 14. Javad H, Akram E, Nasser S et al. Pattern Visual Evoked Potentials in Patients with Type II Diabetes Mellitus; *J Ophthalmic Vis Res*. 2012 Jul; 7(3): 225–230
 15. De-Block C, De-Leeuw H, Van-Gaal L: Autoimmune Gastritis in Type 1 Diabetes: A Clinically Oriented Review. *J Clin Endocrinol Metab* 2008, 93:363-71.
 16. Pflipsen M, Oh R, Saguil A, Seehusen D, Topolski R: The Prevalence of Vitamin B12 Deficiency in Patients with Type 2 Diabetes: A Cross-Sectional Study. *J Am Board Fam Med* 2009, 22:528
 17. Hermann L, Nilsson B, Wettre S: Vitamin B12 status of patients treated with metformin: a cross-sectional cohort study. *British Journal of Diabetes & Vascular Disease* 2004, 4:401.
 18. Nervo M, Lubini A, Raimundo F: Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. *Rev Assoc Med Bras* 2011, 57:46-9.
 19. Kos E, Liszek M, Emanuele M, Durazo-Arvizu R, Camacho P: Effect of metformin therapy on vitamin D and vitamin B12 levels in patients with type 2 diabetes mellitus. *Endocr Pract* 2012, 18:179-84.
 20. Reinstatler L, Qi Y, Williamson R, Garn J, Oakley-Jr G: Association of Biochemical B12 Deficiency with Metformin Therapy and Vitamin B12 Supplements. The National Health and Nutrition Examination Survey, 1999 2006. *Diabetes Care* 2012, 35:327
 21. S, Ainsworth A, Winocour P: Metformin therapy and assessment for vitamin B12 deficiency: is it necessary? *Practical Diabetes* 2011, 28:302-4.
 22. Iftikhar R, Kamran SM, Qadir A et al Prevalence of vitamin B12 deficiency in patients of type 2 diabetes mellitus on metformin: a case control study from Pakistan. *Pan Afr Med J*. 2013 Oct 25;16:67.doi:10.11604/pamj.2013.16.67.2800. eCollection 2013
 23. Ömer Azal Abdullah Özkardeş M. Emin Önde Metin Özata Gökhan Özişik Ahmet Çorakçi M. Ali Gündoğan. Visual Evoked Potentials in Diabetic Patients. *Tr. J. of Medical Sciences*; 139-142-6, 1996.