ejpmr, 2017,4(6), 669-674

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SERUM HOMOCYSTEINE LEVELS IN CELIAC DISEASE PATIENTS

[1]*Dr. Sindhu Sahito, [2]Dr. Abed Elfattah Atieh, [3]Dr. Love Kumar, [4]Dr. Muhammad Fahad Pathan, [5]Usama Abid and [6]Ali Mohsin

[1]*MBBS LUMHS, Jamshoro.
[2]Medical Officer Al-Razi Hospital Jenin.
[3]House Officer LUMHS, Jamshoro.
[4]MBBS LUMHS, Jamshoro.
[5,6]MBBS Students Isra University, Hyderabad.

***Corresponding Author: Dr. Sindhu Sahito**
MBBS LUMHS, Jamshoro.

Article Received on 20/04/2017 **Article Revised on 10/05/2017** **Article Accepted on 30/05/2017**

ABSTRACT

Objective: To assess the serum homocysteine level in Celiac disease patients. **Study Design and Setting:** This was a observational study and was carried out at Department of Medicine and Gastroenterology Unit, Liaquat University Hospital Hyderabad from March 2016 to November 2016. **Material and Methods:** Total 90 cases were selected, out of them 45 cases had celiac disease and 45 were normal. All the diagnosed cases of Celiac disease either gender were incorporated. Careful clinical evaluation including a full medical history and clinical examination were done to confirm the diagnosis. All the participants were informed about advantages and disadvantages. Vein was engorged by a tourniquet applied above cubital fossa.10 ml of blood sample was collected from ante-cubital vein after application of sterilized alcohol swab. 5 ml was put in EDTA containing blood CP bottle and 5 ml in plain glass tube. Serum homocysteine levels were estimated by enzyme linked immunosorbent assay (ELISA) kit. Protein bound Hcy is first reduced to free Hcy, then it is catalyzed by the recombinant methionine, to produced H_2S. The H_2S reacts with chromophore and results are measured at 660nm. Total Hcy levels are measured proportional to the optical density. **Results:** Total 90 cases were compared according to serum homocysteine, out of them 45 were with celiac disease and 45 were normal without significant difference of age and gender. Serum homocysteine was significantly high 14.54±11.76 µM/L in cases having celiac disease as compare to controls 6.77±2.87 µM/Lvp-value of 0.0001. Serum homocysteine as categories of normal, mild elevation, moderate elevation and severe elevation. Mild and moderate elevations of serum Hcy in controls and cases were noted as 3 (6.6%) 0 (0%) vs. 11 (24.4%) and 8 (17.7%) respectively p-value =0.0001. **Conclusion:** We concluded that elevated serum homocysteine was significantly high in the in patients having Celiac disease and prevalence of elevated homocysteine was found in 42.2%.

KEYWORDS: Frequency, serum homocysteine, celiac disease.

INTRODUCTION

Gastrointestinal tract (GIT) is digestive tube which commences at the oral cavity and terminates at the anus. Small intestine is part of GIT next to stomach and precedes the large intestine. Small intestine comprises of duodenum, jejunum and ileum. Physiologically, small intestine is an important part of GIT where most of digestion and absorption occurs altogether.[1] However, primary function is absorption which occurs solely in the small intestine however, digestion is also major function of small intestine.[2] Histologically, the surface mucosa is composed of simple columnar epithelium. Mucosa epithelial cells show small microscopic finger like projections called villi. Whole inner lining of small intestine shows villi. Mucosa epithelial cells show further subdivisions of villi as microvilli. The villi and microvilli increase the total absorptive surface area of the small intestine which is essential to its physiology of digestion and absorption.[3] Villi and microvilli may be damaged in various disease processes of small intestine, this result in incomplete digestion and absorption – termed as malabsorption. One of such disease process of small intestine is known as the celiac disease.[4] The World data shows a prevalence of 1-2% of Celiac disease. Celiac disease is the commonest disorder of absorption. Celiac disease is genetically inherited chronic disorder in which villi and microvilli are perished. Female to male ratio of Celiac disease is approximately 2:1 to 3:1.4.[5] As usual the prevalence of Celiac disease in Pakistan is never estimated and data is lacking.[6] By definition, the Celiac disease is defined as a "chronic, autoimmune mediated enteropathy primarily of small intestine, caused by immune mediated reactions against dietary gluten in genetically predisposed subjects".[7]

Wheat, rye, barley and closely related cereals and grains contain an insoluble form of prolamine poly-peptides which are collectively designated as Gluten.[7,8] In genetically susceptible subjects, the gluten acts as an inciting agent for an inflammatory reaction which is directed against small intestine predominantly centering its upper part. Once a self-directed inflammatory response is initiated, it damages the mucosal surface, culminating eventually into a full blown defective absorptive surface resulting in incomplete absorption of essential nutrients such as iron, vitamins- fat soluble, folic acid and vitamin B12 (cobalamin).[8] Cobalamin is a key micro nutrient in addition to folic acid, needed must for methylation of deoxyribo nucleid acid (DNA). Cobalamin also does play role in other essential metabolic reactions such as the homocysteine metabolism.[9,10] Food of animal origin is the exclusive source of cobalamin for human beings. Daily body requirement is 3 µg and gut absorption is approximately 5 µg depending upon diet. In human beings, the hepatic stores of cobalamin are sufficient enough for 3 -5 years to meet metabolic demands of body. Normal amount of cobalamin which remains in liver approximates 2000-5000 µg in non-vegans.[11] Various cellular enzymes require cobalamin as co-enzyme to catalyze the biochemical reactions.[11] Homocysteine (Hcy) is a sulfur (-thiol) containing amino acid. Hcy is produced by cellular demethylation of methionine. Hcy is transported in circulation bound to plasma proteins in its oxidized form.[13,14] Hcy is converted either into methionine or into cysteine. Major part of Hcy is converted into methionine by re-methylation which is a folate and cobalamin dependent biochemical reaction. This reaction is catalyzed by methionine synthase which needs folate and cobalamin as co-enzymes. In case of folate and cobalamin deficiencies, the Hcy accumulates in blood.[14,15] Hcy is highly reactive and toxic metabolites. Hcy is a risk factor for the cardiovascular disease (CVD).[15-17]

Currently, many patients of Celiac disease are presenting at the Gastroenterology Department of Isra University Hospital, hence there is need to study these patients. The present study is planned to evaluate serum homocysteine level in Celiac disease patients reporting at our tertiary care hospital.

MATERIAL AND METHODS

This was a Observational study and was carried out at Department of Medicine and Gastroenterology Unit, Isra University Hospital Hyderabad from March 2016 to November 2016. Total 90 cases were selected, out of them 45 cases had celiac disease and 45 were normal as:

Group I. Controls- normal healthy subjects (n=45)

Group II. Cases – diagnosed cases of Celiac disease (n=45)

All the diagnosed cases of Celiac disease either gender were incorporated in the study and patients with history of diabetes mellitus, liver cirrhosis, systemic hypertension, renal Disease, pulmonary tuberculosis and other causes of malabsorption were excluded. Written information about the study was provided to each patient at the OPD. All participants of the study underwent careful clinical evaluation including a full medical history and clinical examination to confirm the diagnosis. All the participants were informed about advantages and disadvantages. Vein was engorged by a tourniquet applied above cubital fossa.10 ml of blood sample was collected from ante-cubital vein after application of sterilized alcohol swab. 5 ml was put in EDTA containing blood CP bottle and 5 ml in plain glass tube.

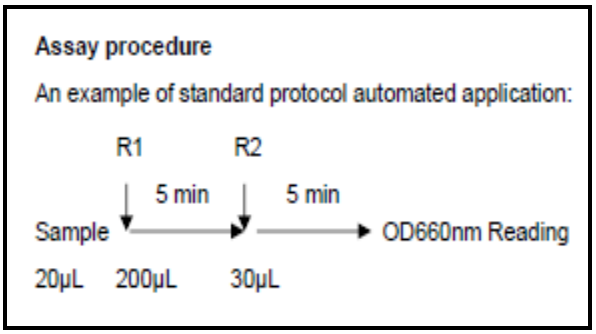
Serum homocysteine levels were estimated by enzyme linked immunosorbent assay (ELISA) kit.

Protein bound Hcy is first reduced to free Hcy, then it is catalyzed by the recombinant methionine, to produced H_2S. The H_2S reacts with chromophore and results are measured at 660nm. Total Hcy levels are measured proportional to the optical density.

Reagent preparation

- Reagent 1 was prepared fresh each time before it was used
- Foil bag contained 1 vial of Hcy enzyme and 1 vial of reducing agent.
- Reducing agent was draw out
- 2 ml Reagent 1 Buffer was added into it
- Stopper replaced immediately
- Mix it gently by inversion for several time
- Allowed to stand for 60 seconds
- Reconstituted solution was transferred back to Reagent 1 buffer bottle
- It was recapped
- Mixed gently
- 2 ml of Reconstituted solution was taken back into the Reducing reagent vial
- Stopper was replaced rapidly
- Vial was rinsed for several times by inversion
- Now transfer the solution back to the Reconstituted solution
- Hcy enzyme was drawn out
- Add 2 ml of Reconstituted solution
- Stopper was replaced immediately
- Gently mixed for several times by inversion
- Allowed to stand for 60 seconds
- Reconstituted solution was transferred back to the Reagent 1 (R1) buffer bottle
- Now put 2 ml of prepared R1 back into the Hcy enzyme
- Stopper was replaced immediately
- Vial was rinsed for several times by inversion
- Rinsed solution was transferred back into the R1
- It was allowed to stand for 10 minutes
- R1 was mixed well before use
- Any foaming was avoided during preparation

— Freshly prepared R1 was sufficient for 30 tests only



Total Hcy levels are measured proportional to the optical density at 660nm.

Data analysis

Data was analyzed by using SPSS 21.0. (IBM, incorporation, USA). Categorical variables like gender were analyzed by Chi square test and results presented as frequency and percentage. Continuous/numerical variables were analyzed by student`s t-test and results presented as Mean ± S.D. P-value of significance will be taken at ≤0.05.

RESULTS

Mean ±SD of controls and cases was noted as 47.53±8.13 and 46.84±7.69 years respectively (t-value 0.41, p-value 0.68). This indicates the cases and controls were age matched. Age of the study subjects is shown in **table 1**. Male and female in cases controls were noted as 37 (82.2%) and 8 (17.7%), & 33 (73.3%) and 12 (26.6%) respectively. (Chi value 1.029, p-value 0.31). This indicates the cases and controls were gender matched also. The gender distribution is shown in **FIG:1**.

Details of serum homocysteine are shown in **table 2 and FIG: 2**. Serum homocysteine in controls was noted 6.77±2.87 µM/L vs. 14.54±11.76 µM/L in cases. Statistically highly significant difference was noted between serum homocysteine of controls and cases as indicated by t value of 44.89 and p-value of 0.0001 as shown in **table: 2**. Serum homocysteine as categories of normal, mild elevation, moderate elevation and severe elevation are shown in **FIG: 2**. Mild and moderate elevations of serum Hcy in controls and cases were noted as 3 (6.6%) 0 (0%) vs. 11 (24.4%) and 8 (17.7%) respectively (Chi value 160, p-value =0.0001).

Table 1. Age distribution of study population (n=90)

	Mean	SD	p-value
Controls	47.53 years	8.13 years	0.68
Cases	46.84 years	7.69 years	

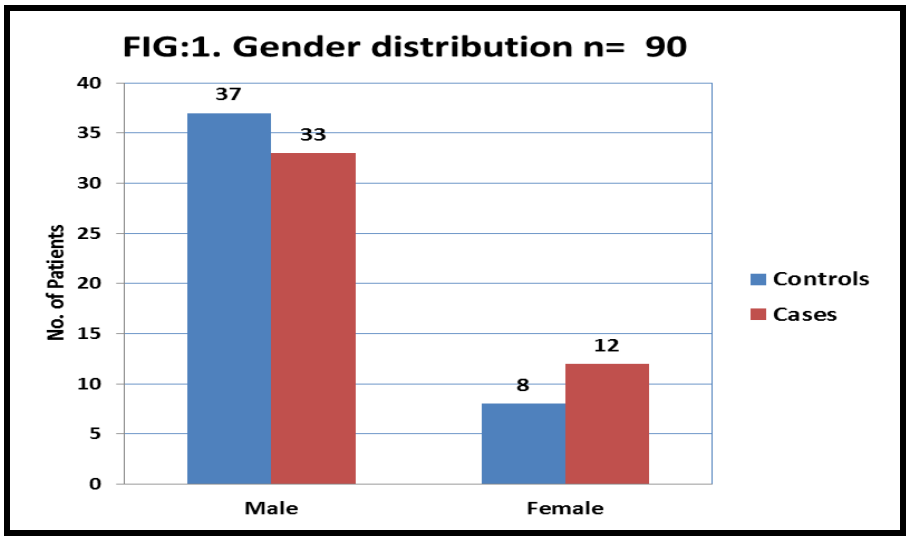
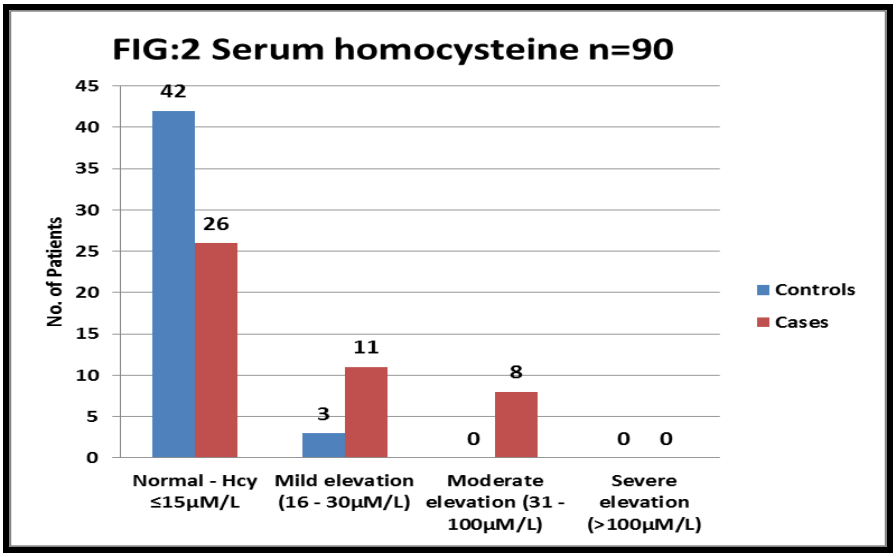


Table 2. serum homocysteine levels of study population (n=90)

	Mean	SD	p-value
Controls	6.77 µM/L	2.87 µM/L	0.0001
Cases	14.54 µM/L	11.76 µM/L	



Graph IV-4. Frequency of homocysteine in controls and cases

DISCUSSION

The present study was conducted at the Department of Medicine and Physiology, Isra University Hyderabad. Isra University caters a large number of patients of gastrointestinal disease as its Gastroenterology Department is one of the busiest medical units equipped with modern facilities of endoscopy. Celiac disease is a common cause of mal absorption. Celiac disease occurs in genetically predisposed subjects and is characterized by partial or complete destruction of intestinal villi. World prevalence of Celiac disease is reported as 1-2%. Male gender is more affected in Celiac disease, with male to female ratio of 2:1 to 3:1.4.[5] Exact prevalence of Celiac disease in Pakistan is not known.[6]

The present study evaluated serum homocysteine, serum cobalamin and red blood cell morphology in diagnosed cases of Celiac disease. Cases and controls were age and sex matched in present study. In present study, the serum homocysteine in elevated in cases, 14.54±11.76 µM/L compared to 6.77±2.87 µM/L in controls. Mild and moderate elevations of serum Hcy in controls and cases were noted as 3 (6.6%) and 0 (0%) compared to 11 (24.4%) and 8 (17.7%) respectively (Chi value 160, p-value =0.0001). Our finding of elevated serum homocysteine in Celiac disease patients is comparable to previous studies.[18,19]

The elevated serum Hcy is reported to be the result of vitamin B complex deficiencies in Celiac disease patients.[20,21] B complex vitamins are essential for the normal Hcy metabolism, which is an intermediate metabolite of methionine amino acid.[22] Inadequate intake of these vitamins is the most common cause of high concentrations of serum homocysteine.[23] About two thirds of hyperhomocysteinemia cases are related to low or moderate serum concentration of these vitamins.[24] High levels of Hcy are regarded as risk factor for the cardiovascular disease (CVD) and cerebro-vascular accident (CVA) or brain stroke.[25] Casella G et al[35] reported that Hyperhomocysteinemia was evident in 32 patients (19.3%), although most of them had moderate levels (mean value 25 mcg/ml; range 15-30). Only one patient had a history of myocardial infarction

In such patients, the vitamin pyridoxine may be deficient; however, the elevated Hcy levels are due to impaired removal by the kidneys.[26-28] Homocystinuria, as a genetic disorder is characterized by elevated serum Hcy, early atherosclerosis and thrombo emboli of veins and arteries predisposes subjects to the CVD and premature coronary artery disease (CAD).[29-31] Hyperhomocysteinemia (HHcy) in celiac patients has been linked to a number of clinical pathologies such as the osteoporosis, abortions and cardiovascular disease.[32-34]

CONCLUSION

We concluded that elevated serum homocysteine was significantly high in the in patients having Celiac disease. Mild and moderate elevated serum homocysteine levels were noted in Celiac disease patients. Frequency of elevated homocysteine was found in 42.2%.

REFERENCES

1. Hall JE. Guyton & Hall Textbook of Medical Physiology, 13th ed. Elsevier Saunders. Philadelphia, Pennsylvania. 2015; 808-18.
2. Helander HF, Fändriks L. Surface area of the digestive tract – revisited. Scand J Gastroenterol, 2014; 49: 681-9.
3. Barret KE, Barman SM, Boitano S, Brooks H. Ganong`s Review of Medical Physiology, 25th ed. M`c Graw Hill Medical Publishing. New York. 2015; 507-8.
4. van der Windt DA, Jellema P, Mulder CJ, Kneepkens CM, van der Horst HE. Diagnostic testing for celiac disease among patients with

abdominal symptoms: a systematic review. JAMA, 2010; 303(17): 1738–46.
5. Babar MI, Ahmad I, Rao MS, Iqbal R, Asghar S, Saleem M. Celiac disease and celiac crisis in children. J Coll Physicians Surg Pak, 2011; 21(8): 487-90.
6. Rashid M, Khan AG. Celiac disease in Pakistan: Challenges and opportunities (Editorial). J Ayub Med Coll Abbottabad, 2009; 21(3): 1-2.
7. Castillo NE, Theethira TG, Leffler DA. The present and the future in the diagnosis and management of celiac disease. Gastroenterol Report 2014: 1–9. URL: http://gastro.oxfordjournals.org/ accessed April 10, 2015.
8. Reilly NR, Fasano A, Green PH. Presentation of celiac disease. Gastrointest Endosc Clin N Am, 2012; 22: 613–21.
9. Adaikalakoteswari A, Jayashri R, Sukumar N, Venkataraman H, Prdeepa R, Gokulakrishan K, et al. Vitamin B 12 deficiency is associated with adverse lipid profile in Europeans and Indians with type 2 diabetes. Cardiovasc Diabetol, 2014; 13: 129.
10. McNulty H, Pentieva K, Hoey L, Ward M. Homocysteine, B-vitamins and CVD. Proc Nutr Soc., 2008; 67(2): 232–7.
11. Linker CA, Damon AE. Blood disorders. In: Mc Phee SJ, Papadakis MA, Rabow MW (ed) *Current medical diagnosis and treatment*. 54th edition. Mc-Graw Hill companies, Inc. New York. 2015; 1161- 1211.
12. Stabler SP. Vitamin B12 deficiency. N Engl J Med, 2013; 368: 149-60.
13. Guo H, Chi J, Xing Y, Wang P. Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. Indian J Med Res. 2009; 129(3):279–84.
14. Baszczuk A, Kopczynski Z. Hyperhomocysteinemia in patients with cardiovascular disease. Postepy Hig Med Dosw, 2014; 68: 579.
15. Curro M, Gugliandolo A, Gangemi C, Risitano R, Lentile R, Caccamo D. Toxic effects of mildly elevated homocysteine concentrations in neuronal-like cells. Neurochem Res., 2014; 39: 1485–95.
16. Shenov V, Mehendale V, Prabhu K, Shetty R, Rao P. Correlation of serum homocysteine levels with the severity of coronary artery disease. Ind J Clin Biochem, 2014; 29(3): 339–44.
17. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutrition J, 2015; 14: 6.
18. Valente FX, do Nascimento-Campos T, de Sousa Morases LF, Hermsdorff HHM, de Morais Cardoso L, Pinheiro-Sant`Ana HM, et al. B vitamins related to homocysteine metabolism in adult celiac disease patients: a cross sectional study. Nutrition J., 2015; 14: 110.
19. De Marchi S, Chiarioni G, Prior M, Arosio E. Young adults with coeliac disease may be at increased risk of early atherosclerosis. Aliment Pharmacol Ther, 2013; 38: 162-169.
20. Hopman EG, Le Cessie S, Von Blomberg BM, Mearin ML. Nutritional management of the gluten-free diet in young people with celiac disease in The Netherlands. J Pediatr Gastroenterol Nutr, 2006; 43: 102–8.
21. Hallert C, Svensson M, Tholstrup J, Hultberg B. B vitamins improve health in patients with coeliac disease living on a gluten-free diet. Aliment Pharmacol Ther. 2009; 29: 811–6.
22. Hadithi M, Mulder CJ, Stam F, Azizi J, Crusius JB, Peña AS, et al. Effect of B vitamin supplementation on plasma homocysteine levels in celiac disease. World J Gastroenterol. 2009; 15: 955–60.
23. Humphrey LL, Fu R, Rogers K, Freeman M, Helfand M. Homocysteine level and coronary heart disease incidence: a systematic review and meta analysis. Mayo Clin Proc. 2008; 83: 1203–12.
24. Voutilainen S, Virtanen JK, Rissanen TH, Alfthan G, Laukkanen J, Nyyssönen K, et al. Serum folate and homocysteine and the incidence of acute coronary events: the Kuopio Ischaemic Heart Disease Risk Factor Study. Am J Clin Nutr. 2004; 80: 317–23.
25. Shenov V, Mehendale V, Prabhu K, Shetty R, Rao P. Correlation of serum homocysteine levels with the severity of coronary artery disease. Ind J Clin Biochem, 2014; 29(3): 339–44.
26. Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation and cardiovascular disease. World J Cardiol, 2014; 6(6): 462–77.
27. Veeranna V, Zalawadiya SK, Niraj A, Pradhan J, Ference B, Burack RC, et al. Homocysteine and reclassification of cardiovascular disease risk. J Am Coll Cardiol, 2011; 58: 1025–33.
28. Schaffer A, Verdoia M, Cassetti E, Marino P, Suryapranata H, Luca GD. Relationship between homocysteine and coronary artery disease. Results from a large prospective cohort study. Thromb Res., 2014; 134: 288–93.
29. Pang X, Liu J, Zhao J, Mao J, Zhang X, Feng L, et al. Homocysteine induces the expression of C - reactive protein via NMDAr-ROS-MAPK-NF-κB signal pathway in rat vascular smooth muscle cells. Atherosclerosis, 2014; 236: 73–81.
30. Okura T, Miyoshi K, Irita J, Enomoto D, Nagao T, Kukida M, et al. Hyperhomocysteinemia is one of the risk factors associated with cerebrovascular stiffness in hypertensive patients, especially elderly males. Naturecom Sci Rep., 2014; 4: 5663.
31. Zhang S, Yong-Yi B, Luo LM, Xiao WK, Wu HM, Ye P. Association between serum homocysteine and arterial stiffness in elderly: a community-based study. J Geriatr Cardiol, 2014; 11: 32–8.
32. Tyagi N, Vacek TP, Fleming JT, Vacek JC, Tyagi SC. Hyperhomocysteinemia decreases bone blood flow. Vasc Health Risk Manag, 2011; 7: 31–5.
33. Bergen NE, Jaddoe VW, Timmermans S, Hofman A, Lindemans J, Russcher H, et al. Homocysteine and folate concentrations in early pregnancy and the

risk of adverse pregnancy outcomes: the Generation R Study. BJOG, 2012; 119: 739–51.
34. De Marchi S, Chiarioni G, Prior M, Arosio E. Young adults with celiac disease may be at increased risk of early atherosclerosis. Aliment Pharmacol Ther, 2013; 38: 162–9.
35. Casella G, Bassotti G, Villanacci V, Di Bella C, Pagni F, Corti GL, Sabatino G, Piatti M, Baldini V. Is hyperhomocysteinemia relevant in patients with celiac. World J Gastroenterol. 2011 Jun 28; 17(24): 2941-4.