ORIGINAL ARTICLE

Clinical profile and serum homocysteine, Vitamin B12 levels and MTHFR gene polymorphism in vitiligo

Krishna Deb Barman,[1] MD, Priyanka Rangappa Kuri,[2] MD, Lekshmi P Kumar,[3] MD
Anjali Bagrodia,[4] MD

[1]Dir Prof, Dept of Dermatology, Maulana Azad Medical College & Lok Nayak Hospital, Delhi, India
[2]Consultant Dermatologist, Bengaluru, India
[3]Specialist, Dept of Dermatology, Lok Nayak Hospital, Delhi, India
[4]Senior Resident, Maulana Azad Medical College, Delhi, India

ABSTRACT

Background: Vitiligo is an acquired depigmenting disorder of multifactorial etiopathogenesis. MTHFR gene polymorphisms, altered homocysteine levels and vitamin B12 deficiency was found to be associated with vitiligo in several studies.

Aims and Objectives: The study was conducted to ascertain the MTHFR gene polymorphisms and to evaluate vitamin B12 and homocysteine levels in association with clinical profile of vitiligo patients.

Methods: Vitamin B12 levels were estimated in all of 40 patients and 40 controls using electro chemiluminescence immunoassay. Similarly, serum homocysteine levels were estimated by ELISA. MTHFR gene polymorphisms were studied by AS-PCR technique.

Results: In our study, serum vitamin B12 levels were found in the range from 105.6 pg/ml to 559 pg/ml in vitiligo patients (mean-232.20 pg/ml) and 167.8 pg/ml to 901.0 pg/ml in controls (mean- 492.94 pg/ml) with statistical significance as p-value<0.001. Serum homocysteine levels were in the range of 6.4 mmol/l to 23.4 mmol/l in vitiligo cases (mean 14.24 mmol/l) while the levels varied from 4.2 mmol/l to 20.4 mmol/l (mean-10.28 mmol/l) in controls with p value <0.001 and statistically significant. The AC genotype of rs1801131was more in cases than controls. The odds ratio for AC genotype was 1.8. However, there was no significant correlation between serum homocysteine level and MTHFR gene polymorphism. There was a significant increase in serum homocysteine levels which paralleled the decrease in serum vitamin B12 levels.

Conclusion: In this study we have concluded that there is an increase in serum homocysteine levels in vitiligo patients, which may be due to decrease in serum vitamin B12 levels rather than MTHFR gene polymorphism. Though MTHFR gene polymorphism was higher in cases than controls it did not correlate with serum homocysteine levels.

INTRODUCTION

Vitiligo is an acquired pigmentary disease that presents with characteristic depigmented macules. It is a multifactorial disorder which ultimately leads to destruction of melanocytes.[1-5] Extensive research over many years has put forth several hypotheses. Among these, the most relevant are autoimmune, neurohumoral, auto cytotoxic and oxidative stress.[1-5] None of the proposed hypotheses definitely explain the different vitiligo phenotypes. The current consensus favours the autoimmune nature of vitiligo.[5]

There are many comorbidities and associations with vitiligo.[6] The association of vitiligo and pernicious anemia was documented much earlier as a part of autoimmune hypothesis, which showed low levels of folic acid and vitamin B12 in vitiligo patients.[7] It was later established that folate and vitamin B12 deficiency led to increase in serum homocysteine levels.[8-10] Homocysteine

Correspondence: *Dr. Krishna Deb Barman, Dir Prof, Dept of Dermatology, Maulana Azad Medical College & Lok Nayak Hospital, Delhi, India*

mediates melanocyte destruction.[8,9] Methylene-tetrahydrofolate is an enzyme affecting serum homocysteine and methionine metabolisms. In case of genetic polymorphisms MTHFR enzyme activity decreases and failure to synthesize methionine from homocysteine causes homocysteine levels to increase in circulation which adversely affects melanocytes.[10-16] This study was conducted to gain further insight into the effects of the above-mentioned factors in the etiopathogenesis of vitiligo.

MATERIALS AND METHODS

Aims and Objectives: To study the correlation between MTHFR gene polymorphism, serum homocysteine and serum vitamin B12 in vitiligo patients.

Study design: The study was conducted during a period of one and half years extending from November 2015 to December 2017 at Maulana Azad Medical College and associated Lok Nayak Hospital, Delhi, India. After approval from the ethical committee of the institution, the patients and controls were selected. Each subject signed an informed consent before being included in the study.

A sample size of 80 was taken which included 40 cases and 40 controls. Inclusion criteria: Patients clinically diagnosed with vitiligo of age equal to or more than 18 years.

Exclusion criteria: Patients with diseases increasing homocysteine levels such as Hypertension, Diabetes mellitus, cardiovascular disease, kidney failure, deep vein thrombosis, Behcet's disease, psoriasis, and pregnant and lactating women.

Methodology: A detailed history was taken and recorded. Vitiligo disease activity score (VIDA) was used to assess the disease activity.[15] The disease extent was assessed by VASI score, to define the extent of the disease.[16] After an informed consent, 5ml of blood was withdrawn, which was used to estimate serum vitamin B12 level (electro chemiluminescence immunoassay) and serum homocysteine level (ELISA). To assess the polymorphism of the MTHFR gene, DNA extraction from erythrocytes was done by using GeneAid DNA extraction kit. The primers for MTHFR gene rs 1801131 polymorphism were designed using software. MTHFR gene polymorphisms were studied by AS-PCR technique.

Statistical analysis of the results: Statistical software IBM PASW (version 22.0) was used for the entire data analysis.

RESULTS

Mean age of the patients in our study was 30.63 ± 11.38 years and mean duration of the disease was 12.2 ± 9.1 years. The male: female ratio was 1:1. The disease progressed rapidly in 6 cases (15%) while the progression was gradual in 34 cases (85%). History of koebnerization was present in 16 cases (40%). A positive family history of vitiligo was found in 20 (50%) of our cases.

Serum vitamin B12 levels were in range from 105.6 pg/ml to 559 pg/ml with mean value of 232.20 pg/ml ± 111.67 pg/ml in vitiligo cases which was significantly lower than controls (492.94 pg/ml ± 235.74 pg/ml). Normal vitamin B12 ranged from 211 pg/ml to 946 pg/ml as mentioned by the kit. The vitamin B12 level was decreased in 26 (65%) vitiligo cases, while only

7 (17.5%) among controls had decreased value. (Fig. 1)

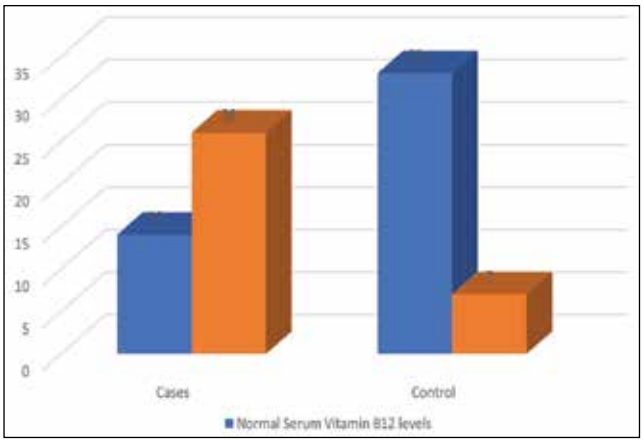

Fig. 1 Distribution of vitamin B12 among cases and controls

Normal homocysteine levels mentioned as per kit was 5 to15 µmol/L. Serum homocysteine levels ranged from 6.4 µmol/L - 23.4 µmol/L in vitiligo cases with a mean value of 14.24 ±4.82 µmol/L, which was significantly higher than controls (10.28±4.29 µmol/L). Out of 40 cases of vitiligo, 23(57.5%) cases had increased serum homocysteine levels, 17(42.5%) cases were within normal range and none had decreased levels, whereas in control group serum homocysteine level was normal in 30(75%) controls, increased in 9(22.5%) and decreased in 1(2.5%) subject. (Fig. 2)

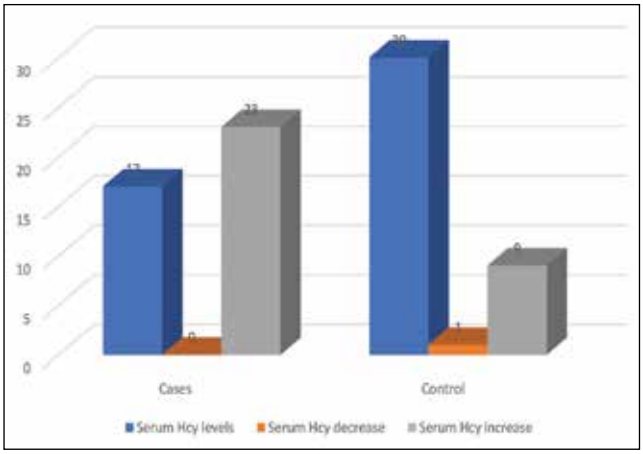

Fig. 2 Distribution of serum homocysteine levels

There was a parallel increase in serum homocysteine level with decrease in serum vitamin B12 level as there was significant negative correlation between serum vitamin B12 and serum homocysteine levels (p value<0.05).

The gene polymorphism AA, A>C, CC were found in 5, 23 and 12 cases out of 40 cases respectively. The same gene polymorphisms in controls were seen in 18, 9 and 3 respectively. Overall, rs1801131 gene polymorphism was statistically significant between cases and controls (p-value <0.001). It was found that rs1801131 A>C allele polymorphism was more in cases than controls, however there was no statistically significant difference of AA or CC allele between cases and controls. The odds ratio for A>C allele was 1.8 , which suggested that there is 1.8 times risk of developing the disease with A>C allele polymorphism. (Fig. 3)

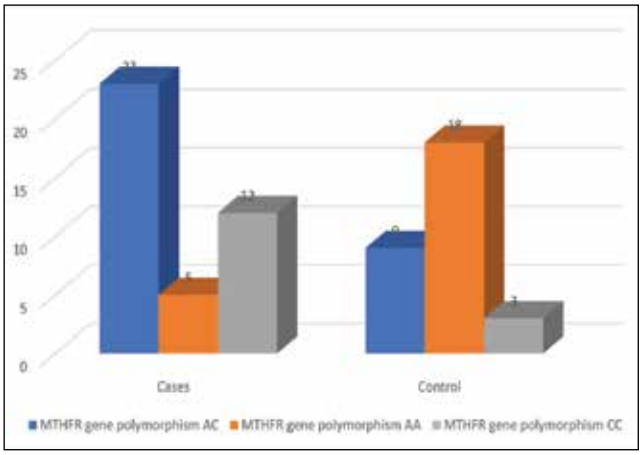

Fig. 3 Distribution of AA, AC and CC genotype

MTHFR gene polymorphism AA, A>C, CC allele did not correlate significantly with serum homocysteine levels (p<0.323). This meant that there was no parallel increase in serum homocysteine levels with polymorphism.

There was a positive significant correlation be-

tween VASI and higher serum homocysteine levels and a negative correlation between serum vitamin B12 and VASI. The correlation of VASI with duration of the disease was non-significant, as p-value was 0.714 (Table 1).

Table 1 Correlation of VASI with clinical and biochemical profile

		VASI	HCY	VIT. B12	Duration	Age of Onset
VASI	Pearson Correlation	1	.763**	-.355*	-0.06	.320*
	p-value		0	0.025	0.714	0.044
	N	40	40(S)	40(S)	40(NS)	40(S)

S-Significant, NS- Non significant

A comparison among the different types of vitiligo in our 40 cases was done. Vitiligo vulgaris was most common as there were 25 cases (62.5%) followed by 6 cases of the acrofacial type (15%) and 5 cases (12.5%) of focal type. There were 2 cases of each segmental and mucosal type (5%). Serum homocysteine levels were significantly higher in patients with vitiligo vulgaris as compared to other clinical types ($p<0.001$). There was no statistically significant relation between serum vitamin B12 and type of vitiligo ($p<0.812$). Though, vitamin B12 was decreased in vitiligo cases having vitiligo vulgaris. Vegetarians constituted 17(42.5%) of cases while non-vegetarians were 23(57.5%). No statistically significant correlation was found between serum homocysteine and vitamin B12 with dietary habits.

DISCUSSION

Ours was a hospital based case control study comparing the serum levels of homocysteine, vitamin B12 and MTHFR gene polymorphism in vitiligo patients with the extent and activity of the disease.

We found that serum homocysteine levels were significantly increased in vitiligo patients compared to controls. This finding was in concordance with several other studies conducted in India as well as other countries.[11-18] It was also found that serum vitamin B12 levels were less than normal range in vitiligo cases in our study. However, no significant correlation in serum vitamin B12 levels and serum homocysteine levels between the case and control group was found by El-Dawela et al, Balci et al, Yasaret al and Kim et al, and the same findings were attributed to regional and ethnic factors.[19,20,21,22]

Homocysteine levels are determined by serum levels of vitamin B12, folic acid and MTHFR gene. Vitamin B12/folate deficiencies or MTHFR gene polymorphism causes elevation of serum homocysteine levels.[8-11,21] Homocysteine has been identified as a circulatory marker of oxidative stress and is a risk factor for vitiligo.[8] Homocysteine induces cell apoptosis via activating ROS and PERK-elF 2alpha-CHOP pathway.[10] Homocysteine also increases the level of IL-6 and activates NF-KB which results in melanocyte destruction.[9,23]

MTHFR gene polymorphism has been attributed in various studies to affect the level of serum homocysteine.[10,11,27] The most common polymorphism observed was C677T (rs1801133).[23] MTHFR 1298 A>C polymorphism is found to be

associated with autoimmune vitiligo.[8,25] MTHFR A1298C (rs1801131) has been identified as the second most frequent mutation seen on the same gene. In this, cytosine (C) substitutes adenine (A) on nucleotide.[27] There was a significant decrease in MTHFR activity in homozygous CC individuals compared to heterozygous (AC) and regular (AA) ones in study by Ozkul et al.[28] In our study it was found that rs1801131 AC allele polymorphism was more in vitiligo cases than in controls. Overall, rs1801131 gene polymorphism was statistically significant between controls and cases. The odds ratio for AC allele was 1.8, which suggested that there is 1.8 times risk of developing the disease with AC allele polymorphism. Our finding was in concordance with Yasar et al whose study among the Turkish population reported that the heterozygote (AC) genotype was significantly higher in vitiligo patients than in controls.[19] J-X Chen et al investigated the associations of the single-nucleotide polymorphisms rs1801133 and rs18011131 in the MTHFR gene with the risk of vitiligo in the Han Chinese population and demonstrated that rs1801133 is associated with a significantly reduced risk of vitiligo, and the T allele of rs1801133 acts as a protective allele.[10] They found no statistical relationship between rs1801131 and vitiligo risk.

MTHFR gene polymorphism AA , A>C, CC allele were not correlated significantly with serum homocysteine levels as per our findings. This means that there is no parallel increase in serum homocysteine levels with polymorphism. Yasar et al also reported similar findings.[21]

Hamidi et al in their study in Iranian population found that C/T+T/T and T/T genotypes of the MTHFR C677T variant were associated with vitiligo onset.[24] T/T genotype reduces the function of the MTHFR enzyme by 60% which predisposes homozygous T/T individuals to elevated homocysteine levels.[24,25,26]

Correlation of serum levels of vitamin B12 and homocysteine with epidemiological and clinical profile and biochemical parameters of study groups:

In our study there was positive significant correlation between VASI and serum homocysteine levels, which denotes that as VASI score increases, there is increase in serum homocysteine levels. There is also a negative significant correlation between serum vitamin B12 and VASI score, which implies that as VASI score increases there is parallel decrease of serum vitamin B12 levels. In concordance with the present study Silverberg et al, El-Dawela et al and Jadeja et al reported a statistically significant association between serum homocysteine levels and extent of disease, but not between folate or vitamin B12 levels and the extent of vitiligo.[8,19,25] Moreover, Silverberg et al strongly recommended the inclusion of homocysteine as a severity marker on initial examination for vitiligo patients.[8] Hasibuan et al in their study in Indonesia and Zaki et al in their study in Egypt did not find any significant relation between serum homocysteine level and VASI score.[29] Chronicity of vitiligo is caused by an uninterrupted ongoing melanocytic injury, either because of the natural course of disease or lack of treatment, which results in extensive depigmentation (greater VASI score). Since chronicity of disease is associated with a long standing insult to melanocytes, both se-

rum homocysteine levels, and VASI scores were found to be significantly raised in patients with long standing disease. Shaker et al and Balci et al could not detect any correlation of either homocysteine, folic acid, or vitamin B12 levels with the extent of vitiligo.[15,20]

In our study serum homocysteine levels were significantly higher in patients with vitiligo vulgaris as compared to other clinical types of vitiligo. However, there was no association of vitamin B12 levels with the type of vitiligo. Most of the studies have not commented upon such association, but Kim et al have reported of not finding any correlation between folic acid and vitamin B12 levels with the type of vitiligo.[22]

No significant association between serum homocysteine level or vitamin B12 level and dietary habits of the patients were observed in our study. This is in accordance with previous studies.[30] Dietary habit may influence homocysteine level because vitamin B12 is mainly present in animal proteins. One of the reasons for almost similar homocysteine or vitamin B12 levels in all the groups in the present study could be the low frequency of intake of meat by our study subjects. It has been established that subtle changes suggestive of a functional intracellular deficiency of vitamin B12 and folic acid occur in many adult patients in the presence of minimal biochemical changes.[20,22,31] In this respect, serum vitamin concentrations have relatively poor sensitivity and specificity in detecting subjects with subtle changes suggestive of vitamin deficiency.[21] The metabolites involved in enzymatic reactions dependent on vitamin B12, folate, vitamin B6 and MTHFR levels have been found to be sensitive estimates of both functional and intracellular deficiencies of these vitamins. Homocysteine especially is widely regarded as a reliable indicator for this purpose.[19] Vitamin B12 can also be regarded as a reliable indicator as there was significant correlation with increase in homocysteine levels and higher VASI score with decrease in serum vitamin B12 levels.

Therapeutic implications of this observation would require further prospective randomized control studies with vitamin B12, and folic acid supplementation in generalized and universal vitiligo.

CONCLUSION

Hence, in this study we would conclude that there is an increase in serum homocysteine levels in vitiligo patients, which may be due to decrease in serum vitamin B12 levels rather than MTHFR gene polymorphism. Though the MTHFR gene polymorphism was significantly higher in cases than in controls it did not correlate with serum homocysteine levels. Increase in serum homocysteine levels cause increase in toxic metabolites and inhibition of tyrosinase which lead to loss of melanocytes. Further studies involving larger sample sizes are recommended to evaluate serum homocysteine, serum vitamin B12 and MTHFR gene polymorphism in vitiligo. It was concluded that the study population was deficient in vitamin B12. Supplementation of vitamin B12 may be helpful to decrease the effect of homocysteine on melanocytes.

REFERENCES

1. Alikhan, A., Felsten, LM, Daly M, Petronic-Rosic

V. Vitiligo: a comprehensive overview Part 1. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. J Am Acad Dermatol. 2011; 65(3):473-91.
2. Laddha NC, Dwivedi M, Mansuri MS et al. Vitiligo: interplay between oxidative stress and immune system. Exp Dermatol. 2013; 22:245-50.
3. Yaghoobi R, Omidian M, Bagherani N. Vitiligo: A review of the published work. J Dermatol. 2011; 38(5):419-31.
4. Gauthier Y, Cario Andre M, Taieb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Research. 2003; 16:322-32.
5. Bergquist C, Ezzedink K. Vitiligo: A review. Dermatology 2020; 236:571-92.
6. S Gill L, Zarbo A, Isedeh P, Jacobsen G, Lim HW, Hamzavi I. Comorbid autoimmine diseases in patients with vitiligo: A cross-sectionl study. J Am Acad Dermatol 2016; 74(2):295-302.
7. Grunnet I, Howitz J, Reymann F, Schwartz M. Vitiligo and pernicious anemia. Arch Dermatol. 1970; 101:82-85.
8. Silverberg JU, Silverberg NB. Serum homocysteine as a biomarker of vitiligo vulgaris severity: a pilot study. J Am Acad Dermatol. 2011; 64:445-47.
9. Agarwal S, Mendiratta V, Chander R, Jain A, Yadav P. Study of serum levels of vitamin B, folic acid and homocysteine in vitiligo. Pigment Int. 2015; 2:76-80.
10. Chen JX, Shi Q, Wang XW, Guo S, Dai W, Li K et al. Genetic polymorphisms in the methylenete trahydrofolate reductase gene (MTTHFR) and risk of vitiligo in Han Chinese populations: a genotype-phenotype correlation study. Br J Dermatol 2014: 170(5):1092-99.
11. El-Tahlawi S, Abdel Halim DM, El-Hadidi H, Fawzy MM, Hegazy RA, Ezzat M et al. Estimation of Homocysteine level and Methylenetetrahydrofolate Reductase (MTHFR)Gene and Cystathionibe B Synthase (CBS) Gene Polymorphisms in Vitiligo Patients. Skin Pharmacol Physiol. 2020; 33:38-43.
12. Chen J, Zhuang T, Chen T , Tian Y, Yi X, Ni Q et al. Homocysteine induces melanocytes apoptosis via PERF-elF2α-CHOP pathway in vitiligo. Clin Sci. 2020; 134:1127-41.
13. Singh S, Singh U, Pandey SS. Increased level of serum homocysteine in vitiligo. J Clin Lab Anal. 2011; 25:110-12.
14. Singh S, Singh U, Pandey SS. Serum folic acid, vitamin B12 and homocysteine levels in Indian vitiligo patients. EDOJ. 2012; 8:1-7.
15. Shaker OG. El-Tahlawi SM. Is there a relationship between homocysteine and vitiligo? A pilot study. Br J Dermatol. 2008; 159:720-24.
16. Karadag AS, Tutal E, Ertugrul DT, et al. Serum holotranscobalamine, vitamin B12, folic acid and homocysteine levels in patients with vitiligo. Clin Exp Dermatol. 2012; 37:62-64.
17. Hamzavi I, Jain H, McLean D, Shapiro J, Zeng H, Lui H. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. Arch Dermatol. 2004; 140(6):677-83.
18. Agarwal S, Ojha A, Gupta S. Profile of vitiligo in Kumaun region of Uttarakhand, India. Ind J Dermatol. 2014; 59(2):209.
19. El-Dawela RE, Abou-elfetouh S. Relationship between Homocysteine, Vitamin B12, Folic acid Levels and Vitiligo. J Appl Sci Res. 2012; 8:5528-35.
20. Balci DO, Yonden Z, Yenin JZ, Okumus N. Serum homocysteine, folic acid and vitamin B12 levels in vitiligo. Eur J Dermatol. 2009; 19:382-83.
21. Yasar A, Gunduz K, Onur E, Calkan M. Serum homocysteine, vitamin B12, folic acid levels and methylenetetrahydrofolate reductase (MTHFR) gene polymorphism in vitiligo. Dis Markers. 2012; 33:85-89.
22. Kim SM, Kim YK, Hann SK. Serum levels of folic acid and vitamin B12 in Korean patients with vitiligo. Yonsei Med J. 1999; 40:195-98.
23. Hasibuan DR, Putra IB, Jusuf NK. Correlation between Serum Homocysteine and Vitilgo Area Scoring Index. J Med Sci. 2017; 5:332-34.
24. Hamidi BA, Namazi N, Amoli MM, Amani M, Gholami M, Youseffian L. Association of MTHFR C677T polymorphism with elevated homocysteine level and disease development in vitiligo. Int J Immunogenet. 2020; 47:342-50.
25. Koubaa N, Hammami S, Nakbi A, Ben Hamda K, Mahjoub S, Kosaka T, et al. Relationship between thiolactonase activity and hyperhomocysteinemia according to MTHFR gene polymorphism in Tunisian Behcet's disease patients. Clin Chern Lab Med. 2008; 46(2):187-92.
26. Jadeja SD, Mansuri MS, Singh S, Patel H, Marfatia YS, Begum R. Association of elevated homocysteine levels and Methylenetetrahydrofolate reductase

(MTHFR)1298 A>C polymorphism with Vitiligo susceptibility in Gujarat. J Derm Sci. 2018; (90):112-22.

27. Friedman G, Goldschmidt N, Friedlander Y, Ben-Yehuda A, Selhub J, Babaey S, et al. A common mutation A1298C in human methylenetetrahydrofolate reductase gene: association with total plasma homocysteine and folate concentrations. J Nutr. 1999; 129(9):1656-61.
28. Ozkul Y, Evereklioglu C, Borlu M, Taheri S, Calis M, Dundar M, et al. 5,10-Methylenetetrahydrofolate reductase C677T gene polymorphism in Behcet's patients with or without ocular involvement. Br J Ophthalmol. 2005; 89:1634-37.
29. Zaki AM, Abdo HM, Ibrahim IM, Ibrahim AEKI. Serum homocysteine and vitiligo. The Gulf J Dermatol Venereol. 2014; 21(2):15-20.
30. Savage DG, Linmenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. Am J Med. 1994; 96:239-46.
31. Song MS, Hann SK, Ahn PS, 1m S, Park YK. Clinical study of vitiligo: comparative study of type A and B vitiligo. Ann Dermatol. 1994; 6:22-30.