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Original Article

Fingolimod and changes in hematocrit, hemoglobin and red blood cells of patients with multiple sclerosis

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Abstract: Introduction: Fingolimod is the first oral drug approved by Food and Drug Administration (FDA) of United States for treating patients with relapsing-remitting multiple sclerosis (RRMS). Fingolimod acts by immunomodulation but there are still much remained about its different effects. Objectives: The aim of this study was to evaluate the changes in hematocrit (Hct), hemoglobin (Hb), and red blood cells (RBC) of patients with MS under treatments with fingolimod. Methods: A total number of 66 MS patients were included to our study based on certain exclusion criteria and eligibility for fingolimod oral treatment. Hct, Hb, and RBC were measured for each patient before drug administrations. Patients were treated with Fingolimod. 5 mg daily and after three months of treatments, measurements of Hct, Hb, and RBC were performed. Data were analyzed using SPSS software version 24. Results: Amounts of Hct, Hb, and RBC were significantly decreased in this patient cohort. Hematocrit was decreased in all patients. Hemoglobin levels were significantly decreased in the female cohorts. Such decreases for male patients were insignificant. Red blood cell counts were also significantly decreased in patients. Conclusion: Accumulating line of evidence had surveys on different side effects of fngolimod but here we indicated that fingolimod will also decrease amounts of Hct, Hb, and RBC which could result further problems in patients susceptible to other diseases.

Keywords: Multiple sclerosis, red blood cells, hemoglobin, hematocrit

Introduction

Multiple sclerosis (MS) is recognized as a disease of young adults with inflammatory basis which have impacts on central nervous system (CNS) [1]. MS is known to cause demyelination and by this means, different signs and symptoms occur [2]. Unfortunately, MS cannot be recognized only according to these signs and symptoms and most of the time, different imaging modalities should help [3]. So far, there is much to discover about MS pathogenesis but it has been proven that inflammation and immune system play a pivotal role in the disease pathogenesis and both inner factors and environmental factors can also affect the disease [4-6]. Based on autoimmune basis of MS pathogenesis, different therapeutic modalities have been used for the disease which mostly influence on immune system [7]. These drugs are mostly injectable and are associated with different infusion related side effects and complications. Besides, patient's compliance for drugs plays an important role in effectiveness of the therapeutic method. Fingolimod (FTY720) is the first oral drug for MS which was approved by American Food and Drug Administration (FDA) in 2010 for treatment of relapsing- remitting MS (RRMS) [8]. Fingolimod is an oral immunomodulatory drug, which helped MS patients to increase their compliance of drugs usage and most importantly, despite other injectable drugs, there is no need to administer the drug under direct observation of health care providers [9]. The mechanism of action of Fingolimod is by down- regulation of sphingosine 1-phosphate (S1P) receptors [10]. These receptors are mostly expressed in brain and cause lymphocytes to leave the lymphoid tissues and enter the circulation. Fingolimod can cross blood brain barrier (BBB) and by this means, it inhibits lymphocytes presence in brain tissue and as a

result, it inhibits further CNS demyelination which is helpful in MS amelioration [1, 11]. Fingolimod has been proven to be effective in curing RRMS in different clinical trials. Like any other drug, fingolimod is also associated with some side effects. Most important of all are dose dependent bradycardia, blood pressure effects, macular edema and possible infection risks [12, 13]. The mechanism of cardiac effects of fingolimod is thought to be due to protection role of S1P receptors in cardiovascular system which interferes in promoting regular heart rhythm [14].

Fingolimod has also been proven in different animal models of MS. Administration of fingolimod to animal with experimental autoimmune encephalitis (EAE), a model of MS, had resulted to prevent development of EAE features [15]. Effects of fingolimod on different cell population especially lymphocytes had been greatly reviewed and it has been documented that fingolimod can cause lymphopenia [16]. Fingolimod has also shown to prevent lymphocytic infiltration even in animal models of intracerebral hemorrhage [17]. There have been some reports about the effects of fingolimod on different cells. For example it has been indicated that fingolimod can cause a state of low platelet [1]. Effects of fingolimod on other blood cells can give us the knowledge to a better understanding of its side effects. However, fingolimod's effect on red blood cell count, hematocrit, and hemoglobin needs to be established as well. This study analyzed the effects on hematocrit, hemoglobin, and red blood cell count in MS patients placed on oral fingolimod treatment for three months. To our knowledge, this is the first patient study analyzing fingolimod's effects in these three areas.

Methods and materials

This cohort study was performed on 78 patients (14 men and 64 women) all with a diagnosis of relapsing remitting multiple sclerosis (RRMS). All were residents of Isfahan, which is located in central Iran. All cases were recruited from Multiple Sclerosis clinic in Al-Zahra hospital, Isfahan. The diagnosis of MS was established according to the McDonald's criteria by a qualified neurologist [18]. Furthermore, other laboratory and clinical history was assessed for supporting their diagnosis. To determine the degree of disability of MS patients the Expanded Disability Status Scale (EDSS) was used by trained evaluators. Within two weeks after the study, all patients underwent pretreatment evaluation to take their demographic data, complete neurologic and medical history, establish findings from their neurologic examination, and record any previous treatment. Because all diagnoses of MS are registered in the IMSS, we also used some previous data in this registry system. The inclusion criteria of study were: 1) a definite diagnosis of MS, 2) EDSS less than 6 and 3) being free of other disorders that can increase or decrease the chances of anemia and polycitmy. Afterwards, the aspects of the study were explained to all the MS patients and consultations were made with their neurologists. Patients were registered in the study and we obtained written informed consent from each participant.

At baseline, before initiation of treatment with fingolimod, 3 ml of peripheral blood samples of each MS patient was collected in tubes containing EDTA as an anti-coagulant. All samples were collected in one day during the morning. Blood samples were prepared and the levels of hematocrit (Hct), hemoglobin (Hb), and red blood cells (RBC) were determined using an automated cell counter according to the manufacturer's instructions. All MS patients continued intake of fingolimod as their current treatment during the three months period of study. During this time neurologists screened all cases for possible fingolimod complications. If complications were deemed severe enough, fingolimod was ceased and the patient was excluded from the study. In this manner, 12 MS patients (3 men and 9 women) were excluded from study due to severe complication after fingolimod initiation. After three months of follow up blood samples from remaining participants were obtained and Hct, Hb, and RBC were measured according to the above-mentioned method (using automated cell counter).

All statistical analysis was performed by SPSS for hardware (version 20.0; SPSS, Chicago, IL). Smirnov Test-sample Kolmogorov-One was used to show normal distribution of data. Furthermore, paired sample T-test and independent sample T-test were other modes of analyses used in this study. All tests were two-tailed and $P<0.05$ was considered a significant threshold. All data were reported as a mean (±SD) and number (percent).

Table 1. Amounts of Hct before and after fingolimod therapies

	Male (Mean ± SD)	Female (Mean ± SD)	Total (Mean ± SD)
Hct1 (%)	45.8000 ± 4.02815	39.3077 ± 3.30750	40.6365 ± 4.08658
Hct2 (%)	43.9273 ± 3.79713	38.0712 ± 2.60455	39.7889 ± 3.45707
Hct1-Hct2 (%)	1.49273 ± 3.81610	0.89654 ± 3.64486	0.99762 ± 3.95398
95% CI	-0.95660-3.90206	-0.12252-1.79559	0.07775-1.81749
P-Value	0.307	0.096	0.023

Table 2. Amounts of Hb before and after fingolimod therapies

	Male (Mean ± SD)	Female (Mean ± SD)	Total (Mean ± SD)
Hb1 (g/dl)	14.8000 ± 1.31833	12.7891 ± 1.15881	13.1242 ± 1.39759
Hb2 (g/dl)	14.5273 ± 1.06592	12.4909 ± 1.00633	12.8303 ± 1.26527
Hb1-Hb2 (g/dl)	0.24273 ± 1.13057	0.29718 ± 1.00747	0.28394 ± 1.01979
95% CI	-0.49680-1.03225	0.04582-0.57054	0.06324-0.54463
P-Value	0.642	0.012	0.012

Results

Of the 78 patients included in our study, 66 of them were able to finish the three months therapeutic course with fingolimod. The levels of Hct, Hb, and RBC were determined for all patients and for men and women separately.

Hematocrit: Data analysis for the Hct level of the 66 patients before treatments (Hct1) indicated a mean level of 40.63 ± 4.08% for all patients and the same analysis after three months of study (Hct2) yielded 39.78 ± 3.45%, which indicates a significant decrease in Hct level (*P*-Value = 0.02). Furthermore, the mean Hct level was analyzed for women and men before and after treatments. These analyses indicated Hct level of 39.30 ± 3.30% as Hct1 and 38.07 ± 2.60% as Hct2 for women, which indeed yield an insignificant decrease (*P*-Value = 0.09) in Hct level for women treated with fingolimod. The same analyses for men were performed showing an Hct level of 45.80 ± 4.02% and 43.92 ± 3.79% before and after therapeutic period respectively. These levels showed a statistically insignificant (*P*-Value = 0.30) decrease in Hct level for these patients. These data are summarized in **Table 1**.

Hemoglobin: After blood samples were taken from patients used for measuring Hb level, analyzed data indicated a slightly considerable decline in the mean level of Hb among all study population. The mean Hb levels before initiating treatments and after three months of treatments were 13.12 ± 1.39 g/dl and 12.83 ± 1.26 g/dl respectively. This indicates a slightly considerable decline (*P*-Value = 0.01). The same analyses were performed to determine the Hb level before and after this three months fingolimod therapy in both women and men. Data showed an Hb level of 12.78 ± 1.15 g/dl before the study was initiated in women and an Hb level of 12.49 ± 1.00 g/dl after fingolimod therapy. This demonstrates a considerable decline in Hb level among these patients (*P*-Value = 0.01). The Hb level average was 14.80 ± 1.31 g/dl before treatments in men and afterwards 14.52 ± 1.06 g/dl showing an insignificant decrease in Hb level (*P*-Value = 0.64). This information is summarized in **Table 2**.

Red blood cells: RBC counts were performed by an automated cell counter for all patients and the results were analyzed. These results demonstrated RBC count of 4.84 ± 0.52 Mil/mm^3 before fingolimod was administered for patients and after three months using fingolimod, an RBC count of 4.65 ± 0.46 Mil/mm^3. Analyses demonstrated a statistically considerable decline in RBC count among patients (*P*-Value = 0.00). In women before treatments the RBC count yielded 4.61 ± 0.41 Mil/mm^3 and after treatments it reached 4.47 ± 0.45 Mil/mm^3 indicating a considerable decrease (*P*-Value = 0.02). The same analyses for men demonstrated a significant (*P*-Value = 0.011) decrease in RBC during three months fingolimod study. Before the study period began the RBC count was 5.35 ± 0.61 Mil/mm^3 and after treatments it was 4.93 ± 0.36 Mil/mm^3 (*P*-Value = 0.02). These data are summarized in **Table 3**.

Discussion

This three months study included 66 RRMS patients who were able to complete the required fingolimod treatments. The three end-point measures were hematocrit level, hemoglobin level, and red blood cell count. In the entire cohort hematocrit, hemoglobin and red blood cells

Table 3. Amounts of RBC before and after fingolimod therapies

	Male (Mean ± SD)	Female (Mean ± SD)	Total (Mean ± SD)
RBC1 (Mil/mm^3)	5.3536 ± 0.61105	4.6135 ± 0.41007	4.8427 ± 0.52799
RBC2 (Mil/mm^3)	4.9391 ± 0.36564	4.4746 ± 0.45122	4.6557 ± 0.46971
RBC1-RBC2 (Mil/mm^3)	0.40455 ± 0.44313	0.12885 ± 0.39058	0.19698 ± 0.41023
95% CI	0.10684-0.71225	0.04011-0.24758	0.09367-0.29030
P-Value	0.021	0.023	0.002

were all significantly decreased. Hematocrit was insignificantly decreased in both female and male cohorts separately. Hemoglobin levels were only significantly decreased in the female cohort, while insignificantly decreased in males. Red blood cell counts were significantly decreased in both the male and female cohorts. Overall, these results suggest a considerable decrease in hematocrit, hemoglobin, and red blood cell populations amongst RRMS oral fingolimod users, even after such a short period as three months. The effects of fingolimod on different types of blood cells including lymphocytes and platelets has been well studied [1, 19] but here in this paper we had a survey on the influence of this drug on RBC, Hct and Hb. There have been different lines of evidence, indicating a decreased lymphocytic count following fingolimod therapy which is indeed, the main basis of fingolimod effects. But so far, there has been no documented study on the effects of fingolimod on RBC, Hct and Hb of patients under treatments. On the other hand, some case reports or article could be discussed about the effects of fingolimod on cell blood counts. For instance, Lysandropoulos and colleagues have reported a case of sever auto-immune hemolytic anemia in a patient under fingolimod treatments [20]. The patient had an Hb level of 6.0 g/dl. In this paper we showed that fingolimod therapies could be associated with decreased RBC count, Hct and Hb. This finding can be considered as important evidence in patients with abnormal laboratory data before fingolimod initiation. In the study by Ontaneda and colleagues, that evaluated effect of treatment with fingolimod on the clinical findings, normal CBC was in the 77.3% and abnormal CBC was 22.7% of patents [21]. These results are in line with our study and indicates that some cautious must be considered in patients susceptible to hematologic issues for fingolimod treatments. On the other hand, some conflicting reports are found. As an example, in a study, van Rensburg and colleagues indicated that MS is a chronic disease and can associate with anemia associated with chronic disease [22]. But we must add that our cases had normal Hb levels before fingolimod initiation and also, we had a survey on the trend of Hb following fingolimod therapies. Another case report study reported that fingolimod can induce retinal hemorrhage following fingolimod therapy and reported that hemoglobin was in the normal range [23]. All these data are somehow in line with our study and emphasis on the different effects on fingolimod on cell counts.

Conclusion

Taken together, in this study we indicated that fingolimod has the potential to affect cell counts and decrease RBC, Hct and Hb in patients. We also emphasis on the different possible hematologic side effects of fingolimod therapies and suggest that such complications must be considered in susceptible patients.

Disclosure of conflict of interest

None.

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