

Peripheral blood monocytes in multiple sclerosis exacerbations

Temel Tombul¹, Omer Anlar², Hayrettin Akdeniz³

ABSTRACT

Objectives: Monocytes (MO), macrophages, and microglia have a central role in the central nervous system inflammation of multiple sclerosis (MS). During clinical activity in MS, MO activation markers increase and some interleukins and tumor necrosis factor-alpha levels are elevated. Our aim was to determine levels of absolute MO count and percentage in peripheral blood of MS patients during the attacks.

Methodology: We assessed the percentage of MO by examining the blood smears in 28 patients with definite MS, in 20 patients with acute cerebrovascular disease (CVD) and in 20 healthy control subjects.

Results: The mean value of absolute MO count in MS patients, CVD and control groups were as 606.67 ± 170.52 , 746.50 ± 414.76 and 360.00 ± 109.54 respectively. The mean values of MO percentage in MS patients, CVD and control group were $8.34 \pm 2.61\%$, $5.56 \pm 2.48\%$ and $5.36 \pm 1.50\%$ respectively. The mean percentage of MO was significantly elevated in MS patients compared with the both groups of CVD and control ($P < 0.001$).

Conclusion: Our results suggest a possible role of an increase in MO activation in the acute exacerbations of Multiple Sclerosis.

KEY WORDS: Peripheral blood, Monocytes, Multiple sclerosis, Exacerbation.

Pak J Med Sci January - March 2011 Vol. 27 No. 1 73-76

How to cite this article:

Tombul T, Anlar O, Akdeniz H. Peripheral blood monocytes in multiple sclerosis exacerbations. Pak J Med Sci 2011;27(1):73-76

INTRODUCTION

Monocytes (MO), macrophages, and microglia have a central role in the central nervous system (CNS) inflammation of multiple sclerosis (MS). During MS attacks, T lymphocytes and monocyte-derived macrophages gain entry to the CNS and form perivascular infiltrates, a process which is accompanied by enhanced permeability of the blood-brain barrier.^{1,2} The presence of activated monocyte-derived macrophages, filled with myelin debris, is related to disease activity and axonal damage.³ The key role for monocytes was also illustrated in the animal model for MS, experimental allergic encephalomyelitis (EAE). In this model, clinical signs and formation of perivascular infiltrates were completely abolished after the depletion of peripheral monocytes and macrophages.^{4,5}

MO are important in the earliest events in MS. Peripheral blood MO secrete prostaglandins before MS attacks. During clinical activity MO activation

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* Received for Publication: June 29, 2010

* Accepted: October 15, 2010

markers increase, and interleukin (IL)1 and tumor necrosis factor-alpha levels are elevated.⁶ Monocytes/macrophages secrete inflammatory and potentially cytopathic mediators such as TNF⁷ and cytokines.⁸ Patients with progressive MS have a high percentage of Interleukin 12 producing MO in the blood compared to normal individuals.⁹ Interleukin-12 is a major proinflammatory heterodimeric cytokine that may play a role in the pathogenesis of MS¹⁰ and of experimental autoimmune encephalomyelitis.¹¹ Regulation of MO inflammatory gene expression may be relevant to the pathogenesis of MS.¹² Blood-borne T cells and MO/macrophages also constitute the major cell types in the perivascular infiltrates characteristic for MS.¹³ Moreover, migration of T cells across membranes, representing the blood brain barrier is facilitated by monocytes.¹⁴

All these data indicate that MO may contribute to the pathological anatomical features observed in the CNS of patients with MS.⁵ The normal percentage of MO is about 4-5% in adult persons.¹⁶ There are not enough studies about the absolute count of peripheral MO in relapsing-remitting (RR) MS patients in the literature. The aim of this study was to determine levels of MO percentage in peripheral blood of MS patients during the attacks.

METHODOLOGY

We investigated peripheral blood from 28 patients aged between 18 and 52 years (18 female, 10 male) with definite RR-MS diagnosed according to the criteria of Mc Donald¹⁷ during exacerbation period and from 20 healthy control (10 female, 10 male) and 20 stroke subjects aged between 16 and 55 years (12 female, 8 male). Because the leukocytes in peripheral blood could be elevated as an inflammatory

response to any acute cerebral disease, we also assessed these parameters in a group of patients with acute cerebrovascular disease (CVD).

We detected the absolute MO count and the percentage of MO by full automatic blood counter machine (counter) and examined the blood smears of patients group and control group subjects. All of the MS patients were treated with prednisolone. However, the serum samples were obtained before corticosteroid therapy was started in each patient. None of the patients had relapses precipitated by febrile episodes.

We used student-t (independent) statistical test. P values less than 0.05 were considered as significant. Variance analysis technique (ANOVA) was used for identifying the difference between the genders and groups of patients with stroke in terms of the absolute count and percentage of monocyte, and as a result of variance analysis, DUNCAN multiple comparison test was used for dual comparison of the mean values of the groups.

RESULTS

There was not a statistical difference of age and sex between the patient and control groups ($p > 0.05$).

Monocyte counts in peripheral blood: The mean value of absolute MO count in MS patients, CVD and control groups were 606.67 ± 170.52 , 746.50 ± 414.76 and 360.00 ± 109.54 respectively (Table-I). There was no statistically significant difference between MS and CVD groups in terms of average of absolute monocyte count according to Duncan multiple comparison test. Absolute monocyte counts were, however, significantly increased in MS and CVD groups compared with control group ($P < 0.001$) (Figure-1).

Table-I: Statistical parameters of the value of monocyte count and percent in the groups of MS, CVD and the controls. Different letters (A or B) show statistically difference for mean values.

Parameters	Group	N	mean	SD	Minimum	Maximum
Monocyte count	MS	28	606.67 ^B	170.52	300.00	900.00
	CVD	20	746.50 ^B	414.76	230.00	1530.00
	Control	20	360.00 ^A	109.54	100.00	500.00
Percentage of monocyte	MS	28	8.34 ^B	2.61	3.90	14.00
	CVD	20	5.56 ^A	2.48	2.07	9.20
	Control	20	5.36 ^A	1.50	3.00	7.60
Monocytes (sum of square)	MS	28	24.38 ^B	3.55	17.32	30.00
	CVD	20	26.32 ^B	7.53	15.17	39.12
	Control	20	18.72 ^A	3.19	10.00	22.36

MS: multiple sclerosis, CVD: cerebrovascular disease
 Different letters (A or B) show statistically difference for mean values.

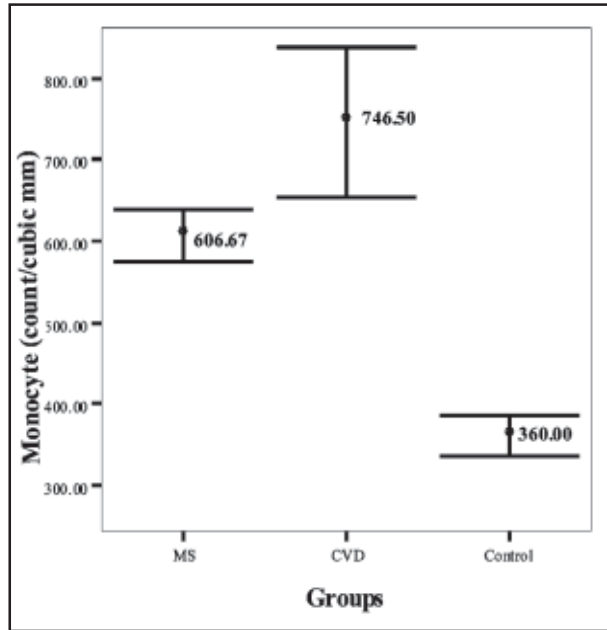


Fig-1: Monocyte count in the groups of MS, CVD and control.

MS: multiple sclerosis, CVD: cerebrovascular disease.

Monocyte percentage: The values of mean percentage of MO in MS patients, the patients with acute stroke and control group were $8.34 \pm 2.61\%$, $5.56 \pm 2.48\%$ and $5.36 \pm 1.50\%$ respectively. There was no significant difference between CVD group and controls in terms of mean percentage of monocyte in peripheral blood. The values of mean percentage of MO in MS patients was, however, significantly increased compared with the both groups of CVD and control ($P < 0.001$) (Figure-1).

DISCUSSION

The potential importance of blood MO in MS is suggested by their potent ability to secrete numerous immunoregulatory cytokines.¹⁵ Most of the studies describe the functional activity of MO in MS. Especially, the studies about EAE suggest that the infiltration of monocytes into the brain parenchyma is essential for the development of new lesions in EAE and MS. The exact mechanism by which monocytes cross the blood-brain barrier is largely unknown.^{9,10} In this study, we show that monocytes are increased in patients with MS during the acute phase, regardless of age and gender. The normal percentage of MO is about 4-5% in adult persons.¹⁶ In our study, absolute monocyte count in peripheral blood was elevated in the patients with stroke as well as in the MS group, whereas there was no increase in the value of MO percentage in stroke patients (5.56%) and controls

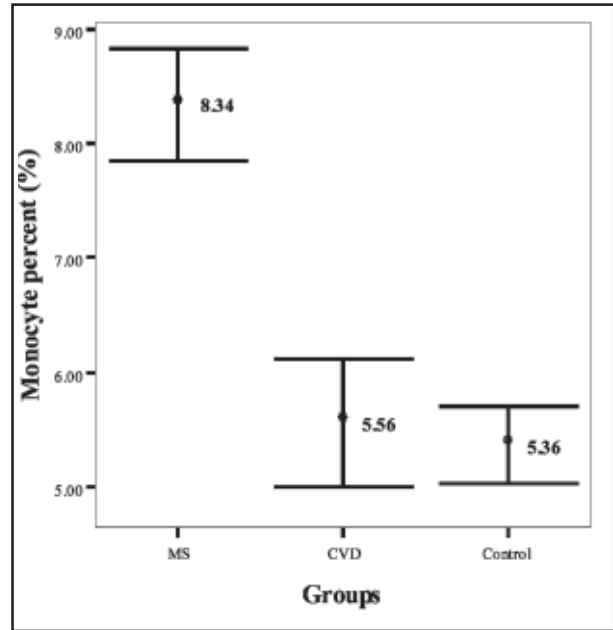


Fig-2: Monocyte percent in the groups of MS, CVD and control.

MS: multiple sclerosis, CVD: cerebrovascular disease.

(5.36%). The elevation of absolute count of monocyte in both groups of MS and CVD may be related to an inflammatory reaction during the acute phase of illness. However, the stroke patients did not show an elevation in monocyte ratio. Therefore, we suggest that such increase of absolute monocyte count and percentage may only be related to an immune-inflammatory process during the acute MS exacerbation.

In a study from Germany, the authors found that the percentage of HLA-DR (+)-monocytes was increased in female MS patients.¹⁸ Another study demonstrated that MO constitute the only peripheral blood cell population showing an increased burst activity in MS patients.¹⁹ A study from Sweden demonstrated that IL-15 positive peripheral blood mononuclear cells were elevated in patients with MS compared to healthy controls.²⁰ On the other hand, an information regarding IL-12 in MS reveals a link between MRI disease activity and elevated percentage of IL-12-producing monocytes.²¹

To conclude, in our study we think that the changes in the percentage of MO in MS exacerbations support the evidence for an immunoregulatory defect in this disease. Our results suggest a possible role of elevated rate of MO in the acute exacerbation of MS. This rate may indicate a high activity level of these immune cells in the course of the disease. However, further studies with on a much wider scale of series,

should be conducted in order to determine the relationship between the percentage of MO and the inflammatory cytokines in various clinical subgroups.

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