

## ORIGINAL ARTICLE

## QUANTITATIVE AND QUALITATIVE CHANGES IN LEUKOCYTES OF PSORIATIC PATIENTS

Mahesar SM, Mahesar H\*, Khand AA\*

Department of Biochemistry Liaquat University of Medical and Health Sciences, \*Department of Physiology University of Sindh Jamshoro, Pakistan

**Background:** Psoriasis is a disease concerned with inflammation and scaling of skin. In psoriasis, cells of the skin come on surface quickly before their complete maturation. In psoriatic patients, T-cells produce an abnormally large amount of toxic chemicals and cause inflammation. This study was undertaken to find out values of prognostic significance for worsening of the disease at early stage and to evaluate the changes (quantitative and qualitative) occurring in white blood cells of psoriatic patients. **Methods:** A total of 158 subjects, 79 psoriatic patients (44 males and 35 females) and same numbers of normal control volunteers were recruited. Total and Differential Leukocyte Counts (TLC and DLC) were determined. Morphological examination was also undertaken. All results of patients were compared with normal control volunteers. **Results:** In 47.7% male and 54.2% female patients TLC was higher than controls while variation in differential count was observed in 61.3% male and 62.8% female patients. Overall, neutrophils in 45% patients, basophils in 30.3%, eosinophils in 65.8%, and monocytes in 15% of patients were elevated. In 77.2% psoriatic patients, lymphocytes were decreased. In volunteers total and differential leukocyte counts were within normal range. Total leukocyte count in normal males was  $5,136 \pm 31$ , and in psoriatic male subjects it was  $10,498 \pm 43$ , and it was  $5,023 \pm 35$  against  $11,390 \pm 31$  in normal versus psoriatic females ( $p < 0.001$ ). **Conclusion:** Total leukocyte count was elevated in psoriatics while on DLC neutrophils, eosinophils and neutrophils were significantly raised where as lymphocytes were significantly decreased in psoriatic patients. Morphological changes were also noted.

**Keywords:** White blood cells, T-lymphocytes, Immunity, Psoriasis, Inflammatory disease, Total Leukocyte Count, Differential Leukocyte Count

## INTRODUCTION

Psoriasis is a chronic, auto-immune skin disorder with coetaneous manifestations. Association of several immunological abnormalities with psoriasis is documented.<sup>1</sup> Normally, for the movement of the skin cells from the site of origin to skin surface, about one month is required. In psoriasis, it may take only 3 to 6 days. There are several variations of psoriasis but the most common type is chronic plaque psoriasis that is characterised by red patches covered by a silvery, flaky scales.<sup>2</sup> It happens when skin cell division regulating factors are impaired that causes rapid proliferation of keratinocytes and result in the inflammation.

Leukocytes produce antibodies to foreign antigens.<sup>3,4</sup> When an antigen enters to the body, T-cells provide immune response by which T-cell activation results.<sup>5</sup> The activated T-cells begin overproducing cytokines and direct the B-cells to produce auto-antibodies. When blood vessels increase flow for nourishing this skin, it causes reddish inflammation. In the resulting autoimmune process, auto-antibodies destroy normal skin cells and if remain in circulation continue to mount an immune attack against these cells.<sup>6</sup>

Psoriatic plaques are highly resistant to fungal, bacterial and viral infections.<sup>7</sup> Development of psoriasis is combination of genetic and environmental factors.<sup>8</sup> Researchers have reported that psoriasis may be caused

by 9 gene mutations.<sup>9</sup> Mutations on genes cause certain cells to function differently.<sup>10</sup> It is demonstrated that psoriasis appears to be associated with an increased cardiovascular mortality and morbidity.<sup>11</sup> In psoriasis the Langerhan's cells are under the influence of cytokines relapsed by keratinocytes.<sup>12</sup> These cells migrate from the skin to nearby lymph nodes, where they interact with T-cells.<sup>1</sup> The most frequent extracutaneous medical problem in psoriasis is inflammatory bowel disorder.<sup>14</sup> Abnormal epidermal differentiation and hyper-proliferation in psoriatic patients is also documented.<sup>15,16</sup>

The inflammatory cell infiltrate may contain many neutrophils in the epidermis, but a more consistently finding is the presence of T-cells found in the epidermis and dermis accompanied by rise in number of dermal cells, mast cells and macrophages.<sup>17</sup>

Approximately 2–3% of the population worldwide is affected by psoriasis. It can prevail in any race, however, epidemiologic studies have shown 1.5–3% prevalence in western European and Scandinavian populations<sup>18</sup>, 2–3% in Caucasians<sup>19</sup>, about (4.8%) in Norway, 3% in United States, and the highest prevalence (12%) is reported in Arctic Kasach'y.<sup>20</sup> Lower rates have been reported among Japanese and Inuit populations.<sup>15</sup> In 10–30% of patients there can also be nail dystrophy accompanied by

psoriatic arthritis.<sup>21</sup> Psoriasis most commonly develops between of 15–25 years of age and it develops in both genders equally.<sup>22</sup>

The present study was carried out to find out values of prognostic significance for worsening of the disease at early stage and to evaluate the changes (quantitative and qualitative) occurring in white blood cells of psoriatic patients.

## MATERIAL AND METHODS

The samples were collected from 158 volunteers with age range 5–68 years. Psoriatic patients were 44 males and 35 females. Same number of normal controls was included. Indoor (Skin wards) and Outdoor patients (OPD) patients were recruited from Liaquat University of Medical and Health Sciences Hospital Hyderabad and Jamshoro. Controls were selected from amongst the normal family members of the psoriatic patients.

Laboratory work was conducted in the Department of Physiology, University of Sindh Jamshoro. Volunteers were divided into 3 age groups (5–15, 16–40 and 41–68 years). For determination of Total Leukocyte Count (TLC) and Differential Leukocyte Count (DLC), 5 ml of venous blood was taken from the antecubital vein. The TLC was performed on a Neubar's chamber, and DLC under oil immersion lense ( $\times 100$  power) after staining the fresh smear with Leishman's stain.<sup>24</sup>

## RESULTS

In 21 (47.7%) male patients TLC was higher than controls while variation in DLC was observed in 27 (61.3%) male patients. In 19 (54.2%) female patients higher TLC was observed, and in 22 (62.8%) variation in DLC was observed. In 36 (45%) patients neutrophils, in 24 (30.3%) basophils, in 12 (15%) monocytes, and in 52 (65.8%) patients eosinophils were elevated. In 61 (77.2%) psoriatic patients lymphocytes count was decreased. In volunteers total and differential leukocyte counts were within normal reference range (Table-1).

Mostly leukocytes were filled with toxic granulation while some of the polymorph nuclear leukocytes were hyper-segmented. Neutrophils and eosinophils were scattered and varied in size and shape. Lymphocytes showed increased size average 6–9  $\mu\text{m}$ .

**Table-1: Total and differential leukocyte count in control and psoriatic patients (Mean $\pm$ SD)**

Variable	Males		Females	
	Control (n=44)	Patients (n=44)	Control (n=35)	Patients (n=35)
TLC/mm <sup>3</sup>	5136 $\pm$ 31	10498 $\pm$ 43	5023 $\pm$ 35	11390 $\pm$ 31
Neutrophils (%)	56.8 $\pm$ 4.5	70.8 $\pm$ 3.4	59.7 $\pm$ 4.7	73.4 $\pm$ 4.8
Lymphocytes (%)	29.4 $\pm$ 2.4	19.6 $\pm$ 3.6	28.2 $\pm$ 3.4	27.4 $\pm$ 5.5
Eosinophils (%)	4.3 $\pm$ 0.05	8.5 $\pm$ 0.2	4 $\pm$ 0.2	8.2 $\pm$ 0.2
Monocytes (%)	3 $\pm$ 0.2	4 $\pm$ 0.1	3 $\pm$ 0.2	4 $\pm$ 0.1
Basophils (%)	0.3 $\pm$ 0.06	3.4 $\pm$ 0.05	0.4 $\pm$ 0.02	4.1 $\pm$ 0.07

## DISCUSSION

Lymphopenia is reported at the development of psoriasis in a study by Jeffrey *et al.*<sup>25</sup> Same authors have further commented that in initial stages of a pustular flare the leukocyte count raises to 25,000–40,000/mm<sup>3</sup>, the lymphocyte count drops from a normal level to absolute lymphopenia. Such 'lymphocyte eclipse', they believe, is a reliable sign of impending pustular activity. A significant decrease in T-lymphocyte count while no change in B-lymphocyte count was also observed in another study where the authors stated that decrease in T-cells was found to be directly proportional to the extent of skin involvement.<sup>26</sup>

Development of peripheral eosinophilia may suggest the significant roles of eosinophils in the pathogenesis of the psoriasis. However, it is revealed from a recent study that eosinophilia is not the complete criterion for diagnosing a disease or specific medical condition without considering signs or symptoms, other diagnostic investigations and case history.<sup>27</sup> Our findings are consistent with a study conducted in 1998, which found activation of monocytes in psoriatic patients and confirmed the overproduction of cytokines in peripheral monocytes.<sup>28</sup> Our findings are also consistent with results of Zheng and Mrowietz<sup>29</sup> who have reported the significant increase in the percentage of monocytes in psoriatic patients. They are of the view that the differential phenotype pattern of surface molecules on monocytes in psoriasis may reflect an abnormal monocyte maturation/differentiation state. This may explain the functional abnormalities of monocytes in psoriatic patients. It is reported that neutrophil activating factor mediates migration of monocytes, neutrophils and T-lymphocytes from the vascular endothelium to the epidermis.<sup>30</sup>

Increased adherence and chemotaxis of normal human polymorphonuclear cells by sera from psoriasis patients has been reported.<sup>31</sup> Higher TLC in psoriatic patients is also reported.<sup>32</sup> In their opinion such increase counts from higher neutrophil count.

In our study changes in size and shape of neutrophils, monocytes and eosinophils of psoriatic patients were observed. A significant increase in the percentage of monocytes in the differential count and differential phenotype pattern of surface molecules on monocytes in psoriatic patients is also reported earlier.<sup>29</sup>

Mostly leukocytes were filled with toxic granulation while some of the polymorph nuclear leukocytes were hyper-segmented. Neutrophils and eosinophils were scattered and varied in size and shape. Lymphocytes showed increased size average 6–9  $\mu\text{m}$ . This finding of present study is consistent with earlier reported results who have commented that most probably lymphocytes increase in size in inflammatory conditions.<sup>33,34</sup>

The activated lymphocytes migrate to epidermis and activate keratinisation process.<sup>35</sup> Neutrophil activating factor mediates migration of monocytes, neutrophils and T-lymphocytes from the vascular endothelium to the epidermis.<sup>30</sup>

Activation of T-cells is involved in interleukin enhancer binding factor (ILF) resulting in inflammatory proliferative characteristics of lesional skin.<sup>36</sup> Human lymphocyte and monocyte proliferation in psoriasis is also reported.<sup>36</sup> Biologics are defined as therapeutic agents produced by organisms through the use of recombinant biotechnology. Regarding pathogenesis of psoriasis several hypotheses are being proposed. Psoriatic plaque represents deregulated growth and inflammation that never develops into malignant clones of keratinocytes, melanocytes, or T-cells.<sup>37</sup> Early therapeutic strategies targeted the hyperplastic epidermis following the lead of oncologists to arrest keratinocyte growth using antiproliferative agents such as arsenic and later methotrexate. Other theories implicated psoriatic fibroblasts<sup>38</sup> neutrophils, mast cells, nerve cell endings, endothelial cells, T-lymphocytes<sup>24-39</sup> and specifically, clonal expansion of T-cells.<sup>34-40</sup> Researchers have observed high numbers of T-cells in psoriatic plaques.<sup>41</sup>

## CONCLUSION

We cannot draw conclusion at large owing to small number of samples, but statistically it can be concluded that peripheral blood eosinophilia seems to be associated with severe forms of psoriasis. This finding may suggest that the eosinophils have significant role in pathogenesis of these types of psoriasis.

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**Address for Correspondence:**

**Hidayatullah Mahesar**, Department of Physiology, University of Sindh, Jamshoro, Pakistan.

**Email:** hidayat\_mahesar@yahoo.com