

# Clinical study and assessment of leukocyte phagocytic function in children with atopic dermatitis in Qassim region of Saudi Arabia

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## ABSTRACT

**Objective:** Atopic dermatitis (AD) is a skin disorder clinically seen in the pediatric population. It is well recognized that patients with AD have an increased susceptibility to cutaneous colonization and infection with bacteria, fungi, and viruses. This study was undertaken to investigate the phagocytic activity and chemotactic response of mononuclear and polymorphonuclear leukocytes in severe AD patients.

**Methods:** A total of 50 children with severe AD were selected according to severity scoring of AD (the SCORAD index) and 30 healthy children of same age and sex were also selected as controls. The mononuclear and neutrophilic leukocytes were separated and the phagocytic ingestion of zymosan particles was determined. Migration distance in response to bacterial lipopolysaccharide chemotactic factor was also determined. Immunological disturbance in AD patients was determined by sandwich enzyme-linked immunosorbent assays for total serum immunoglobulin E (IgE), complement 3 (C3) C4.

**Results:** Of 50 AD patients with severe disease activity, 36 patients (72%) showed reduction in mononuclear and neutrophilic phagocytic activity. Children with AD had higher levels of total serum IgE, C3, and C4 compared to healthy children ( $P < 0.01$ ).

**Conclusions:** The study results demonstrated an inhibition in the chemotactic response and phagocytic activity by mononuclear and/or neutrophilic leukocytes in severe AD patients. We further observed an involvement of perturbed complement system in patients with AD. Hence, we clearly showed that AD is exacerbated with compromised immunological response, especially the innate immune response.

**Keywords:** Atopic dermatitis, chemotaxis, mononuclear leukocytes, neutrophils, phagocytic function

## Introduction

Atopic dermatitis (AD) is a chronic skin disorder with a high prevalence in developed countries and is more common in children as compared to adults.<sup>1,2</sup> Up to 95% of AD begins below the age of 5 years.<sup>3</sup> Although etiology of AD is not completely understood, immunologic, genetic, and environmental factors are reported to be involved.<sup>4,5</sup> It is well established that AD is associated with immune dysregulation and epidermal barrier dysfunction.<sup>6</sup> The imbalance of T helper type 2 (Th2) and Th1 pathways and their associated inflammatory mediators offer as one of the possible pathogenic mechanisms in AD.<sup>6,7</sup> Alterations in T-cells and their associated signaling events during the acute and chronic phases of AD may cause variations in disease presentation.<sup>6,7</sup> It is also reported that AD patients have an increased susceptibility to cutaneous colonization with bacteria, fungi, and viruses.<sup>6</sup>

Mononuclear and polymorphonuclear leukocytes participate in the innate defence, acting quickly against different agents. These leukocytes initially show chemotactic activity through migration toward the chemotactic factors and then to the area where the immune response takes place.<sup>8</sup> This further leads to phagocytosis of pathogenic organisms by leukocytes which includes ingestion and digestion of pathogenic organisms, with subsequent elimination of their inactivated products. Moreover, impairment of polymorphonuclear leukocytes reduces the defence against pyogenic bacteria such as streptococci, serratia, escherichia, pseudomonas, and aspergillus.<sup>8</sup> In case of mononuclear leukocytes disorders, infections involving intracellular organisms are more frequently observed, such as those caused by mycobacterium, candida, gypseum, tonsurans, and viruses in general.<sup>8</sup>

In this study, we investigated the phagocytic activity and chemotactic response of mononuclear and polymorphonuclear

leukocytes in 50 children with severe AD. We have also shown the abnormal functioning of immune response in AD patients, which further supports the dysfunctionality of AD patients' leukocytes.

## Methods

### Selection of AD patients

Fifty children diagnosed with AD from history and clinical examinations were selected from Qassim University pediatric and dermatology clinics. At the same time, normal children ( $n = 30$ ) of comparable age and sex who came for regular check-ups were also selected from the same clinics and were used as controls. The age of patients and controls ranged from 1 to 15 years. The inclusion criteria for patient's selection were: Patients recently diagnosed with AD with age range from 1 to 15 years and from Qassim region of Saudi Arabia. The exclusion criteria were patients with another comorbid skin disease or nonspecific dermatitis and patients with a history of prolonged use of systemic steroid therapy or other immunosuppressive therapy.

### Preparation of leukocytes for assessment of phagocytic activity

Using the standard procedures, neutrophils were prepared through spontaneous sedimentation at a temperature of 37°C, whereas mononuclear cells were prepared by the Ficoll-Hypaque gradient as described previously.<sup>9</sup> To evaluate phagocytic ingestion, a stock of  $2 \times 10^6$  cells/ml was prepared and following assays were performed in Leighton tubes:

1. PhagoPhagocytes were incubated with  $10^8$  particles of zymosan/ml;
2. Same concentration of phagocytes and zymosan were incubated with 200 ml of homologous serum pool (HS); and
3. Phagocytes, zymosan, and 200 ml of autologous serum (AS).

After 2 hours of incubation with 5% CO<sub>2</sub> at a temperature of 37°C, numbers of phagocytes presenting three or more phagocytic vacuoles were counted within a fixed number of 200 phagocytes.<sup>9</sup>

### Assessment of leukocytes chemotactic activity

For the assessment of chemotactic activity, the same method described above was used: Incubated phagocytes were treated with lipopolysaccharide. The chemotactic activity was determined according to the distance of cellular migration, measured in micrometers as described previously.<sup>9</sup>

### Enzyme-linked immunosorbent assays (ELISAs) for immune functions

Total immunoglobulin E (IgE) levels were measured in the serum samples by human IgE specific sandwich ELISA according to the manufacturers' instructions (cat. # 20783-

72876, Gen Way Biotech, CA, USA). Human complement 3 (C3) levels were measured in the serum samples by human C3 sandwich ELISA according to the manufacturers' instructions (cat. # LS-F4278-1, Life Span Bio Sciences, Washington, USA). Whereas, human C4 levels in the serum samples were measured by Human C4a Sandwich ELISA according to the manufacturers' instructions (cat. # LS-F26723-1, Life Span BioSciences, Washington, USA).

### Statistical analysis

Statistical analysis was performed using the SPSS program version 16.0 (SPSS Inc., Chicago, IL, USA) as described previously.<sup>10</sup> The comparisons were performed using one-way ANOVA analysis followed by Tukey's post-hoc analysis and the value of  $P < 0.05$  was considered statistically significant. Results are expressed as mean±standard deviation (SD).

## Results

### Demographic and hematologic characterization of AD patients

Children with AD ( $n = 50$ ) included 28 males and 22 females. Their mean age and SD was  $11.90 \pm 1.93$  years. Children in control group comprised of 30 healthy children (19 males, 11 females). Their mean age and SD was  $11.67 \pm 1.62$  years. There was no statistically significant difference in the demographic and hematologic characterization of AD patients and healthy controls ( $P > 0.05$ ) except eosinophil counts. There was the statistically significant difference in eosinophil counts ( $P < 0.05$ ) between the study group and controls. The complete demographic and hematologic characterization of AD patients and healthy controls are summarized in Table 1.

### Chemotaxis and phagocytosis of mononuclear cells and neutrophils in AD patients

As shown in Figure 1, there was a significant decrease in AD patients' mononuclear leukocytes' chemotaxis ( $P < 0.05$ )

**Table 1:** Demographic and hematologic characterization of AD patients and healthy controls

Parameters	AD patients ( $n=50$ )	Controls ( $n=30$ )	<i>P</i> value
Age (years)	11.90±1.93	11.67±1.62	0.581
Sex (M/F)	28M/22F	19M/11F	-
RBC ( $10^6/\mu\text{L}$ )	4.22±0.47	4.26±0.49	0.711
Hb (g/dL)	10.49±0.97	10.54±1.02	0.838
WBC ( $10^3/\mu\text{L}$ )	10.77±2.97	10.85±3.16	0.905
Platelets ( $10^3/\mu\text{L}$ )	$2.33 \times 10^2 \pm 46.7$	$2.33 \times 10^2 \pm 49.9$	0.976
Lymphocytes (%)	31.36±6.50	32.90±5.42	0.279
Monocytes (%)	4.02±1.94	3.43±1.63	0.170
Eosinophils (%)	7.26±5.16	3.12±2.42	0.000

n: Number, M: Males, F: Females, RBC: Red blood cells, Hb: Hemoglobin, WBC: White blood cells

and mononuclear phagocytosis ( $P < 0.05$ ) as compared to chemotaxis and phagocytosis of normal human mononuclear leukocytes. Moreover, neutrophils from AD patients also showed similar results as of mononuclear leukocytes. There was a significant decrease in AD patients' neutrophils' chemotaxis ( $P < 0.05$ ) and phagocytosis ( $P < 0.05$ ) as compared to the chemotaxis and phagocytosis of healthy children's neutrophils (Figure 2).

### Total serum, IgE and complement levels in AD patients

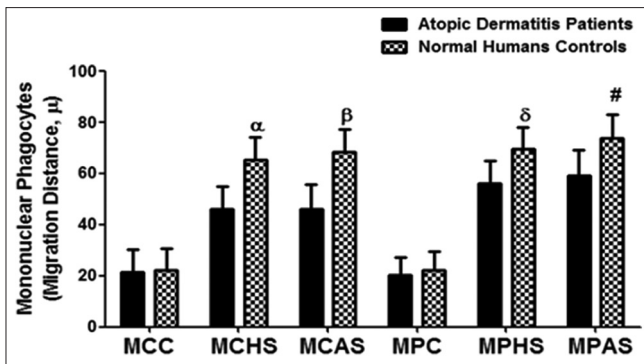
Total serum IgE levels were significantly higher in AD patients compared with healthy controls ( $P < 0.0001$ ). The average IgE

levels ( $\pm$ SD) in patients' sera and of healthy children were  $515.2 \pm 82.24$  and  $151.5 \pm 44.04$  IU/ml, respectively (Figure 3a). We also determined human C3 and C4 levels in same patients' groups and found that AD patients had significantly higher levels of C3 and C4 as compared to controls ( $P < 0.01$ ). The average human C3 levels ( $\pm$ SD) in patients' sera and of healthy children were  $266.2 \pm 54.64$  and  $123.9 \pm 45.57$  mg/dl, respectively (Figure 3b). The average human C4 levels ( $\pm$ SD) in patients' sera and of healthy controls were  $43.1 \pm 9.13$  and  $30.8 \pm 5.79$  mg/dl, respectively (Figure 3c).

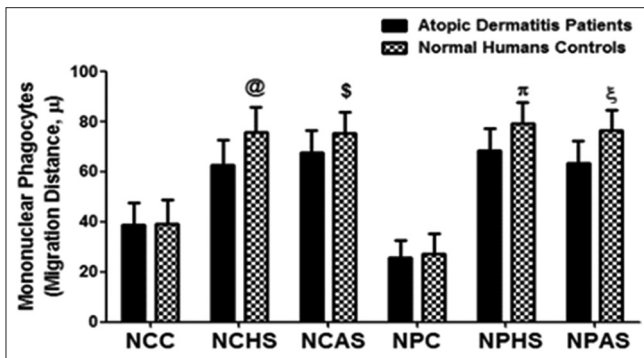
### Discussion

For the last decade, it is well known that AD is complicated by recurrent bacterial and viral infections that, when left untreated, can lead to significant complications. These microbial infections include *Staphylococcus aureus* skin infections, eczema herpeticum, eczema vaccinatum, and eczema coxsackium.<sup>11</sup> It is well established that the defects in skin barrier reduces the antimicrobial peptides activity and increases skin pH. Under such circumstances, Th2 cells become a potential contributing factor for the risk of skin infections in patients with AD.<sup>11,12</sup> Not only this, under such conditions, bacterial virulence (such as methicillin-resistant *S. aureus*) produces super-antigens that further increase these skin infections in AD patients.<sup>12</sup> It is also suggested that *Staphylococcus epidermidis* or other coagulase-negative staphylococci may play an important role in controlling *S. aureus* skin infections in AD. Furthermore, genetic variants in the innate immune response may predispose AD patients to increased risk of viral skin infections. A common staphylococcal toxin,  $\alpha$ -toxin, may also play a role in enhancing herpes simplex virus skin infections in AD.<sup>5,11-13</sup> Numerous cofactors, such as compromised skin barrier function, dysfunctionality of the immune system and complex genetic factors may contribute in the development of AD.<sup>4,14</sup> Within these multiple co-factors, macrophages play a key role in enhanced susceptibility to skin infections and act as an important factor in AD pathogenesis.<sup>14</sup> However, the exact mechanism of macrophages activation in AD pathogenesis is still not completely known.

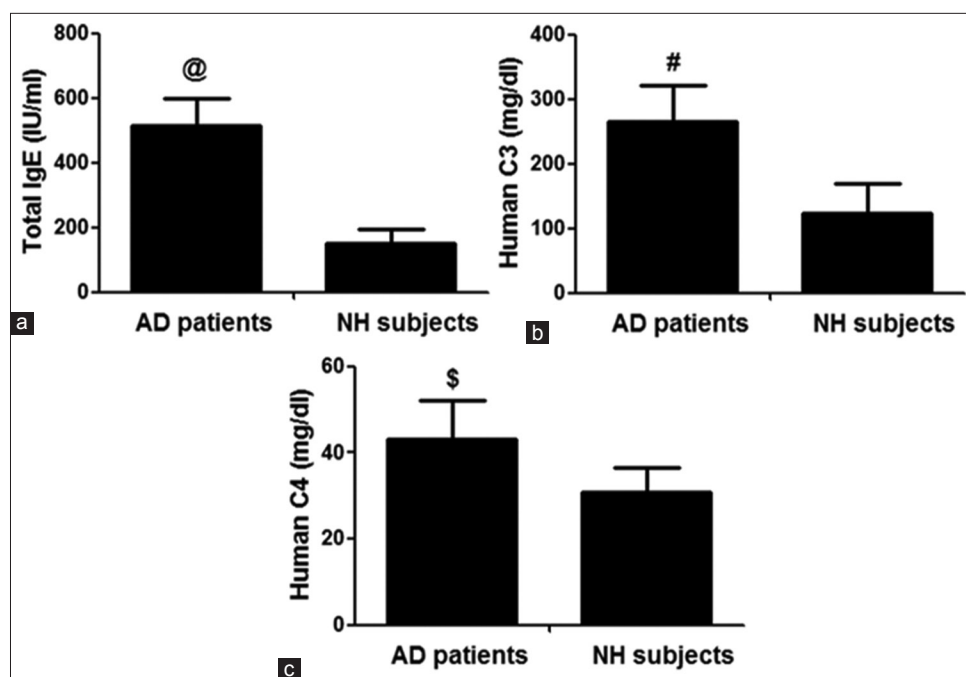
In an innate defense system, it is well documented that the mononuclear leukocytes and polymorphonuclear neutrophils participate in defense against infecting agents. These leukocytes initially show chemotactic migration toward the area of infection.<sup>9,15</sup> In AD patients, high frequency of infections indicates that there exist immune related disorders, possibly involving the modifications of these leukocytes. Forte *et al.*<sup>15</sup> showed a deficiency in the activity of mononuclear leukocytes, but only in five AD patients. In another study, Forte *et al.* showed that there was an inhibition in the phagocytic activity of mononuclear leukocytes and neutrophils derived from 19 AD patients, in which, 14 (73.68%) showed a reduction in the neutrophilic and mononuclear phagocytic activity, whereas two (1.53%) patients presented a reduction in the activity of both



**Figure 1:** Chemotaxis and phagocytosis of mononuclear leukocytes in atopic dermatitis (AD) patients and controls. Percentage of mononuclear leukocytes, out of a fixed number of 200, which showed phagocytosis of 3 or more zymosan particles. <sup>α</sup> $P < 0.05$  versus AD patients mononuclear-leukocytes chemotaxis homologous serum; <sup>β</sup> $P < 0.05$  versus AD patients mononuclear-leukocytes chemotaxis autologous serum; <sup>δ</sup> $P < 0.05$  versus AD patients mononuclear-leukocytes phagocytosis homologous serum; <sup>#</sup> $P < 0.05$  versus AD patients Mononuclear-leukocytes phagocytosis autologous serum. MCC: Mononuclear-leukocytes chemotaxis control; MPC: Mononuclear-leukocytes phagocytosis control



**Figure 2:** Chemotaxis of neutrophils in atopic dermatitis (AD) patients. Percentage of neutrophils, out of a fixed number of 200, which showed phagocytosis of 3 or more zymosan particles. <sup>@</sup> $P < 0.05$  versus AD patients neutrophils chemotaxis homologous serum; <sup>\$</sup> $P < 0.05$  versus AD patients neutrophils chemotaxis autologous serum; <sup>π</sup> $P < 0.05$  versus AD patients neutrophils phagocytosis homologous serum; <sup>ξ</sup> $P < 0.05$  versus AD patients neutrophils phagocytosis autologous serum. NCC: Neutrophils chemotaxis control; NPC: Neutrophils phagocytosis control



**Figure 3:** Immunological investigations in atopic dermatitis patients. Immunoglobulin E, @ $P < 0.0001$  versus normal human controls; complement 3, # $P < 0.001$  versus normal human controls; complement 4, \$ $P < 0.01$  versus normal human controls

phagocytes.<sup>9</sup> Melezyńska-Matej *et al.* reported decreased phagocytic activity in 12 AD patients out of 18 studied patients.<sup>16</sup> In the present study, we have investigated leukocytes' phagocytic activity in 50 severe AD patients. Our data demonstrated that there has been a reduction in the phagocytic activity of mononuclear leukocytes in AD patients compared with the mononuclear leukocytes of normal human controls. Neutrophils from our AD patients also showed a similar reduction in the phagocytic activity compared with the neutrophils obtained from healthy children.

The complement system plays a vital role in the innate defense against all commonly occurring pathogens. Activation of complement leads to robust and efficient proteolytic cascade, which results in the killing of pathogens as well as in the generation of the classical inflammatory regulatory responses through the production of pro-inflammatory mediators. Now it is well-established that complement is a functional bridge of innate immune response that allows a host to defend against pathogenic challenges.<sup>17</sup> Therefore, we measured C3 and C4 in our AD patients and compared them with the control group. Our data showed that both human C3 and C4 levels were significantly higher in our AD patients than healthy children in control group. These results further support our findings of phagocytic activity of leukocytes in our AD patients.

## Conclusion

Mononuclear and/or polymorphonuclear leukocytes from severe AD patients showed a reduction in chemotactic response

and phagocytic activity. This may link with the compromised complement system in patients with AD. Hence, in the present work, we clearly showed that AD is exacerbated with compromised immunological response, especially the innate immune response.

## Acknowledgments

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