*Original Article*

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# Evaluation of Oxidative Stress and Erythrocyte Properties in Children with Henoch-Shoenlein Purpura

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## Abstract

***Objective:*** Pathogenesis of Henoch-Schönlein purpura (HSP) is not clearly defined. The present study was conducted to investigate the alterations in erythrocyte deformability and oxidative stress in HSP and to examine the possible relationship between erythrocyte deformability and organ involvement in this disease.

***Methods:*** Plasma malondialdehyde (MDA) levels, total antioxidant status (TAS), erythrocyte deformability and aggregation were measured in 21 children with HSP at the disease onset and during the remission period in comparison with healthy subjects.

***Findings:*** HSP patients at the active stage had significantly higher MDA and lower TAS levels ($P$<0.05). Erythrocyte deformability was decreased at the active-stage and increased again at the remission period of HSP ($P$<0.05). Erythrocyte deformability was significantly decreased at four different shear stresses in patients with gastrointestinal system or renal involvement; and decreased at six different shear stresses in patients with gastrointestinal system, and renal involvement compared to the patients without organ involvement ($P$<0.05). No significant difference was observed in aggregation parameters ($P$>0.05).

***Conclusion:*** The present findings emphasize the association between impaired erythrocyte deformability and organ involvement in HSP.

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***Key Words:*** Erythrocyte Aggregation; Erythrocyte Deformability; Henoch-Schönlein Purpura; Malondialdehyde; Oxidative Stress

## Introduction

Henoch-Schönlein purpura (HSP) is the most common vasculitis of childhood characterized by involvement of skin, joints, gastrointestinal system and kidneys. Its clinical manifestations are due to generalized vasculitis including small vessels. Immunoglobulin A (IgA) mediated inflammatory process resulting from immune complex reaction is regarded as the basic pathology of HSP[1-3]. Immune complexes deposited on small vessels activate inflammatory cells including polymorphonuclear leukocytes, monocytes, macrophages and lead to production of reactive oxygen species (ROS), which provoke oxidative stress and lipid peroxidation[4]. ROS are produced in small amounts during oxygen metabolism and cleared away by antioxidant mechanisms. Increase of oxidants and decrease of antioxidants cause oxidative stress eventually causing tissue damage.

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HSP is one of such diseases where oxidative stress is important[4-8].

Blood flow, deformability and aggregability of red blood cells are the main components of hemorheology. In large blood vessels, a basic component is the blood flow. In microcirculation, where cells must deform to pass through narrow capillaries, deformability and aggregation of red blood cells (RBCs) are major determinants of resistance to flow. Ability of the entire RBC to deform is of crucial importance for performing its function of oxygen delivery and it is also a determinant of cell survival time in the circulation[9]. Products of lipid peroxidation change membrane functions by constituting cross-bonds between skeletal proteins of erythrocyte membrane and leading to polymerization of proteins, potassium outflow from cell and dehydration of erythrocytes. Exposure of RBCs to oxidative stress also has an influence on both the extent and strength of aggregation[10].

Erythrocyte deformability plays an important role in blood circulation; facilitates flow of 8 µm-diameter erythrocyte through 2-3 µm-diameter capillaries[11]. Although accumulating data in literature, shows alterations of RBC deformability and aggregation in diseases such as Behçet's disease, diabetes mellitus, hypertension, peripheral and coronary artery diseases[12-14], no study has yet evaluated possible changes of erythrocyte deformability and aggregation in HSP.

Therefore, the aims of the present study were; *i)* to examine the possible alterations in erythrocyte deformability and aggregation in HSP, *ii)* to observe the relationship between these alterations and oxidative stress indices and, *iii)* to investigate the association between erythrocyte deformability and organ involvement in HSP.

## Subjects and Methods

A total of 21 patients with HSP were investigated. The diagnosis of HSP was based on the criteria of the American College of Rheumatology[15]. All patients had characteristic purpura and at least one more organ involvement. None of the patients was receiving any drug known to interfere with erythrocyte properties and oxidant/antioxidant status on admission. Children who had received nonsteroidal anti-inflammatory drugs were excluded from the study. There was no other systemic disease, which could affect erythrocyte properties and oxidant/antioxidant status. The following laboratory investigations were performed: white blood cell (WBC), hemoglobin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), antistreptolysin O (ASO) titer, prothrombin time, activated partial thromboplastin time, fibrinogen, electrolytes, glucose, urea, creatinine, total protein, albumin, total cholesterol, triglycerides, IgA, complements 3 and 4 levels, rheumatoid factor (RF), antinuclear antibody (ANA), anti-double-stranded deoxy-ribonucleic acid (anti-DNA), throat culture, stool guaiac test and abdominal ultrasonography.

The patients were divided into subgroups according to the presence or absence of gastrointestinal system, joint, or renal involvements. Oral or intravenous prednisolone (1-2 mg/kg/day) was given to the patients with GIS, renal involvement. Renal biopsy was not performed in any patient. All patients exhibited uneventful and complete clinical recovery.

The control group consisted of 21 voluntary, age and gender matched healthy children with no history of hypertension, renal or cardiac diseases. Children in the control group had normal physical examination and biochemical analysis values. This study was approved by the local ethical committee. The study protocol was explained and written informed consent obtained.

Venous blood samples were collected twice from all patients: First, in the active stage at presentation of HSP disease and second, in the remission period when the clinical and laboratory signs of disease activation disappeared after 10-12 weeks. Erythrocyte deformability and aggregation were measured within 1-3 hours after blood collection. Plasma obtained by centrifugation was stored at -70$^0$C until laboratory analysis of oxidative stress parameters.

Lipid peroxidation levels were monitored by malondialdehyde (MDA). The measurement of MDA level, the end product of lipid peroxidation, is a well-known method to detect the rate of lipid peroxidation, which is induced by reactive oxygen species[16]. Serum MDA levels were estimated using thiobarbituric acid (TBA) method described

by Asakawa and Matsushita[16]. Serum MDA values were calculated using the extinction coefficient of MDA-TBA complex (532 nm=1.56×105 mol/cm) and expressed as nmol/ml. Plasma total antioxidant status (TAS) was determined by automatized colorimetric method developed by Erel et al[17]. The measurement of TAS is a reasonable way to assess the antioxidant system. The results were expressed as mmolTrolox eq/L and the precision of this assay is excellent, error being lower than 3%.

**Measurement of erythrocyte deformability:**

RBC deformability (i.e., the ability of entire cell to adapt a new configuration when subjected to applied mechanical forces) was determined by laser diffraction analysis using an ektacytometer (LORCA, RR Mechatronics; Hoorn, The Netherlands). The system has been described elsewhere in detail[18]. On the basis of the geometry of the elliptical diffraction pattern, an elongation index (EI) was calculated for 9 shear stresses between 0.3 and 30 pascal (Pa) as: EI = ($L$ -$W$)/($L$+$W$), where $L$ and $W$ are the length and width of the diffraction pattern. An increased EI at a given shear stress indicates greater cell deformation and hence greater RBC deformability. All measurements were carried out at 37°C.

**Measurement of the erythrocyte aggregation:**

RBC aggregation was also determined by LORCA as described elsewhere[19]. The measurement is based on the detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a computer-assisted system at 37˚C. Back-scattering data were evaluated by computer and aggregation index (AI), aggregation half time ($t_{1/2}$) which shows the kinetics of aggregation and amplitude (AMP) which is a measure for the total extent of aggregation were calculated on the basis that there is less light back-scattered from aggregating red cells. Hematocrit of the samples used for aggregation measurements was adjusted to 40% and blood was fully oxygenated.

**Statistical analysis:**

All statistical analyses were performed using Systat statistical software (version 16.0; SPSS Inc, Chicago, IL, USA). Student t-test or chi-square test was used for comparison of study groups and subgroups depending on data distribution pattern and sample size. Paired sample's t test was used to determine the differences between active and remission stages of HSP. Correlation between MDA and TAS was evaluated with Pearson's correlation test. Level of statistical significance was $P$<0.05.

## *Findings*

The mean age of the HSP patients and control group were 7.5±2.6 years (3-13.6) and 8.0±2.3 years (3.6-12), respectively. There were no significant differences between the groups in age or male-to-female ratio (8:13 and 9:12, respectively).

All patients had skin involvement and 17 (80%) joint involvements, 12 (55%) GIS involvement and 10 (45%) renal involvements. Nine (42%) patients had both renal and GIS involvements. At the onset of the disease, five (50%) of the ten patients with renal involvement had only microscopic hematuria, whereas 4 patients (40%) suffered from both microscopic hematuria and nephritic proteinuria and only one patient (10%) had nephritic proteinuria. Hypertension and impaired renal functions were not detected. All of the patients with GIS involvement had abdominal pain, five had vomiting, and four had GIS bleeding. All of the patients recovered on follow-up. The interval between symptoms and remission period was 56.2±13.1 (40-93) days.

WBC, CRP, and IgA levels were significantly higher, albumin level was significantly lower in active-stage HSP patients than in controls ($P$<0.05). WBC and CRP reduced to a level comparable to the control values in remission period ($P$<0.05). There were no significant differences between groups in terms of hemoglobin, glucose, total cholesterol, triglycerides and fibrinogen levels, which could affect erythrocyte deformability or aggregation ($P$>0.05) (Table 1). Decrease in complement 3, 4, and positivity of RF, ANA, Anti-DNA were not detected in patients. ASO was elevated in seven (33%) patients.

MDA levels of the HSP patients in active stage were significantly higher than those in remission

**Table 1:** Comparison of biochemical and oxidative stress parameters of the groups

| Variable | HSP active (n=21) | HSP remission (n=21) | Control (n=21) | *P.* value* | *P.* value† | *P.* value‡ |
|---|---|---|---|---|---|---|
| **Hemoglobin (g/dl)** | 12.7 (1.0) | 12.9 (0.8) | 12.7 (0.68) | NS | NS | NS |
| **WBC (x10³/mm³)** | 10.2 (2.7) | 8.5 (2.2) | 8.2 (2.8) | <0.05 | <0.05 | NS |
| **CRP (mg/dl)** | 1.19 (1.61) | 0.26 (0.76) | 0.21 (0.21) | <0.05 | <0.05 | NS |
| **ESR (mm/h)** | 20.9 (13) | 15.2 (5.3) | 18.5 (12.2) | NS | NS | NS |
| **Total protein (g/dl)** | 7.1 (0.6) | 7.3 (0.4) | 7.6 (0.4) | <0.05 | NS | <0.05 |
| **Albumin (g/dl)** | 4.2 (0.3) | 4.4 (0.2) | 4.6 (0.1) | <0.05 | <0.05 | <0.05 |
| **Glucose (mg/dl)** | 96.2 (10.6) | 95.5 (9.3) | 93.8 (7.4) | NS | NS | NS |
| **Creatinine (mg/dl)** | 0.45 (0.1) | 0.5 (0.1) | 0.4 (0.1) | NS | NS | NS |
| **Total cholesterol (mg/dl)** | 139.3 (22.5) | 144.7 (23.5) | 144.5 (27.4) | NS | NS | NS |
| **Triglyceride (mg/dl)** | 108.6 (58) | 90.3 (31) | 87.4 (30.4) | NS | NS | NS |
| **Fibrinogen (mg/dl)** | 379.4 (105.6) | 313.3 (83.2) | 335.4 (74.4) | NS | NS | NS |
| **IgA (mg/dl)** | 210 (95.5) | 179.5 (69.1) | 123.1 (50.3) | <0.05 | <0.05 | <0.05 |
| **MDA (nmol/ml)** | 9.3 (3.6) | 3.6 (1.9) | 5.8 (2.4) | <0.05 | <0.05 | <0.05 |
| **TAS (mmolTroloxEq/l)** | 1.285 (0.36) | 1.567 (0.45) | 1.681 (0.43) | <0.05 | <0.05 | >0.05 |

*: between the active stage of HSP patients and control group; †: between the active stage and remission period of HSP
‡: between the remission period of HSP and the control group; HSP: Henoch-Schönlein purpura, WBC: White Blood Cell, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate, MDA: Malondialdehyde, TAS: Total Antioxidant Status, NS: Non-significant

period and controls ($P$<0.05). TAS levels in the active-stage of HSP were lower than in controls and increased in the remission period ($P$<0.05). HSP group exhibited similar TAS levels in the remission period with the control group (Table 1). No significant difference was observed between MDA and TAS levels of the HSP patients with gastrointestinal, renal and joint involvement in both the active-stage and remission period. Positive correlation was detected between MDA and TAS levels of HSP patients in the remission period ($P$=0.05, $r$=0.456).

*Erythrocyte deformability indices:* RBC deformability (i.e., the elongation index EI) was reduced in all shear stresses except the lowest one (0.3 Pa) at the active-stage of HSP patients compared to healthy controls, the difference was significant for all shear stresses except 0.53 Pa ($P$<0.05). EI was higher in all shear stresses in the remission period than the active-stage values with significant differences for all shear stresses ranging from 0.53 to 9.49 Pa ($P$<0.05) (Table 2).

When the relationship between organ involvement and erythrocyte deformability was evaluated; EI of four different shear stresses of HSP patients with GIS involvement (3 Pa, 5.33 Pa, 9.49 Pa, 16.87 Pa), four different shear stresses of HSP patients with renal involvement (0.95 Pa, 1.69 Pa, 3 Pa, 5.33 Pa), and six different shear stresses of HSP patients with both GIS and renal involvement (0.95 Pa, 1.69 Pa, 3 Pa, 5.33 Pa, 9.49 Pa, 16.87 Pa) were significantly lower than those

**Table 2:** Comparison of the erythrocyte elongation indexes of the groups

| Shear stress (Pa) | HSP active (n=21) | HSP remission (n=21) | Control (n=21) | *P.* value* | *P.* value† | *P.* value‡ |
|---|---|---|---|---|---|---|
| 0.3 | 0.042 (0.01) | 0.047 (0.01) | 0.035 (0.007) | <0.05 | NS | <0.001 |
| 0.53 | 0.064 (0.01) | 0.074 (0.02) | 0.071 (0.01) | NS | <0.01 | NS |
| 0.95 | 0.141 (0.02) | 0.154 (0.03) | 0.156 (0.01) | <0.01 | <0.05 | NS |
| 1.69 | 0.242 (0.02) | 0.257 (0.03) | 0.262 (0.01) | <0.01 | <0.05 | NS |
| 3 | 0.343 (0.02) | 0.357 (0.03) | 0.366 (0.01) | <0.001 | <0.05 | NS |
| 5.33 | 0.429 (0.02) | 0.442 (0.03) | 0.454 (0.01) | <0.001 | <0.05 | NS |
| 9.49 | 0.496 (0.02) | 0.507 (0.02) | 0.519 (0.01) | <0.001 | <0.05 | NS |
| 16.87 | 0.555 (0.02) | 0.561 (0.02) | 0.572 (0.01) | <0.01 | NS | NS |
| 30 | 0.601 (0.01) | 0.602 (0.03) | 0.612 (0.009) | <0.05 | NS | NS |

*: between the active stage of HSP patients and control group; †: between the active stage and remission period of HSP
‡: between the remission period of HSP and the control group; HSP: Henoch-Schönlein purpura, NS: Non-significant

**Table 3:** Erythrocyte elongation indexes of HSP patients with organ involvements

| Gastrointestinal involvement | | | |
|---|---|---|---|
| Shear stress(Pa) | (+) n=12 | (-) n=9 | *P.* value |
| 0.3 | 0.044 (0.01) | 0.042 (0.08) | NS |
| 0.53 | 0.059 (0.01) | 0.070 (0.01) | NS |
| 0.95 | 0.134 (0.01) | 0.150 (0.01) | NS |
| 1.69 | 0.234 (0.02) | 0.252 (0.02) | NS |
| 3 | 0.334 (0.02) | 0.354 (0.02) | <0.05 |
| 5.33 | 0.421 (0.02) | 0.439 (0.01) | <0.05 |
| 9.49 | 0.488 (0.01) | 0.507 (0.02) | <0.05 |
| 16.87 | 0.548 (0.01) | 0.564 (0.02) | <0.05 |
| 30 | 0.596 (0.02) | 0.608 (0.01) | NS |
| **Renal involvement** | | | |
| Shear stress (Pa) | (+) n=10 | (-) n=11 | *P.* value |
| 0.30 | 0.042 (0.01) | 0.041 (0.09) | NS |
| 0.53 | 0.058 (0.01) | 0.069 (0.01) | NS |
| 0.95 | 0.131 (0.01) | 0.149 (0.01) | <0.05 |
| 1.69 | 0.231 (0.01) | 0.251 (0.02) | <0.05 |
| 3 | 0.331 (0.02) | 0.353 (0.01) | <0.05 |
| 5.33 | 0.418 (0.02) | 0.438 (0.01) | <0.05 |
| 9.49 | 0.486 (0.02) | 0.505 (0.01) | NS |
| 16.87 | 0.546 (0.02) | 0.562 (0.01) | NS |
| 30 | 0.595 (0.02) | 0.606 (0.01) | NS |
| **Renal and Gastrointestinal involvement** | | | |
| Shear stress (Pa) | (+) n=9 | (-) n=8 | *P.* value |
| 0.30 | 0.042 (0.01) | 0.042 (0.08) | NS |
| 0.53 | 0.057 (0.01) | 0.071 (0.01) | NS |
| 0.95 | 0.130 (0.01) | 0.153 (0.01) | <0.05 |
| 1.69 | 0.232 (0.02) | 0.256 (0.01) | <0.05 |
| 3 | 0.333 (0.02) | 0.359 (0.01) | <0.05 |
| 5.33 | 0.421 (0.02) | 0.445 (0.01) | <0.05 |
| 9.49 | 0.489 (0.02) | 0.512 (0.01) | <0.05 |
| 16.87 | 0.549 (0.02) | 0.570 (0.01) | <0.05 |
| 30 | 0.598 (0.02) | 0.612 (0.01) | NS |

NS: Non-significant

of the HSP patients with no organ involvement (P<0.05) (Table 3). These differences were not detected for joint involvement (*P*>0.05). $t_{1/2}$ were 25.7±4.5, 65.5±15.1, 2.84±4.5, respectively at the active stage of HSP. These parameters were 24.4±3.3, 66±7.1, 1.98±0.7 in the remission period and 25.6±2.9, 64.9±5.6, 2.1±.5 in the control group. No significant alterations were observed between groups in aggregation parameters (*P*>0.05).

## *Discussion*

HSP is a generalized systemic inflammatory vasculitis affecting small vessels. HSP has many different forms of pathogenesis and oxidative damage may have a role too[20]. Release of reactive oxygen species from the inflammatory cells provokes oxidative stress and causes tissue damage. Oxidative damage has been suggested to play a role in the pathogenesis of HSP as well as other inflammatory diseases[4-8].

In the present study, oxidative stress and erythrocyte deformability parameters were examined in HSP patients first in the active stage and then in the remission period in comparison with a healthy control group. Oxidative stress was increased and RBC deformability was decreased at different shear stresses in HSP patients in the active stage. Higher MDA and lower TAS levels were found in the active-stage of HSP compared to the control group. Considerable decreases in MDA and increases in TAS levels were detected in the

remission period. The higher levels of MDA in the active stage suggest that lipid peroxidation plays an important role in the pathogenesis of HSP and may explain the decrease of TAS as a result of antioxidant consumption at higher oxidant stress levels. Moreover, we found a positive correlation between TAS and MDA levels in the remission period. This finding may be an evidence for the compensatory response of the antioxidant system to increased oxidative stress at lower oxidant stress levels. The present findings are in line with those of previous clinical studies evaluating role of oxidative stress in the pathogenesis of HSP[4-8].

HSP is spontaneously resolved within 4 weeks; however, severe GIS and renal involvement are the most important causes of morbidity and mortality[1]. Therefore, detection of predictive markers of GIS and renal involvement is very important for timely treatment and maintenance. Demircin et al[5] reported increased MDA levels in HSP patients with renal involvement. The present findings are parallel to those studies[6,7] reporting no differences in oxidant/antioxidant status in HSP patients in regards with organ involvement.

Oxidative stress can damage many cell and tissue types, with RBCs being one of the most susceptible cells. Products released during lipid peroxidation are known to decrease erythrocyte deformability by polymerization of membrane proteins such as spectrin and constituting cross-bonds between the proteins[21]. Erythrocyte deformability reduces as a consequence of these changes[22]. Previous studies suggested that an increase in oxidative stress causes reduction in erythrocyte deformability[22,23]. Usküdar et al[14] reported decreased erythrocyte deformability in Behçet's disease, which is a systemic inflammatory vasculitis. Erythrocyte deformability is a major determinant of microcirculation and blood viscosity. To our knowledge, erythrocyte deformability in HSP patients has not been evaluated before. In the present study, we investigated the mean EI value of erythrocytes in HSP patients, not only in active-stage but also in remission period, at nine different shear stresses ranging between 0.3 to 30 Pa. Our findings suggest a decrease in erythrocyte deformability in the active-stage of patients and an increase in the remission period.

Early diagnosis of organ involvement in HSP patients, particularly predictive markers of GIS and renal involvement causing morbidity and mortality is of the most importance for the successful treatment and maintenance[1]. Onset age ≥7 years, relapse, persistent abdominal pain and persistent purpura over 1 month, factor XIII activity <80% of the normal range and central nervous system involvement are risk factors of renal involvement[24,25]. The present study revealed significantly reduced erythrocyte deformability at four different shear stresses in HSP patients with GIS or renal involvement. On the other hand, erythrocyte deformability was significantly reduced at six different shear stresses in the HSP patients with GIS and renal involvement. These findings suggest that reduced erythrocyte deformability may increase the risk for GIS and renal involvement through the hemorheological changes especially in small vessels.

RBC aggregation is the reversible adhesion of adjacent erythrocytes. The physiological importance of RBC aggregation in circulation is its tendency to alter the local blood viscosity in low shear flow and to disturb the passage in capillary circulation through the formation of sludge. Plasma fibrinogen concentration is one of the major determinants of erythrocyte aggregation[26]. The present findings did not reveal significant alterations in erythrocyte aggregation and plasma fibrinogen concentration.

## Conclusion

The present findings suggest an association between erythrocyte deformability and oxidative stress in HSP and to our knowledge; this is the first study in the literature. RBC deformability may be used in follow-up of HSP patients. RBC deformability has a potential to be used as a new predictive risk factor to determine organ involvement in HSP. Further studies with larger number of patients with serious organ involvement are warranted to better clarify this issue.

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### *Authors Contribution*

D. Gürses and N. Parlaz: Concept / Design
N. Parlaz, G. Erken: Acquisition of Data
D. Gürses, M. Bor-Küçükatay and V. Küçükatay: Data Analysis / Interpretation
D. Gürses and N. Parlaz: Manuscript Preparation
D. Gürses, M. Bor-Küçükatay and V. Küçükatay: Critical Revision of the Manuscript
All authors approved final version of the paper.

***Conflict of Interest:*** None

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