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Effect of stress on erythryocytic osmotic fragility in healthy individuals

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Abstract

Background: The aim of the study was to examine relationship between severity of stress and onset/completion of erythrocyte haemolysis in normal healthy individuals.

Materials and Methods: The study was conducted in Physiology department, Deccan College of Medical Sciences, Hyderabad, after approval from the Institutional Review Board. The study included 50 healthy individuals of both the sexes aged between 20 to 50 years. Individuals working for minimum 8 to 10 hours a day were included and individuals suffering from hypertension, obesity, diabetes mellitus, musculo-skeletal disorders, sickle cell disease and goitre were excluded. Osmotic fragility was determined by collecting 2ml of blood from each individual and stored in EDTA tubes. A drop of blood was added to saline solutions of different tonicity arranged in 12 test tubes. The test tubes were seen for onset and end of hemolysis after an hour.
Stress was measured by Cohen's perceived stress questionnaire and individual were divided into 3 groups viz. low, moderate and high stress categories as per their scores.

Results: Findings were analysed by student's t-test and completion of hemolysis was earlier in severe stress group compared to mild and moderate. There is no significant difference between males and females regarding point of onset of hemolysis (p=0.41) and end of hemolysis (p=0.37). Stress increases fragility and it was found that RBC fragility was significantly related to severity of stress. Fragility is not affected by gender.

Conclusion: RBC fragility was significantly related to severity of stress and it was found that fragility is not affected by gender.

Keywords: Osmotic Fragility, Erythrocyte, Hemolysis, Stress, Cohen's Stress Scale.

Introduction

The erythrocyte or red blood cell (RBC) membrane accounts for 1 percent of total weight of the red cell, yet it plays an important role in the maintenance of erythrocyte integrity. The red cell membrane and its skeleton provide the erythrocyte the flexibility, durability, and tensile strength. It maintains a non-reactive exterior so that erythrocytes do not adhere to endothelial cells or aggregate and occlude the microcirculation.(1) The unique non-nucleated erythrocyte has an important and distinguishing feature of undergoing passive deformations during repeated passage through the narrow capillaries of microvasculature whose diameter is smaller than RBC itself, throughout its 120 day life span. RBC deformability is related to RBC osmotic fragility (Clark et al., 1983). The degree of squeezing of RBC depend mainly on membrane elasticity. Therefore, the decrease of the RBC membrane elasticity will led to the increase of the blood capillary resistance for RBC passage.(9)

Stress is one of the important cause of disease in our modern world. Every challenge to the mind and body creates a demand on the adrenal glands, creating stress. As a result adrenal glands produce high cortisol and place us into a "fight or flight" syndrome in which RBCs have to play an important role Stress effects deformability and fragility of RBCs which in turn affects macro and micro circulation. Literature is scant regarding studies about effect of stress on fragility; therefore we have undertaken this brief study.

The present study was planned to explain the relationship between osmotic fragility of an erythrocyte and severity of stress in healthy individuals. Osmotic fragility determines the rate of hemolysis of erythrocytes.

The osmotic fragility test is for measuring erythrocyte resistance to haemolysis when exposed to varying levels of dilution of a saline solution. When erythrocytes are exposed to a hypotonic environment, water enters the cell and causes swelling and then lysis. The classic osmotic fragility test was originally described by Parpart(7) et al (1947) and involves a series of solutions which are prepared with tonicity ranging from 0.1%-0.9%. Small amount of fresh blood is added to each tube. Tonicity at which onset of hemolysis and end of hemolysis occur are noted.

The osmotic fragility depends upon the ratio between surface area and volume of erythrocyte. In a disease such as hereditary spherocytosis, erythrocytes have a smaller ratio of surface area to volume and are thus more susceptible to osmotic stress, as opposed to the increased resistance characteristic of thalassemia, iron deficiency anemia, or any other condition that would cause an increased surface area to volume ratio.(4)

Materials and Methods

The study was conducted in Physiology department, Deccan College of Medical Sciences, Hyderabad.

The study included 50 healthy volunteers of both sexes, aged between 20 to 50 years. Study was approved by Institutional Review Board and written informed consent was taken from each volunteer.

Inclusion criteria: Individuals selected were not suffering from any ailments and leading a normal healthy life style and working for minimum 8 to 10 hours a day.

Exclusion criteria: Individuals not suffering from diseases like hypertension, obesity, diabetes, mellitus, musculo-skeletal disorders, sickle cell disease and goitre.

Anthropometric clinical measures: Bodyweight, height, body mass index and Blood pressure were note.

Sample collection: Blood samples of individuals were collected after minimum work schedule of 8 to 10 hours a day. 2 ml of blood was collected under aseptic precaution from cubital vein from each subject and stored in EDTA tubes. RBC osmotic fragility was performed as described by Parpart[7] et al.

Method: For assessing osmotic fragility, 12 tubes are arranged in the rack, with decreasing tonicity from left to right. Different concentration saline solutions are prepared by mixing the required number of drops of 1% NaCl solution and distilled water. A drop of blood was added to each tube, mixed with the saline and tubes were allowed to stand for an hour without shaking. The results were read as follows:

1) Test tubes in which no hemolysis has occurred, RBC will be seen as a red drop,
2) Proceeding from left to right 1st tube with some hemolysis indicating onset of haemolysis, saline will be tinged red with hemoglobin and RBCs as drop at the bottom of the tube,
3) Further proceeding towards right the test tube in which hemolysis is complete, saline will be uniformly red with no red mass at the bottom. The osmotic fragility test is useful because it is inexpensive and causes minimum discomfort to the subject.[2]

In normal healthy individuals osmotic hemolysis begins at about 0.48% and ends at about 0.36% NaCl solution. In spherocytosis emolysis may begin early at about 0.7% and end at 0.45%. In thalassemia hemolysis is late and values may be as low as 0.36% and 0.24% respectively.[2]

Stress was measured by Cohen's perceived stress questionnaire[3] which was given to each subject after collection of blood sample, questionnaire had a set of 10 questions and stress scores were categorized as follows:

1) 1-13: average stress
2) 13-20: medium stress
3) > 20: high stress

Statistical methods: Data is presented as mean and standard deviation (mean±SD). Means are compared between groups by student 't' test. A p value of < 0.05 was considered statically significant. Statistical analysis was carried out in the present study by using SPSS 17 version software.

Results

Table 1: Male Female Ratio

Gender	Number of cases (50)	Percentage	Stress score
Female	29	58.0	17.24±6.33
Male	21	42.0	17.48±4.31

Table 1 shows the ratio between male and females and the stress scores were found to be similar in both the sexes (*p*=0.87).

Table 2: Effect of gender and stress on fragility (Mean values)

	Stress score	Onset of hemolysis	End of hemolysis
Female	17.24±6.33	0.49±0.04	0.38±0.31
Male	17.48±4.31	0.48±0.25	0.38±0.32
p	0.87	0.41	0.37

There was no significant difference between males and females regarding point of onset of hemolysis (*p*=0.41) and also end of haemolysis (*p*=0.37).

Table 3: Stress groups

	Cohen's score	N
Mild	10.72	11
Moderate	16.89	27
Severe	24.42	12

The group of moderate stress was dominating with 54% cases.

Table 4: Stress and onset of hemolysis

	Mild	Moderate	Severe
N	11	27	12
Mean stress score	10.73±3.74	16.89±2.22	24.42±3.53
Mean tonicity	0.48±0.03	0.48±0.02	0.51±0.04
r	-0.41	0.28	0.82
p	<0.005	<0.005	<0.005

- Onset of hemolysis is earlier in severe stress group and correlates well with the stress score (*r*=0.82).
- There is inverse correlation between mild stress group and hemolysis of erythrocytes.

Table 5: Stress and end of hemolysis

	Mild	Moderate	Severe
N	11	27	12
Mean stress score	10.73±3.74	16.89±2.22	24.42±3.53
Mean tonicity	0.38±0.03	0.37±0.02	0.40±0.04
R	-0.66	0.26	0.56
R	<0.005	<0.005	<0.005

- Stress increases fragility.
- Completion of hemolysis was earlier in severe stress group compared to mild and moderate and there is an inverse relationship between mild stress group and end of fragility of erythrocytes.

Table 6: Range between onset and end of hemolysis

	Mild	Moderate	Severe
Onset	0.48+/-0.03	0.48+/-0.02	0.51+/-0.04
End	0.38+/-0.03	0.37+/-0.02	0.40+/-0.04
Range	0.1	0.11	0.11

The range between onset and end of haemolysis was similar in all the three stress groups.

Discussion

Stress effects the RBC fragility by several mechanisms e.g. hormonal (cortisol and catecholamines), metabolic free radicals) and circulatory (vasoconstriction, etc.). In the present study we determined the osmotic fragility of the RBCs in different stress groups i.e. mild, moderate and severe. We found that the group of moderate stress is dominating with 54% cases, which reveals that majority of the population may be under moderate stress regularly in day to day life.

Stress is actually a normal part of life. At times, it serves a useful purpose. Stress can motivate you to get that promotion at work, or run the last mile of a marathon. But if you don't get a handle on your stress and it becomes long-term, it can seriously interfere with your job, family life, and health.

In our study we also found that stress scores were similar in males and females (p=0.87). This is surprising as there goes a common belief that stress affects men more than females. There is no significant difference between males and females regarding point of onset of haemolysis (p=0.41) as well as end of haemolysis (p=0.37).

Regarding the effect of stress on the fragility it was found that stress increases fragility but the effects are different according to the level of stress. The onset and completion of haemolysis is earlier in severe stress group and there is a good correlation between the stress score and onset of fragility. Cortisol and epinephrine are released due to stress which causes the liver to produce more glucose. The released blood sugar levels may cause peroxidation of unsaturated lipids of membrane which can result in the inactivation of enzymes and cross linking of membrane lipids increasing fragility and cellular lysis of red blood cells and untimely cell death. The stress due to high glucose concentration causes damage to the erythrocyte membrane proteins, even in relatively short exposure times. Sometimes mild metabolic stress may cause greater increase in osmotic fragility in erythrocytes.[(8)] Thus, in this study we have another interesting point that in mild stress group though the fragility is increased but there is a negative correlation between the stress score and onset as well as end of haemolysis. The range between onset and completion of haemolysis is similar in all the three groups.

Stress factors have been shown to impair activity of antioxidant ascorbic acid[(6)] and depletion of some other antioxidant could increase the vulnerability of tissues and cellular components to reactive oxidation.

All the above points indicate that the gender effect is not important in determining the fragility but effect of stress score is significant.

Everyone has different stress triggers. Work stress tops the list, according to surveys. Forty percent of U.S. workers admit to experiencing office stress, and one-quarter say work is the biggest source of stress in their lives.[(5)]

Causes of work stress include:

- Being unhappy in your job
- Having a heavy workload or too much responsibility
- Working long hours
- Having poor management, unclear expectations of your work, or no say in the decision-making process
- Working under dangerous conditions
- Being insecure about your chance for advancement or risk of termination
- Having to give speeches in front of colleagues
- Facing discrimination or harassment at work, especially if your company isn't supportive

Other examples of life stresses are:

- **Family:** The death of a loved one, Divorce, Getting married
- **Financial:** Low income, extravagancy, high expenditure etc.
- **Medical:** Chronic illness or injury, Emotional problems (depression, anxiety, anger, grief, guilt, low self-esteem),
- **Traumatic events:** Such as a natural disaster, theft, rape, or violence against you or a loved one

There are some potential limitations of our study:

1. First the sample size is limited. A more large scale survey is likely to be more accurate with little bias.
2. A more comprehensive grouping as per type of the stress and its relation to fragility will be more useful.
3. Determination of blood indices and correlation with fragility will help for determining the effect of cell size on hemolysis.

Conclusion

RBC fragility is significantly related to severity of stress. Fragility is not related to gender. Moderate stress is dominating in the sample population. Stress though effecting the onset and completion of hemolysis, but it doesn't affect the range between onset and completion of hemolysis. High stress groups must consider stress reduction techniques because high psychological stress is associated with hematological disturbances and is an important risk factor for cardiovascular diseases.

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