International Journal of Research in Medical Sciences
Dervišević E et al. Int J Res Med Sci. 2022 Jun;10(6):1225-1231
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20221476

Original Research Article

Poikilocytotic forms caused by hyperthermia and heat stroke-experimental study on Wistar rats

Emina Dervišević[1]*, Muhamed Katica[2], Sabaheta Hasić[3], Anes Jogunčić[4], Lejla Dervišević[5], Haris Vukas[6], Adis Salihbegović[7]

[1]Department of Forensic Medicine, [2]Department of Pathuphysiological Physiology of Domestic Animals, [3]Department of Medicinal Biochemistry, [5]Department of Anatomy, [6]Department of Forensic Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
[4]Department of Epidemiology, Public Health Institute of Canton Sarajevo, Sarajevo, Bosnia and Herzegovina
[7]Department of Vascular surgery, Cantonal Hospital Zenica, Zenica, Bosnia and Herzegovina

Received: 28 March 2022
Accepted: 28 April 2022

***Correspondence:**
Dr. Emina Dervišević,
E-mail: emina.dervisevic@mf.unsa.ba

ABSTRACT

Background: The aim of the study was to find out what happens to erythrocytes and their forms during life and after death as a result of high water temperature.
Methods: Heat stress was used on a rat model.to investigate the effects of different temperature intensities (37°C and 44°C) and exposure time (20 min and until the time of death) on erythrocyte morphology. Total of 23 Wistar rats were divided into two groups: 37°C as control group and 44°C as trial groups. The trial groups were classified into antemortem the exposure time of 20 min and postmortem groups exposure time until fatal outcome. The anaesthetised rats were exposed to preheated water using the water bath. May-Grünwald-Giemsa colouring technique was applied on blood samples taken from the abdominal aorta.
Results: Exposure of Wistar rats to water temperature in groups KG37 and G44 led to a significant changes in core temperature. In the control group, the thermoregulatory mechanism established normothermia, and in G44 hyperthermia was detected during 20 minutes of exposure. The frequency of heat stroke in group G44 was 43.8%. Target cells and anulocytes were predominant in antemortem group at 44°C, while anulocytes and spherocytes in postmortem groups 44°C, respectively. Dacryocytes with spherocytes were significantly higher in postmortem group 44°C than in antemortem group 44°C (p=0.002, p=0.017, respectively).
Conclusions: Poikilocytosis is associated with the exposure length and temperature intensity. Following a fatal outcome dacryocytes with spherocytes at 44°C were significantly more than in corresponding antemortem groups.

Keywords: Antemortem, Experimental, Heat stress, Poikilocytosis, Postmortem, Rats

INTRODUCTION

Poikilocytosis is a major deviation from the normal shape of erythrocytes that is evidential of an increased rate of blood destruction.[1] Identification of poikilocytes is an important part of blood smear evaluation because shape changes often are associated with specific diseases, providing clues to underlying pathogenesis and facilitating diagnosis and treatment. It is particularly striking in pernicious anemia and some of the hemolytic anemias, but is also characteristically found in a variety of other conditions, such as environmental stress.[2] It has been realized that the erythrocytes in vivo does not always behave like a perfect osmometer during periods of physiological stress. In normal red blood cells, the biconcave disc shape creates an advantageous surface

area volume relationship, allowing red cells to undergo marked deformation while keeping a constant surface area. Directly or indirectly, membrane integral and skeletal proteins band, actin and spectrin as well as protein interaction play an important role in the regulation of factors that influence cellular deformability.[3] It is evident that there are unfavorable endogenous and exogenous factors that can in certain circumstances alter the original biconcave form of mammalian erythrocytes and thus partially or completely disable its physiological role in gas exchange. Adverse effects of external factors on the shape of erythrocytes are known in the literature, such as toxic effects of aluminum compounds, deficient nutrition with microelements, implant placement during surgical interventions.[4-6] The presence of abnormally shaped erythrocytes is a significant part of blood smear assessment, as shape changes are often associated with specific diseases, providing traces in the background of pathogenesis and facilitating diagnosis and treatment, and in forensics the cause of death. Poikilocytes may be the result of biochemical changes, erythrocyte damage: which shortens the lifespan of erythrocytes and ultimately contributes to anemia.[7-9]

In the world, an increasing number of deaths are caused by hyperthermia and often require forensic expertise. The incidence of heat-related diseases is increasing, and heat stroke is the most severe form associated with mortality and morbidity. Although the pathogenesis of heat stroke is not fully understood, inflammatory cytokines and related proteins, especially heat shock proteins, play a significant role in mediating responses, heat tolerance, and outcomes.

Abnormal core temperature deviations of even a few degrees will trigger the body's thermoregulatory mechanisms, and temperature changes outside the physiological range can prove fatal. Measured body temperature above 42°C leads to cytotoxicity with protein denaturation and impaired deoxyribonucleic acid (DNA) synthesis, resulting in organ failure and neuronal damage.[10]

The physiological body temperature of rats was 35.9-37.5°C.[11]

The body temperature of 40.9°C is the upper limit before the compensating mechanisms are switched on. Studies showed that animal model for inducing rat hyperthermia was comparable to the clinical situation.[12] The model has been shown to be useful for studying the effects of diseases associated with exposure to high ambient temperatures on changes in various organs and systems.

The aim of the present investigation was to study the effects of length and temperature intensity on occurrence of poikilocytes in blood during antemortem and postmortem analysis on Wistar rats.

METHODS

A study has been conducted at Medical faculty Univeristy of Sarajevu, between 04th and 18th May 2020. After animal care Ethics committee approval of the Medical faculty University of Sarajevo (registration number 02-3-4-1253/20), 23 adult albino Wistar rats, both sexes (300-350 g, 3 months old) were used in prospective, controlled, randomized experimental designed type of the study. Animals were housed in central animal care facility under optimal environmental conditions, with 12:12 dark light cycle. Food and water were provided ad libitum. Animals were monitored for appropriate 7 days acclimatization prior to the experiment. The study was conducted in accordance with the Principles of Laboratory Animal Care.[13]

Animals were randomly divided into two groups depending on temperature that they were exposed: control group (n=7) exposed to 37ºC, trial group (n=16) exposed to 44ºC. The trial group was further subdivided due to time of analysis as antemortem group (n=8) with exposure time of 20 minutes and postmortem group (n=8) with exposure until time of death.

On the day of experiment all animals were anesthetized anesthetized with ketamine (1.2 ml/1 kg body weight ±10%) and fixed on a wooden board with the placement of a probe that measured core temperature as evidence of hyperthermia. They were then immersed in a water bath, set temperatures, to chin level, and the temperature was read on a thermometer (Physitemp Thermalert Model TH-8). After the expiration of the given time or death, blood was taken from the abdominal aorta on two slides per each sample and then working materials prepared for microscopy were made. Blood smears were made according to the usual laboratory procedure.[14]

Experimental protocol was performed for each anaesthetized rats sequentially. On each original, stained smear, 2000 erythrocytes were analyzed using a Motic Type 102M light microscope, with a magnification of 1000 X. Poikilocytes were recorded relying on standard morphology, and counting was limited to representative single-layer visual fields, where blood corpuscles did not overlap. Fields on two microscopes were analyzed by two independent researchers and then the mean was calculated. The most representative fields of view are stored in electronic form using the computer software Motic Images Plus 2.0.

The number and type of poikilocytes is expressed as a percentage for each morphological form of red blood cells. Poikilocytosis was classified semiquantitatively according to similar studies, following criteria: non-existent (0%), rare (>0.05-0.5%), mild (>0.5-3%), moderate (>3-10%), or expressed (>10%).[15] The normality of the distribution of poikilocyte data was tested by histogram and Shapiro-Wilk test. The Mann Whitney test was used to test the differences between the

two groups, with incorrect data distribution, while the Kruskal Walis test was used to compare three or more groups. Category variables are represented by frequency as an absolute number and/or in percentages. The accepted level of significance of the difference is p<0.05. IBM Statistical package for social sciences (SPSS) computer program (SPSS; statistical package for Social Sciences) version 25.0 and Excel package MS Office 2016 were used for statistical analysis.

RESULTS

the basal temperature of the rats was 38.25±0.12°C and that it decreased to 37.75±0.38°C after 20 minutes of water exposure of 37°C, p=0.044 (Table 1).

Table 1: Mean values of the rats body temperature from repeated measurements in the control group.

KG37				
T(°C)	X	±SD	95% IP	
			DG	GG
T-b	38.25	0.12	38.13	38.37
T-u	38.17	0.23	37.95	38.39
T-20	37.75	0.38	37.40	38.11

KG37- control group of rats exposed to water temperatures of 37˚C; T (°C)- Temperature in Celsius degrees; X- mean value; ±SD-standard deviation; IP-confidence interval; DG-lower limit; GG- upper limit; T-b- bazal temperature; T-u- rat immersion temperature; T-20-temperature in 20th minute.

Table 2: Mean values of rat body temperature at repeated measurements in G44.

G44				
T(°C)	X	±SD	95% IP	
			DG	GG
T-b	38.02	0.55	37.72	38.32
T-u	38.67	0.90	38.19	39.15
T-20	43.09	0.70	42.71	43.46

G44- group of rats exposed to water temperatures of 44˚C; T (°C)- Temperature in Celsius degrees; X- mean value; ±SD-standard deviation; IP- confidence interval; DG- lower limit; GG- upper limit;;T-b- bazal temperature; T-u- rat immersion temperature; T-20- temperature in 20th minute.

The lowest temperature of rats in group 44 was basal temperature, and the highest temperature after 20 minutes of exposure during repeated measurements was 38.02±0.55°C versus 43.09±0.70°C, and the difference of all three groups was p<0.0005 (Table 2).

The mean values of the measured body temperatures of rats depending on the group are presented in Table 3.

Frequency analysis of rats with a body temperature of 40.05◦C and higher was found to be highest in seven G44 rats. No statistically significant difference was found in the frequency of rats with heat stroke p=0.053 (Table 4).

Table 3: Mean values of measured body temperature of rats of experimental groups at four time points.

T (°C)	Group	x̄	±SD	95% IP	
				DG	GG
T-b (°C)	KG37	38.25	0.12	38.13	38.37
	G44-AM	37.96	0.66	37.40	38.51
	G44-PM	38.08	0.46	37.69	38.47
T-u (°C)	KG37	38.17	0.23	37.95	38.39
	G44-AM	38.9	1.12	37.95	39.84
	G44-PM	38.45	0.61	37.93	38.96
T-20 (°C)	KG37	37.57	0.38	37.40	38.11
	G44-AM	43.00	0.81	42.32	43.67
	G44-PM	43.18	0.62	42.66	43.70
T-s (°C)	KG37	34.45	2.4	32.19	36.72
	G44-AM	39.00	1.20	37.98	40.01
	G44-PM	44.02	0.38	43.70	44.34

T (°C)- Temperature in Celsius degrees; X- mean value; ±SD-standard deviation; IP- confidence interval; DG- lower limit; GG- upper limit;T-b- bazal temperature; T-u- rat immersion temperature; T-20- temperature in 20th minute T-s- temperature in moment of death; KG37- control group of rats exposed to water temperatures of 37˚C; G44-AM- antemortem group of rats exposed to water temperatures of 44˚C (exposure length 20 minutes); G44-PM- postmortem group of rats exposed to water temperatures of 44˚C (length of exposure to death).

Table 4: Frequency of heat stroke in experimental groups.

Heart stroke	Groups			Total
		KG37	G44	
No	N	7	9	16
	%	100.0	56.3	60.5
	% of total number	18.4	23.7	60.5
Yes	N	0	7	7
	%	0.0	43.8	39.5
	% of total number	0.0	18.4	39.5

There is a statistically significant difference between both the control group and G44 in ovalocytes, dacryocytes, anulocytes, spherocytes, reticulocytes, and Target cells (Table 5). Table 6 shows the poikilocytotic forms between the antemortem and postmortem groups at 44°C. When comparing antemotrem and postmortem rats exposed to a water temperature of 44°C, a significant

difference in dacryocytes and spherocytes was observed (Table 6, Figure 1 and 2).

Table 5: Differences in poikilocytotic forms between antemortems group and control groups.

	A:Temperature 37°C			B:Temperature 44°C			
	Med	Per 25	Per 75	Med	Per 25	Per 75	p A v B
Dacryocytes	1.0	0.0	2.0	5.00	2.00	9.0	0.038
Anulocytes	1.0	0.0	3.0	47.0	25.0	74.0	0.001
Echinocytes	0.0	0.0	1.0	0.00	0.00	15.0	0.079
Stomatocytes	1.0	0.0	2.0	17.0	6.00	35.0	0
Ovalocytes	1.0	0.0	2.0	3.00	2.00	3.00	0.038
Schizocytes	0.0	0.0	2.0	1.00	1.00	2.00	0.079
Acantocytes	0.0	0.0	0.0	0.00	0.00	0.00	0.636
Spherocytes	1.0	0.0	2.0	2.00	1.00	15.0	0.007
Reticulocytes	1.0	1.0	1.0	1.00	1.00	4.0	0.02
Target cells	1.0	0.0	1.0	12.0	3.0	24.0	0.02

Variables are represented as median value with interquartile range. P A v B was tested with Kruskall Wallis H test, differences between two groups were tested with Mann Whitney U test. P – probability with p<0,05 deemed as significant

Table 6: Differences in poikilocytotic forms between antemortem and postmortem group at 44°C.

Variables	Antemortem			Postmortem			P value
	Temperature od 44°C			Temperature od 44°C			
	Median	Per 25	Per 75	Median	Per 25	Per 75	
Dacryocytes	5	2	9	16	8	19	0.002*
Anulocytes	47	25	74	100	28	123	0.165
Echinocytes	0	0	15	7	1	13	0.318
Stomatocytes	17	6	35	15	8	26	1.000
Ovalocytes	3	2	3	3	1	10	0.902
Schizocytes	1	1	2	1	1	2	0.535
Acantocytes	0	0	0	0	0	1	0.383
Spherocytes	2	1	15	46	25	54	*0.017
Reticulocytes	1	1	4	4	1	10	0.383
Target cells	12	3	24	1	1	2	0.053

Note: *-represents a significant difference between groups.

DISCUSSION

Heat stroke is a condition of the body in which the body's compensatory mechanisms of internal temperature control are weaken. Combined with stressors such as physical labor, fluid loss, weakness, and medical pathological conditions, heat stroke culminates and can take the form of mild heat exhaustion to potentially fatal heat stroke.

There is an increase in body core temperature, rapid heart rate and sweating, and a very pronounced form can result in heat stroke, a condition involving human defense mechanisms including activation of pro-inflammatory and inflammatory cytokines. Incidence data downplay the severity of the condition because it is often not diagnosed properly due to inadequate definition.

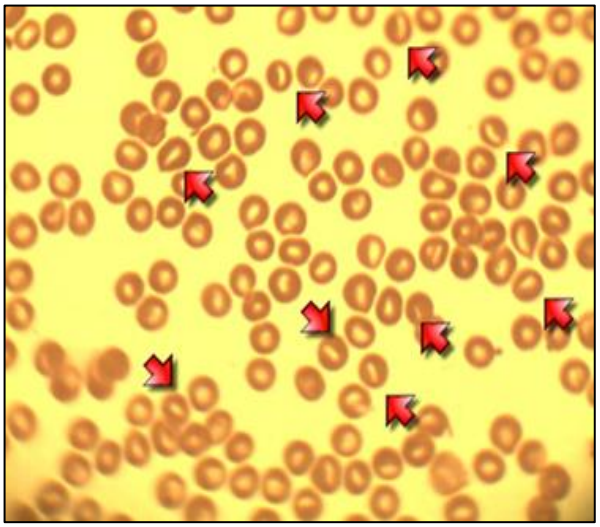

Figure 1: Poikilocytic forms of RBC, peripheral blood smear, magnification 1000x, G44-AM; red arrow: stomatocytes.

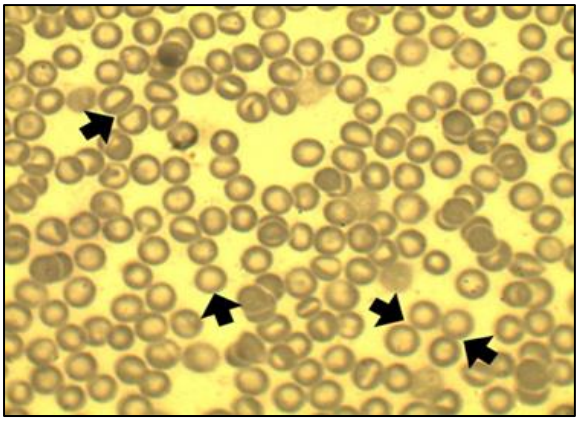

Figure 2: Poikilocytic forms of RBC, peripheral blood smear, magnification 1000x, G44-PM; B- black arrow: anulocytes.

The condition can cause nervous system dysfunction, myocardial infarction, renal failure, and muscle dysfunction and is defined as a multisystem disorder of the body.[17] A condition that leads to death in 10-50% of cases, and the cardiovascular abnormalities that accompany it are hypotension, electrolyte disturbance, malignant arrhythmia, ischemia, depression of heart function with global hypokinesia.[18] Understanding the physiological and behavioral response of animals, including humans, to elevated ambient temperature has gained in critical importance with global warming and with the expectation of an increase in the number and severity of heat waves.[19]

As a fatal condition, heat stroke is characterized by an elevated core temperature of 40.05°C and above, secondary due to prolonged exposure to high ambient temperature.[16]

In our study, the thermoregulatory response of the rat organism was monitored by exposing the rats to a water temperature of 37˚C-KG37 and 44˚C-G44. A change in core temperature was recorded using an oesophageal probe placed in anesthetized rats. According to the literature, the physiological body temperature of rats is 37.5-37.7°C, and the goal was to develop a model of hyperthermia and record the frequency of core temperature ≥40.05°C which is the temperature threshold for heat stroke and see the impact temperatures on the state of the organism through compensatory mechanisms projected in the development of red blood cell shape.[20]

Analysis of the mean values of rat body temperature measured at four time points showed that the basal temperatures of rats in the groups did not differ significantly ($p>0.005$), which indicates the physiological condition and the absence of pathological processes in rats before starting the experiment. The basal temperature of rats in the control group was 38.25±0.12°C and decreased after 20 minutes of water exposure of 37°C, which indicates physiologically adaptable mechanisms of heat distribution in the body. In both groups, a significant difference was found in the temperature of the nucleus at immersion, after 20 minutes and at death, which indicates the influence of exposure to water temperature on the increase in body temperature of rats using control mechanisms. After 20 minutes of exposure, the highest temperature of rats of group G44 (43.09±0.7°C) was observed, according to KG37 (37.75±0.38°C). These results indicate that exposure to water temperature led to hyperthermia and heat stroke in the G44 group.

The frequency of heat stroke in the G44 group was 43.8%. Rats exposed to a water temperature of 44°C had a survival time of 4.14 minutes, so a short period of time caused that although insignificantly we have a larger number of group 44 rats that did not reach the heat stroke temperature threshold. In the study by Quinn et al it is pointed out that many studies use predefined temperatures to confirm heat stroke, which was done in our study, and it is considered that interindividual variation in response excludes the use of predefined maximum temperature jump as the main indicator of heat stroke severity.[20] The correlation between the rise in body temperature and water temperature is shown in the study by Hori et al. in healthy adult subjects.[21] In their study, the internal temperature reached values of 40°C after 10 minutes of exposure. The results of the study by Hori et al. suggest that the degree of damage depends on both the temperature of the environment to which the body is exposed and the length of the body's exposure to that environment.[21] Norloei et al showed that chronic exposure to heat stress causes an increase in the serum osmolality and a decrease in mean cell volume and the white blood cells in the exposed group compared with the nonexposed group.[22] Although the red blood cells count in the exposed group decreased as well, it was not statistically significant.

Twenty minutes exposure of Wistar rats to water of 44°C induced poikilocytosis. Poikilocytosis was associated with the exposure length and temperature intensity. The most distinct shapes of poikilocytes found in the postmortem group of rats were “expressed” dacriocytosis and “expressed” spherocytosis. Their presence was “expressed” in our study. In G44 and control group statistically significant difference was in almost all poikilocytic shapes, which indicated that high temperature induces poikilocytosis following 20 min until death exposure to the high temperature. The present finding was as results of Lucijanović et al., pointing that poikilocytosis was associated with the temperature to which rats were exposed, but also with the length of exposure.[23]

Dacriocytes and spherocytes numbers were significantly higher in postmortem than in antemortem group 44°C. The high temperature causes protein denaturation with cell malfunction, loss of membrane integrity and finally cell death. Studies in cell lines and animal models suggest that heat directly results in tissue damage. The severity of

the damage depends on the critical thermal maximum, a term that tries to quantify the level and duration of temperature increase that causes tissue damage to begin.[24] Few studies have been conducted on the impact of heat stress on oxidative stress indices and hematological parameters.[25, 26]

A review by Horv´ath and Babinszky stated that heat stress can lead to harmful impacts on birds including increase in the production of reactive oxygen species, the formation of malondialdehyde as an indicator for lipid peroxidation, and decreased vitamin concentrations.[27] Choi and Pai showed that although short-term exposure to heat stress does not have an effect on erythrocyte count, hemoglobin concentration, and mean corpuscular hemoglobin, under the same conditions, it does increase mean cell volume and hematocrit.[28]

Specific poikilocytes are associated with diseases and noted in the rat, but may also be observed during the evaluation of rat blood in toxicity studies. In these studies the most common forms of poikilocytes are echinocytes and acanthocytes. Red blood cell fragments (schistocytes) are observed in hemolytic processes such as those associated with Heinz body formation, osmotic shock, and disseminated intravascular coagulation. Rarely, red cell shapes such as stomatocytes, target cells, ghost cells, spherocytes, and others were observed by Car et al and Spahić et al.[29, 30]

It is difficult to compare the results of studies about chronic and acute exposure to heat, because the differences in the degrees of adaption to heat exposure in the subjects can cause differences in the results. Our findings indicate that prolonged exposure to heat stress can be a risk factor for abnormal structural changes in erythrocytes and concludes that acute heat stress evokes a series of drastic changes in the animal's hematological functions.

CONCLUSION

Poikilocytosis is associated with the exposure length and temperature intensity. Following a fatal outcome dacryocytes with spherocytes at 44°C were significantly more than in corresponding antemortem groups.

ACKNOWLEDGEMENTS

This methodology was part of a research doctoral thesis on "Forensic meaning of Heat Shock Protein 70 and Troponin I for terminal hyperthermic miocardial injury in rats" by Emina Dervišević. The thesis was successfully defended at the Faculty of Medicine of the University of Sarajevo (Sarajevo, Bosnia and Herzegovina) in May 2021.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Bandaru SS, Gupta V. Poikilocytosis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2021.
2. An X, Schulz VP, Mohandas N, Gallagher PG. Human and murine erythropoiesis. Curr Opin Hematol. 2015;22:206-11.
3. De Franceschi L, Bosman GJ, Mohandas N. Abnormal red cell features associated with hereditary neurodegenerative disorders: the neuroacanthocytosis syndromes. Curr Opin Hematol. 2014;21(3):201-9.
4. Bosman GJCGM. Disturbed Red Blood Cell Structure and Function: An Exploration of the Role of Red Blood Cells in Neurodegeneration. Front Med (Lausanne). 2018;16(5):198.
5. Vittori D, Garbossa G, Lafourcade C, Perez G, Nesse A. Human erythroid cells are affected by aluminium. Alteration of membrane band 3 protein. Biochim Biophys Acta. 2002;1558:142-50.
6. Katica M, Gradascevic N. Hematologic profile of laboratory rats fed with bakery products. IJRG. 2017;5(5):221-31.
7. Christopher MM, Hawkins MG, Burton AG. Poikilocytosis in rabbits: Prevalence, type, and association with disease. PLoS One. 2014;9(11):e112455.
8. Kuhn V, Diederich L, Keller TCS. Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. Antioxid Redox Signal. 2017;26(13):718-42.
9. Yoshida T, Prudent M, D'alessandro A. Red blood cell storage lesion: causes and potential clinical consequences. Blood Transfus. 2019;17(1):27-52.
10. Lepock JR. Cellular effects of hyperthermia: relevance to the minimum dose for thermal damage. Int J Hyperthermia. 2003;19(3):252-66
11. Delibegovic S, Koluh A, Cickusic E, Katica M, Mustedanagic J, Krupic F. Formation of adhesion after intraperitoneal application of TiMesh: experimental study on a rodent model. Acta Chir Belg. 2016;116(5):293-300
12. Režić-Mužinić N, Mastelić A, Benzon B, Mrkotić A, Mudnić I, Grković I et al. Expression of adhesion molecules on granulocytes and monocytes following myocardial infarction in rats drinking white wine. PLoS One. 2018;13(5):196.
13. De Labra C, Pardo-Vazquez JL, Cudeiro J, Rivadulla C. Hyperthermia-Induced Changes in EEG of Anesthetized Mice Subjected to Passive Heat Exposure. Front Syst Neurosci. 2021;15:709337.
14. National Institutes of Health. Principles of Laboratory Animal Care. National Institutes of Health publication No. 86-23. Bethesda, MD: National Institutes of Health. 1985.

15. Zini G, d'Onofrio G, Briggs C, Erber W, Jou JM, Lee SH, et al. International Council for Standardization in Haematology (ICSH) recommendations for identification, diagnostic value, and quantification of schistocytes. Int J Lab Hematol. 2012;34:107-16.
16. Suzuki M, Hori S. Experimental investigation in rats to identify the cause of sudden death during bathing in Japan. AMS. 2014;1(2):101-4
17. Nzvere FP, Tariq E, Nishanth K, Arshid A, Cancarevic I. Long-Term Cardiovascular Diseases of Heatstroke: A Delayed Pathophysiology Outcome. Cureus. 2020;12(8):e9595.
18. Rajan R, Amin O, Gaballa A, Dashti R, Jarallah MA. Heat Stroke Manifesting as Cardiac Arrest and Multi-Organ Failure. JCR. 2017;7:264-66.
19. Barney CC, Kuhrt DM. Intermittent heat exposure and thirst in rats. Physiol Rep. 2016;4(8):e12767.
20. Delibegovic S, Koluh A, Cickusic E, Katica M, Mustedanagic J, Krupic F. Formation of adhesion after intraperitoneal application of TiMesh: experimental study on a rodent model. Acta Chir Belg 2016;116(5):293-300
21. Quinn CM, Duran RM, Audet GN, Charkoudian N, Leon LR. Cardiovascular and thermoregulatory biomarkers of heat stroke severity in a conscious rat model. J Appl Physiol. 2014;117(9): 971-8.
22. Hori S. Report of the research on actual situation and preventive strategies of bathrelated fatalities, Health and Labor Sciences Research Grants: comprehensive research on life-style related disease including cardiovascular diseases and diabetes mellitus. 2014;118.
23. Norloei S, Jafari MJ, Omidi L, Khodakarim S, Bashash D, Bagher M et al. The effects of heat stress on a number of hematological parameters and levels of thyroid hormones in foundry workers. Int J Occup Saf Erg. 2017;23(4):481-90.
24. Lucijanić M. Analiza gena i proteina signalnih puteva Wnt i Sonic Hedgehog u primarnoj i sekundarnoj mijelofibrozi (dissertation). Medicinski Fakultet Sveučilišta u Zagrebu, Zagreb. 2017;p19.
25. Bruneaux M, Visse M, Gross R, Pukk L, Saks L, Vasemagi A. Parasite infection and decreased thermal tolerance: impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. Functional Ecology. 2017;31(1):216-26.
26. Rabeiy RE. Evaluation of indoor heat stress on workers of bakeries at Assiut City, Egypt. International Journal of Environmental Science and Technology. 2019;16(6): 2637-42.
27. Horv´ath M, Babinszky L. Impact of selected antioxidant vitamins (vitamin A, E and C) and micro minerals (Zn, Se) on the antioxidant status and perfor mance under high environmental temperature in poultry. A review. Acta Agriculturae Scandinavica, Section A- Animal Science. 2018;68(3):152-60.
28. Choi JW, Pai SH. Changes in hematologic para meters induced by thermal treatment of human blood. Annals of Clinical and Laboratory Science. 2002;32(4):393-8.
29. Car BD, Bounous DI. Clinical Pathology of the Rat. In: The Laboratory Rat. 2th ed. American College of Laboratory Animal Medicine. 2006;127-46.
30. Spahić E, Katica M, Jogunčić A, Katica A, Hasić S. Impact of temperature and the length of exposure on morphological characteristics of erythrocytes in antemortem and postmortem analysis: Experimental study on Wistar rats. Kafkas Univ Vet Fak Derg. 2020;26(6):771-6.

Cite this article as: Dervišević E, Katica M, Hasić S, Jogunčić A, Dervišević L, Vukas H et al. Poikilocytotic forms caused by hyperthermia and heat stroke-experimental study on Wistar rats. Int J Res Med Sci 2022;10:1225-31