Hematological reference intervals of some snake species in Turkey

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Received: 11.05.2009

Abstract: In this study, some blood parameters were studied in 7 distinct snake species (Dolichophis caspius, Eirenis modestus, Malpolon monspessulanus, Natrix natrix, Platyceps rubriceps, Telescopus fallax, and Typhlops vermicularis) of the families Colubridae and Typhlopidae, collected from various regions of Turkey. A total of 7 hematologic parameters, namely red blood cell count (RBC), white blood cell count (WBC), hematocrit value (HCT), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were examined. It was detected that Platyceps rubriceps had the highest erythrocyte count and the lowest MCV value among the species examined, Telescopus fallax had the lowest erythrocyte count and hematocrit value, and Eirenis modestus had the highest hematocrit and MCV values. With this study, the variations observed in the blood measurements and counts of snakes were also observed in clinical parameters.

Key words: Colubridae, Typhlopidae, Ophidia, hematology, Turkey

Türkiye'deki bazı yılan türlerinde hematolojik referans aralıkları

Özet: Bu çalışmada Türkiye'nin çeşitli bölgelerinden toplanan Colubridae ve Typhlopidae familyalarına ait 7 farklı yılan türünde (Dolichophis caspius, Eirenis modestus, Malpolon monspessulanus, Natrix natrix, Platyceps rubriceps, Telescopus fallax, Typhlops vermicularis) bazı kan parametreleri çalışılmıştır. Hematolojik parametrelerden; eritrosit sayısını (RBC), lökosit sayısı (WBC), hematokrit değeri (HCT), hemoglobin konsantrasyonu (Hb), ortalamaya eritrosit hacmi (MCV), ortalamaya eritrosit hemoglobin konsantrasyonu (MCH), oratalama eritrosit hemoglobin yoğunluğu (MCHC) olmak üzere toplam 7 parametre incelenmiştir. Platyceps rubriceps incelesen diğer türlerden en yüksek eritrosit sayısı ve en düşük MCV değerine, Telescopus fallax türünden en düşük eritrosit sayısı ve hematokrit değerine, Eirenis modestus türünün ise en yüksek hematokrit ve MCV değerine sahip olduğu tespit edilmiştir. Bu çalışma ile yılanların kanlarına ait ölçüm ve sayımlarda görülen varyasyonlar klinik parametrelerde de görülmüştür.

Anahtar sözcükler: Colubridae, Typhlopidae, Ophidia, hematoloji, Türkiye

Introduction

Checking blood parameters in reptiles may guide the evaluation of physiological and health conditions of populations and may be used as an indicator in determining environmental conditions, since species are very sensitive to changes of habitat (Jacobson et
al., 1991; Raphael et al., 1994; Dickinson et al., 2002; Lopez-Olivera et al., 2003). Thus, it is important to examine blood parameters in reptiles and determine the changes in these species from environmental change.

There may be many internal (species, sex, age, and physiological state) and external (season, temperature, habitat, nutritional pattern, and captivity) factors that affect blood parameters. Therefore, it is difficult to determine the reference interval of blood values (Lawrence and Hawkey, 1986; Gottdenker and Jacobson, 1995; Lopez-Olivera et al., 2003). Recently, the majority of the hematologic studies carried out on different reptile species have dealt with blood composition, as well as blood cell counts and sizes (Atatür et al., 2001; Knotkova et al., 2002; Tosunoğlu et al., 2004).

Hematologic studies on different snake species are quite high in number (Dessauer, 1970; Duguy, 1970; Saint Girons, 1970; Wojtaszek, 1991, 1992; Troiano et al., 1997, 1999, 2000; Lamirande et al., 1999). Particularly, the hematologies of venomous species of snakes have been studied by many researchers (Hattingh and Willemsen, 1976; Troiano et al., 1997; Dutton and Taylor, 2003; Allender et al., 2006; Santos et al., 2008). In Turkey, however, the hematologic studies on various snake species are related only to blood cell sizes and counts (Arıkan et al., 2004; Tok et al., 2006; Arıkan et al., 2009). The hematologic studies on snake species living in Turkey are limited only to the morphology and size of blood cells and there is no detailed study on clinical parameters. Thus, the hematologic parameters of some snake species living in Turkey were determined for the first time and interspecies comparison were made.

Materials and methods

A total of 47 specimens, with 1 species (Typhlops vermicularis) belonging to the family Typhlopidae and 6 species (Dolichophis caspius, Eirenis modestus, Malpolon monspessulanus, Natrix natrix, Platyceps rubriceps, and Telescopus fallax) belonging to the family Colubridae, were collected from various localities in Turkey between 2004 and 2009 and were examined in this study. The blood required was taken by means of a heparin-coated hematocrit capillary tube in 1 day via postorbital sinus from the colubrid specimens that had been caught alive in nature and brought to the laboratory (MacLean et al., 1973). The necessary blood samples were taken from etherized Typhlops vermicularis by means of ventricular puncture, via heparinized hematocrit capillaries (Arıkan et al., 2003). Later, the colubrid specimens were released back to the area from which they were collected.

The red blood cell counts (RBC) and white blood cell counts (WBC) were carried out using a Neubauer hemocytometer, where standard Hayem’s solution for red blood cells and Turk's solution for white blood cells were used as a diluting solution.

Hematocrit (HCT) was determined using the microhematocrit method (Tanyer, 1985). The tubes were then spun in a microhematocrit centrifuge for 5 min at 12,000 rpm and the hematocrit (HCT) was calculated with the total blood level divided by the blood cell level. Hemoglobin concentration (Hb) was measured by the Sahli method with a Sahli hemoglobinometer (Tanyer, 1985). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated mathematically, taking the above results into consideration (Tanyer, 1985).

The descriptive statistics and Mann–Whitney U test of the data obtained from our study were performed using SPSS (10.0 for Windows Student Version). Hematological variables were summarized as mean, standard deviation (SD), standard error of the mean (SE), and range. We used a nonparametric test (Mann–Whitney U) for comparison of the species. Results were considered significant at P < 0.05.

Results

When the hematologic values of the snake species were examined, the highest erythrocyte count was found in Platyceps rubriceps, whereas the lowest erythrocyte count was found in Telescopus fallax. The hemoglobin value was detected to be the highest in Natrix natrix and the lowest in Telescopus fallax and Malpolon monspessulanus. The hematocrit value was found to be the highest in Eirenis modestus and the lowest in Telescopus fallax. The MCV value was found
to be the highest in *Eirenis modestus* and the lowest in *Platyceps rubriceps*, and the MCH value was found to be the highest in *Eirenis modestus* and the lowest in *Platyceps rubriceps*. The MCHC value was detected to be the highest in *Dolichophis caspius* and the lowest in *Typhlops vermicularis*. The hematologic values of snake species are given in detail in Table 1.

**Discussion**

Hematologic and biochemical measurements may vary depending on factors such as sex, age, pregnancy, physical exercise, weather, stress, altitude, and captivity (Hartman and Lessler, 1964; Wojtaszek, 1991, 1992; Palenske and Saunders, 2003; Santos et al., 2008). According to Ryerson (1949), the erythrocyte count of reptiles is smaller than that of mammals or birds. Lizards generally have higher erythrocytes counts than snakes, but turtles have the lowest (Duguy, 1970).

Many researchers have stated that there are great intraspecific and interspecific variations of blood cell count in snakes (Duguy, 1970; Saint Girons, 1970; Arıkan et al., 2004; Tok et al., 2006; Arıkan et al., 2009). In this study, it was determined that there were also interspecific variations in clinical values.

Species of 2 different families were examined in this study. Many researchers have found that there was a homogeneous group in snakes, except for *Typhlops vermicularis*, in terms of erythrocyte measurements; the erythrocytes of the species *T. vermicularis* were completely different, small and elongated (Saint Girons, 1970). When the clinical values of both families were compared, the MCHC value was found to be lower in *T. vermicularis* than in *D. caspius* or *T. fallax*. This difference of MCHC was statistically significant (P < 0.05). The blood of *T. vermicularis* contains the same erythrocyte count as other colubrid species, but in a smaller volume, which places the species among snakes most specialized in this respect. The other parameters of colubrid species were similar to *T. vermicularis*. The morphology of erythrocytes was stated to have an oval shape in other ectothermic vertebrates (Arıkan et al., 2004).

The family Colubridae is the most extensive snake group, including semivenomous snakes and semiaquatic snakes. In this study, the semivenomous snake species *T. fallax* was found to have the lowest RBC, Hb, and HCT values in comparison with those of other colubrid species. According to Tok et al. (2006), the shortest erythrocyte belongs to the species *M. monspessulanus*. Nevertheless, clinical results similar to other species were obtained in the semivenomous snake species *M. monspessulanus* in our study. On the other hand, the erythrocyte count was lower in *Malpolon monspessulanus* in comparison with the previous findings of Duguy (1970).

When the semivenomous snake species *Malpolon monspessulanus* and *Telescopus fallax* were compared in terms of hematologic values, almost similar results were obtained. Only the erythrocyte count was higher in the species *M. monspessulanus*, but there was not a statistically significant difference. Only the hematocrit values were found to be significant differences among *Eirenis modestus*, *M. monspessulanus*, and *T. fallax* (P < 0.05). When the data from the semivenomous snake species, *Malpolon monspessulanus* and *Telescopus fallax*, were compared with previous studies on venomous snakes, the erythrocyte count was found to be higher, the leukocyte count, Hb, MCH, and MCHC values were found to be lower, and the HCT and MCV values were found to be similar in comparison with species in the family Crotalidae, namely *Bothrops jararacussu* (Troiano, 2000) and *Bothrops ammodytoides* (Troiano et al., 1999). In the species *Malpolon monspessulanus* and *Telescopus fallax*, the erythrocyte counts were found to be higher than that of the viperid species *Crotalus durissus terrificus* in one study (Santos et al., 2008) but lower than that of *Crotalus durissus terrificus* in a different study (Troiano et al., 1997). Hematological differences in this species depend on seasonal variation (Troiano et al., 1997). In *Malpolon monspessulanus* and *Telescopus fallax*, the hemoglobin value was lower than that of *Bitis arietans* (Hattingh and Willemse, 1976) and *Crotalus durissus terrificus* (Troiano et al., 1997; Santos et al., 2008), whereas the hematocrit values were similar. In semivenomous snakes, the MCV and MCH values were detected to be higher than those of the subspecies *Crotalus durissus terrificus*, while the MCHC value was detected to be lower (Troiano et al., 1997) (Table 2).

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>RBC (1 mm$^3$)</th>
<th>WBC (1 mm$^3$)</th>
<th>Hb (g/dL)</th>
<th>HCT (%)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
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<tr>
<td></td>
<td>N</td>
<td>Mean ± SD (Min-Max)</td>
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<tr>
<td><strong>D. caspius</strong></td>
<td>4</td>
<td>918,500 ± 137,706 (767,000-1,100,000)</td>
<td>3750 ± 1707.8 (4400-8200)</td>
<td>30.75 ± 4.19 (28-37)</td>
<td>339.89 ± 62.55 (263,63-402,17)</td>
<td>101.80 ± 21.08 (78.18-122.55)</td>
<td>29.88 ± 1.74 (28.57-32.41)</td>
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<tr>
<td><strong>E. modestus</strong></td>
<td>10</td>
<td>768,196 ± 226,513.4 (393,333-1,063,000)</td>
<td>5500 ± 476.09 (5000-6000)</td>
<td>35.2 ± 4.76 (30-40)</td>
<td>583.11 ± 270.98 (378,05-889,83)</td>
<td>132.19 ± 52.08 (78.00-217.39)</td>
<td>24.55 ± 7.26 (14.86-33.33)</td>
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<tr>
<td><strong>M. monspessulanus</strong></td>
<td>6</td>
<td>894,444 ± 306,396.2 (406,666-1,310,000)</td>
<td>5466 ± 1410.4 (3400-7200)</td>
<td>25 ± 4.35 (22-30)</td>
<td>359.85 ± 157.46 (255,56-540,98)</td>
<td>96.46 ± 38.63 (61.11-137.71)</td>
<td>27.12 ± 4.29 (23.91-32)</td>
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<tr>
<td><strong>N. natrix</strong></td>
<td>13</td>
<td>846,166 ± 177,583 (513,000-1,266666)</td>
<td>3766 ± 1886 (1600-6500)</td>
<td>31.75 ± 7.72 (19-40)</td>
<td>347.37 ± 55.34 (265,73-440,48)</td>
<td>102.11 ± 26.12 (68.04-153.57)</td>
<td>29.07 ± 3.18 (24.44-34.86)</td>
<td></td>
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<tr>
<td><strong>T. fallax</strong></td>
<td>4</td>
<td>681,750 ± 246,871.6 (380,000-887,000)</td>
<td>5530 ± 183.8 (5400-5660)</td>
<td>22.33 ± 9.86 (11-29)</td>
<td>365.43 ± 116.86 (289,47-500,00)</td>
<td>97.01 ± 36.31 (65.39-148.28)</td>
<td>29.34 ± 1.93 (27.27-31.11)</td>
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<tr>
<td><strong>P. rubriceps</strong></td>
<td>6</td>
<td>1,156,667 ± 104,626.3 (1,040,000-1,300,000)</td>
<td>6080 ± 4136.6 (3000-11,000)</td>
<td>30.4 ± 5.12 (25-38)</td>
<td>268.36 ± 61.93 (215,38-351,85)</td>
<td>73.74 ± 10.64 (61.54-90.38)</td>
<td>28.11 ± 4.32 (21.05-32.86)</td>
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<tr>
<td><strong>T. vermicularis</strong></td>
<td>5</td>
<td>849,333 ± 299,206.1 (520,000-1,166666)</td>
<td>4611 ± 757.4 (3800-5300)</td>
<td>31 ± 3.46 (29-35)</td>
<td>390.97 ± 144.58 (300-557,69)</td>
<td>102.13 ± 33.60 (71.74-138.46)</td>
<td>24.24 ± 1.29 (22.76-25.14)</td>
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In the present study, only the MCV value was found to be low in the semiaquatic snake species *N. natrix* in comparison with *E. modestus* ($P = 0.013$), and there were significant differences in RBC, MCV, and MCH values between *N. natrix* and *P. rubriceps* ($P < 0.05$). When data from the species *Natrix natrix* were compared with previous studies, the erythrocyte count and leukocyte count were found to be lower, the hemoglobin, MCV, and MCH values were found to be higher, and the hematocrit and MCHC values were found to be similar in comparison with a study by Wojtaszek (1991). Similar results were obtained when the erythrocyte count was compared with the findings of Duguy (1970) (Table 2).

A total of 7 species in families Colubridae and Typhlopidae, distributed in Turkey, were examined hematologically and were given as references for the first time. As a result of this study, there was found to be interspecies variations in terms of clinical parameters.

**Acknowledgements**

This study was supported by Çanakkale Onsekiz Mart University, BAP, under Project No: 2009/37.
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References


