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Evaluation of Clinical Signs, Hematological and Biochemical Parameters after Blood Transfusion from Sheep to Goat

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ABSTRACT

Blood transfusion is needed for the treatment of severe anemia. The purpose of this study was to detect clinical reactions, hematological and biochemical alterations after a blood transfusion from sheep to goat. Four Iranian mixed breed male sheep and goats were used in this study. Fifteen milliliters per kilogram of whole blood was taken from the sheep. Blood transfusion to goats was performed after 24 hours. Blood samples were taken from goats at times 0 (10 min before transfusion), 10 min, 3, 6, 12, 24, 48, 72, 96, and 192 hours after blood transfusion. No reactions such as coughing, dyspnea, muscle tremors, salivation, tearing, fever, and ruminal bloating during and after blood transfusion were seen. The respiratory rate significantly increased, 3, 6, and 12 hours after blood transfusion ($p < 0.05$). Hematocrit was significantly reduced 3 hours after blood transfusion ($p < 0.05$). Serum creatinine levels were increased significantly 10 minutes and 3 hours after blood transfusion ($p < 0.05$). Blood urea serum was significantly increased 6 and 12 hours after blood transfusion ($p < 0.05$). Serum calcium levels were increased significantly 72 and 96 hours after blood transfusion ($p < 0.05$). Serum magnesium levels were significantly increased 72 and 96 hours after blood transfusion ($p < 0.05$). In conclusion, temporary and transient changes observed in this study were safe and were not life-threatening for the goats, and the sheep blood can be used in anemic goats for one time.

Introduction

Few studies have been performed on blood transfusions, especially the status of the recipient's response to blood received in ruminants. Even in anemic patients, sometimes this treatment is performed without any appropriate criteria and without considering the possible risks of the reaction due to blood transfusion.[1]

Blood can be obtained from an animal and stored for a period of time for autologous blood transfusion before surgery, but it should be noted that stored red blood cells are always subject to a series of changes due to storage and these changes get worse over time.[2] Storing whole sheep blood in transfusion bags containing citrate, phosphate, dextrose, and adenine as preservatives, for 35 days, increased plasma

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hemoglobin, potassium, and lactate concentrations and decreased sodium, bicarbonate, glucose, and pH.[3] The occurrence of such changes as a result of blood storage is an indicator of the loss of stored blood quality that may play a role in the reactions caused by such blood transfusions.[4,5]

Hunt and Wood reported in 1999 that a reaction to homologous blood transfusions is uncommon in ruminants receiving blood for the first time because these animals have low levels of isoantibodies in their circulatory system.[6] Symptoms of a homologous transfusion reaction seen in ruminants include fever, tachycardia, tachypnea, dyspnea, tremor, pruritus, hematuria, and hemoglobinuria.[7] Non-hemolytic reactions due to fever following blood transfusion are the most common non-lethal blood transfusion reactions that may or may not be associated with clinical symptoms and are divided into two general categories: inflammatory reactions and allergic reactions.[8]

In France, Italy and Australia, dog blood has been used extensively in the treatment of anemic cats.[1] But so far there are no reports of heterologous blood transfusions in ruminants. The present study evaluates the clinical response and the occurrence of possible reactions resulting from heterologous blood transfusion from sheep to goats.

Materials and Methods

In this study, four Iranian mixed breed male sheep with an age of about 2 years and an approximate weight of 50-60 kg as blood donors and four Iranian mixed male goats with an age of about 1 year and an approximate weight of 25-30 kg as blood recipient were used. None of the animals had a history of blood transfusions and had not received any blood-based vaccine. All animals were kept in the same place, fed green alfalfa hay twice a day, and had free access to water. All the animals were carefully examined for heart rate, respiratory rate, rectal temperature and rumen movements before blood sampling and blood transfusion. The whole body were examined for any lesions or presence of external parasites. The feces of all animals were carefully examined for the presence of any parasite eggs and their blood samples were examined for the presence of any blood parasites. After two weeks adaptation period, 15 ml/kg of body weight of the receiving goat (375-450 ml) blood, was collected in the blood bags containing citrate, phosphate, dextrose and adenine (CPDA-1) (Hora Teb Co., Khorramdareh, Iran).[9] Blood samples were refrigerated for 24 hours at 4° C. Prior to blood transfusion, cross match test was performed according to the Michel et al. method.[10]

About half an hour before blood transfusion, the same amount of transferable blood (15 ml/kg) was taken from each goat, in order to prevent false increase in blood volume due to blood transfusion. 30 minutes before the blood transfusion, the blood bag was removed from the refrigerator and stored at room temperature. Blood infusion set with a micropore filter (260 µm) in-line (Medi-life Co., Haryana-India) was used for this purpose.

The blood transfusion rate was 5-6 ml/kg/hour in the first few minutes and then increased to 30-40 ml/kg/hour.[10] During the blood transfusion, the goats were monitored for any adverse reactions such as coughing, dyspnea, muscle tremors, salivation, tearing, fever, and ruminal bloating.[11]

Clinical parameters including heart rate (HR), respiratory rate (RR), rectal temperature (RT), and capillary refill time (CRT); hematological parameters including packed cell volume (PCV), red blood cell (RBC) count, and white blood cell (WBC) count; and biochemical parameters including serum total protein (TP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), creatine kinase (CK), creatinine, blood urea nitrogen (BUN), calcium (Ca), phosphorus (P), and magnesium (Mg) were assayed 10 min before and after blood reinfusion (Br0 and Br, respectively) and 3, 6, 12, 24, 48, 72, 96 and 192 h after blood reinfusion (Br3, Br6, Br12, Br24, Br48, Br72, Br96, and Br192, respectively.[9,14]

Blood samples were collected from the left jugular vein in to the tubes containing ethylenediaminetetraacetic acid (EDTA) for hematological evaluation. PCV was determined by microhematocrit technique. RBC and WBC were determined by microdilution in a Neubauer chamber.[11] Serum samples were separated by centrifugation (3000 rpm for 20 min) for biochemical analysis, using an automatic biochemical analyzer (Technicon RA, 1000, USA). Total protein concentration was determined by the Biuret method. Enzymatic activities of ALP, AST, GGT and CK were determined by International Federation of Clinical Chemistry (IFCC), Deutsche Gesellschaft für Klinische Chemie (DGKC), and Szasz methods, respectively, using commercial kits (Parsazmun, Karaj, Iran). The serum creatinine, urea,

Ca, P, and Mg were determined by the JAFFE, urease-GLDH, cresolphthalein complexone, UV test, and xylidyl blue methods, respectively, using commercial kits (Parsazmun, Karaj, Iran).

Statistical Analysis

The statistical analyses were carried out using the SPSS 11.5 program (SPSS Inc., Chicago, IL, USA.). The paired *t*-test was used for comparison of Br0 with the other time points of blood infusion. All results were displayed as mean ± SEM, and significance was set at $p < 0.05$.

Results

No erythrocyte lysis was seen following cross match test. There was no adverse reaction such as coughing, dyspnea, muscle tremors, salivation, tearing, fever and ruminal bloating following blood transfusion. Data from clinical examination in all time points are presented in Table 1. Respiratory rate (RR) increased significantly from 3 to 12 hours after blood transfusion ($p < 0.05$). There were no significant changes in heart rate (HR), rectal temperature (RT) and capillary refill time (CRT) after blood transfusion ($p > 0.05$) (Table 1).

Data from hematological examination in all time points are presented in Table 2. PCV and RBC levels decreased significantly 3 hours after blood collection ($p < 0.05$). WBC increased significantly immediately after blood transfusion and decreased after 48 hours ($p < 0.05$) (Table 2).

Data from biochemical examination in all time points are presented in Tables 3, 4 and 5. Serum total protein level increased significantly immediately after blood transfusion and decreased 72 hours there after ($p < 0.05$). No significant changes were observed in the activity of serum ALP, AST, GGT and CK enzymes following blood transfusion ($p > 0.05$) (Table 3).

Total serum bilirubin levels in goats increased after blood transfusion but these changes were not significant ($p > 0.05$). Serum creatinine and BUN levels increased significantly ($p < 0.05$) at 6 and 12 hours and ten minutes and 3 hours after blood transfusion respectively (Table 4).

Serum calcium and magnesium concentrations increased significantly ($p < 0.05$) at 72 and 96 hours and 12 and 48 hours after blood transfusion respectively. No significant change in serum phosphorus concentration of goats was observed after blood transfusion ($p > 0.05$) (Table 5).

Discussion

In the goats studied in this study, the respiratory rate increased 3, 6 and 12 hours after blood transfusion ($p < 0.05$). Bovens and Gruffydd (2012) also reported that following the blood transfusion from dog to cat, some cats showed signs of tachypnea, which may be due to mild reactions to blood transfusions.[1] Increased cardiovascular pressure following the blood transfusion can increase pulmonary pressure, dyspnea, pulmonary edema, and possibly death.[12] In the goats studied in this study, the increase in respiratory rate may have been due to reactions to blood transfusions, but these changes were temporary and did not threaten the animal's life. Repeated blood transfusions can be fatal in goats by anaphylaxis. Repeated blood transfusions 6 days after the first blood transfusion in dogs have been led to anaphylaxis.[1]

In the goats studied in this study, no significant changes in rectal temperature were observed following blood transfusion ($p > 0.05$). Heinius *et al*. (2002) also observed that blood transfusions of 15 mg/kg in pigs did not alter body temperature.[13] Bovens and Gruffydd (2012) reported that some cats developed fevers

Table 1. Heart rate (HR), respiratory rate (RR), rectal temperature (RT) and capillary refill time (CRT) before and following blood transition (mean ± SEM).

Time point	HR (bpm**)	RR (rpm***)	RT (°C)	CRT (seconds)
Br0	87.5 ± 6.46	27.25 ± 2.06	39.40 ± 0.14	2.00 ± 0.00
Br	96.25 ± 4.35	33.50 ± 1.00	39.68 ± 0.24	2.00 ± 0.00
Br3	97.25 ± 2.06	34.75 ± 7.09*	39.58 ± 0.15	2.00 ± 0.00
Br6	95.75 ± 4.35	36.25 ± 1.50*	39.73 ± 0.19	2.00 ± 0.00
Br12	94.50 ± 4.20	35.25 ± 3.78*	39.63 ± 0.35	2.00 ± 0.00
Br24	92.25 ± 1.71	33.25 ± 0.96	39.20 ± 0.25	2.00 ± 0.00
Br48	91.75 ± 2.75	30.00 ± 1.63	38.93 ± 0.17	2.00 ± 0.00
Br72	87.25 ± 2.22	27.75 ± 3.10	38.80 ± 0.22	2.00 ± 0.00
Br96	88.25 ± 3.95	25.25 ± 1.26	38.55 ± 0.10	2.00 ± 0.00
Br192	90.00 ± 1.63	27.25 ± 2.75	38.58 ± 0.05	2.00 ± 0.00

In each column, * was significantly differed with Br0 ($p < 0.05$) **beats per minute; *** respiration per minute.

Table 2. Packed cell volume (PCV), red blood cell (RBC) and White blood cell (WBC) before and following blood transition (mean ± SEM).

Time point	PCV (%)	RBC (×10⁶/µl)	WBC (×10⁹/µl)
Br0	34.25 ± 2.50	11.4 ± 0.61	9300 ± 365.15
Br	28.25 ± 2.36	9.41 ± 0.31	11825± 842.12*
Br3	27.25 ± 1.71*	9.08 ± 0.09*	14388± 201.56*
Br6	25.75 ± 0.96*	8.5 ± 0.27*	13975± 170.78*
Br12	24.00 ± 2.71*	8 ± 0.40*	12525± 727.44*
Br24	24.50 ± 3.11*	8.16 ± 0.11*	11575± 722.84*
Br48	25.25 ± 4.27*	8.41 ± 0.70*	10125 ± 650.00
Br72	25.25 ± 3.78*	8.41 ± 0.57*	10500 ± 818.54
Br96	24.25 ± 2.99*	8.08 ± 0.33*	9863 ± 206.65
Br192	27.00 ± 1.14*	9 ± 0.15*	8350 ± 968.68

In each column, * was significantly differed with Br0 (p < 0.05).

following blood transfusions from dogs to cats, which could be due to mild reactions to blood transfusions.[1]

In this study, no significant changes (p > 0.05) were observed in CRT following blood transfusion. This indicates that blood transfusions of 15 mg/kg in goats, did not cause serious damage to the animal's circulation.[14]

In goats studied in this study, PCV decreased significantly (p < 0.05) from 3 hours after blood transfusion. This may be due to hemolytic reactions due to blood transfusions. Acute hemolytic reactions due to antigen incompatibility occur following blood transfusion within 24 hours of blood transfusion. In acute cases, the clinical signs due to intravascular hemolysis include fever, dyspnea, increased heart rate, decreased blood pressure and hemoglobinuria, and laboratory findings include: hemoglobinemia, hemoglobinuria, increased lactate dehydrogenase, hyperbilirubinemia, and decreased haptoglobin in the blood. In the event of kidney damage, the serum creatinine and BUN increases.[15] Extravascular hemolysis due to delayed hemolytic reactions causes clinical signs such as fever and mild jaundice that often is not recognizable.[16] Delayed hemolytic reactions occur at least 24 hours after blood transfusion. These reactions are less severe than acute hemolytic transfusions and may rarely cause symptoms of renal failure.[15] Heterologous blood transfusions from dogs to cats are now performed in cats with acute anemia when compatible blood is not available. However, side effects such as extensive hemolysis and anaphylactic reactions have been reported.[17] Blood group mismatch in ruminants leads to primary hemolysis by the complement reactions.[11] Sarpataki *et al.* (2014) have shown that heterologous blood transfusions of 20 mg/kg from dog to tow European shorthair cat with severe anemia was safe even hematocrit and hemoglobin levels in cats increased.[17] Considering the amount of hematocrit decreased for at least 196 hours following heterologous blood transfusion, it can be concluded that intravascular hemolysis has occurred, and since no hemoglobinuria was observed clinically, such a conclusion can be made. Intravascular hemolysis did not appear to be life-threatening in this condition.

In the goats studied in this study, the total WBC increased significantly (p < 0.05) ten minutes after blood transfusion and decreased 48 hours thereafter (p < 0.05). Nazifi and Mashhadi-Esmaeil (2005) also showed, total WBC and the absolute number of neutrophils increase significantly after blood transfusion. Elevated WBC associated with neutrophilia, lymphopenia, and eosinopenia may be due to animal stress during blood transfusions.[18] Changes in WBC counts can also occur as a result of immune responses associated with blood transfusions and the presence of antibodies against blood cell antigens.[19-21] In one study, both whole blood and blood without WBC were used for autologous blood transfusions in dogs. The results showed that the immune responses

Table 3. Serum total protein (TP) and the activity of aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and creatinine kinase (CK) before and following blood transition (mean ± SEM).

Time point	TP (g/dl)	ALP (U/L)	AST (U/L)	GGT (U/L)	CK (U/L)
Br0	7.80 ± 0.00	380.25 ± 87.37	56.85 ± 3.26	28.25 ± 7.41	92.00 ± 17.15
Br	8.85 ± 0.30*	348.48 ± 95.11	74.08 ± 26.63	21.58 ± 12.11	120.55 ± 23.73
Br3	9.05 ± 0.19*	319.98 ± 68.37	91.50 ± 29.79	22.98 ± 9.65	205.63 ± 93.18
Br6	9.40 ± 0.28*	354.30 ± 86.46	83.40 ± 27.26	29.18 ± 7.46	237.58 ± 129.55
Br12	8.93 ± 0.10*	346.20 ± 90.34	80.33 ± 23.59	28.60 ± 8.92	220.35 ± 167.04
Br24	8.80 ± 0.16*	371.18 ± 93.76	74.98 ± 21.15	27.18 ± 9.68	131.88 ± 52.60
Br48	8.80 ± 0.16*	325.33 ± 84.50	71.90 ± 19.82	28.40 ± 10.60	130.73 ± 9.46
Br72	8.55 ± 0.19*	318.03 ± 98.10	71.03 ± 12.32	27.45 ± 11.05	220.18 ± 165.96
Br96	8.10 ± 0.12	325.33 ± 100.85	69.98 ± 9.65	27.43 ± 13.06	156.03 ± 59.06
Br192	7.90 ± 0.20	350.23 ± 129.76	59.70 ± 8.43	27.70 ± 12.49	97.50 ± 23.10

In each column, * was significantly differed with Br0 (p < 0.05)

Table 4. Serum concentration of total bilirubin, creatinine, and blood urea nitrogen (BUN) before and following blood transition (mean ± SEM).

Time point	Creatinine (mg/dL)	BUN (mg/dL)	Total bilirubin (mg/dL)
Br0	0.73 ± 0.05	30.7 ± 1.72	0.27 ± 0.02
Br	1.65 ± 0.44*	33.25 ± 8.80	0.66 ± 0.50
Br3	1.80 ± 0.14*	40.55 ± 1.34	0.41 ± 0.19
Br6	0.58 ± 0.10	61.93±13.23*	0.42 ± 0.15
Br12	0.43 ± 0.10	66.85±10.86*	0.50 ± 0.10
Br24	0.40 ± 0.08	38.25 ± 5.88	0.39 ± 0.07
Br48	0.43 ± 2.15	34.10 ± 2.15	0.47 ± 0.11
Br72	0.48 ± 0.05	34.00 ± 1.86	0.34 ± 1.86
Br96	0.48 ± 0.05	27.83 ± 2.13	0.57 ± 0.09
Br192	0.48 ± 0.05	28.70 ± 2.14	0.48 ± 0.60

In each column, * was significantly differed with Br0 (p < 0.05).

Table 5. Serum concentration of calcium (Ca), phosphorus (P) and magnesium (Mg) before and following blood transition (mean ± SEM).

Time point	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)
Br0	9.25 ± 0.41	7.9 ± 0.57	2.15 ± 0.27
Br	7.83 ± 0.42	9.9 ± 1.51	2.35 ± 0.13
Br3	7.95 ± 0.50	10.88 ± 0.48	2.60 ± 0.16
Br6	9.8 ± 1.51	8.18 ± 0.95	2.68 ± 0.29
Br12	9.63 ± 0.82	8.13 ± 0.80	2.88 ± 0.51*
Br24	10.73 ± 1.49	8.45 ± 1.64	3.00 ± 0.47*
Br48	11.30 ± 1.77	7.78 ± 1.58	2.68 ± 0.23
Br72	12.40 ± 1.63*	6.85 ± 1.74	2.45 ± 0.19
Br96	12.55 ± 1.93*	7.70 ± 1.02	2.53 ± 0.10
Br192	11.40 ± 0.56	6.45 ± 1.35	2.55 ± 0.24

In each column, * was significantly differed with Br0 (p < 0.05).

were higher in animals that received whole blood.[22] Medical studies have also shown that the total WBC increases 12 hours after blood transfusion and reaches its initial level after 24 hours.[23]

According to table 3, the total protein increased significantly (p < 0.05) from ten minute after blood transfusion and decreased significantly (p < 0.05) 96 hours thereafter. Since 7% of total plasma consist of protein, therefore, blood transfusion may have increased the total protein.[10]

In this study, the activity of some enzymes was evaluated to show any changes in liver function following blood transfusion. No significant changes (p > 0.05) were observed in the activity of ALP, AST, GGT and CK enzymes.

ALP is found in bone, liver, placenta, kidney, and intestinal tissues. Hepatic isoenzyme ALP is found in liver cells and canaliculi membranes. Increased osteoblasts activity also causes an elevation of bone isozyme ALP.[24] Since the variation of ALP activity was minimal and remained within the reference values for goats (93–387 U/L) no hepatic injury has been suggested following blood transfusion.

AST present in all cells of the body but it is essentially used to diagnose liver damages. This enzyme is highly sensitive to liver disorders and since the serum activity of AST did not change significantly following blood transfusion in this study, it can be concluded that heterologous blood transfusion from sheep to goat was not associated with serious liver damage.[24] AST also present in red blood cells, so hemolysis can increase its serum activity.[11] A slight increase of AST in this study, may have been due to intravascular hemolysis.

GGT activity of serum can indicate the health status of bile duct.[24] Since the serum activity of GGT was not affected, it can be concluded that the bile ducts were not damaged following blood transfusion from sheep to goats.

Serum CK activity is the most sensitive indicator of skeletal muscle injury.[25] It increases within a few hours of the onset of skeletal muscle injury and peaks within 6 to 12 hours. If the muscle damage is not progressive, serum CK activity returns to normal within 24 to 48 hours after cessation of injury.[24] No changes in CK activity during blood transfusion indicate that there was no skeletal muscle injury during recumbency. An increased CK activity following blood collection due to recumbency in sheep has been observed.[14] CK is also present in red blood cells, so hemolysis can increase its serum activity.[24] The slight increase in serum CK activity, from 10 minutes after blood transfusion may have been due to intravascular hemolysis.

Serum creatinine levels increased from ten minutes to 3 hours after blood transfusion. BUN concentration also increased significantly from 6 to 12 hours after blood transfusion. In ruminants, BUN concentration do not increase in proportion to creatinine due to urea changes in the rumen.[23] Hemoglobinuria due to an acute hemolytic reaction following a blood transfusion can cause kidney damage (nephrotoxic effects of hemoglobin), so serum creatinine levels and BUN concentration increase steadily.[26] Temporary changes in serum creatinine and BUN concentrations following blood transfusion, represent the restoration of the kidney health.

No significant changes were observed in serum total bilirubin levels following blood transfusion (p > 0.05). The slight increase in serum total bilirubin, may probably due to intravascular hemolysis. Blood

transfusion from dog to cat has slightly increased serum bilirubin 7 days later.[17] Meyer and Harvey (1998) reported that intravascular hemolysis leads to an increase in total serum bilirubin.[27]

In this study, serum calcium and magnesium of goats increased respectively 72 and 96 hours, and 12 and 24 hours after blood transfusion compared to the time before blood transfusion ($p < 0.05$). Similar results have been reported in Sousa 's study.[14] Serum calcium and magnesium concentrations can be elevated in disorders such as renal failure, dehydration, hyperproteinemia, acidosis, and hyperthyroidism.[24] Since the changes in serum calcium and magnesium concentrations was temporary, the possibility of permanent kidney damage is ruled out.

In conclusion, temporary and transient changes in clinical signs, hematological and biochemical parameters observed in this study were safe and heterologous blood transfusions from sheep to goats for the first time had no life-threatening effects on goats so the sheep blood can be used in anemic goats for one time.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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