Effects of erythropoietin on fracture healing in rats

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Fracture healing and the factors affecting it has always attracted researchers' curiosity. Fracture healing is a complex process, local (mechanical, physical, chemical, environmental), and systemic (hormonal factors, vitamins, drugs, systemic growth factors, diseases) factors all play a part at each stage. Although the histopathological process of fracture healing is now almost completely understood, the effects of most of the factors on the fracture-healing event have not yet been absolutely verified. Erythropoietin (EPO) is the primary hormonal stimulant of red blood cells production. It is thought that EPO used in the treatment of anemia requiring symptomatic treatment, and transfusion associated with kidney failure, and that used to prevent anemia in cases receiving chemotherapy may enhance the fracture healing. From our literature search, no review was found on the effects of EPO on fracture healing in rats. We now aim to discuss the effect of EPO on fracture healing.

Twenty female, healthy Sprague-Dawley rats, weighing between 250-300 g, were used. The study protocol was performed in accordance with the Guide for the Care and Use of Laboratory Animals, and was reviewed and accepted by the institutional animal care and usage committee. The right limbs were shaved and cleaned by Betadine solution. The right tibia of each rat was exposed via an antero-medial skin incision under ketamine hydrochloride anesthesia. Following the skin incision the midshaft of the tibia was reached by blunt dissection. The shaft was then osteotomized transversally by a Gigli saw taking care to cause minimal soft tissue injury at the fracture site. An additional longitudinal parapatellar incision was made, and a Kirschner wire was introduced to the intramedullary canal of the tibia for fixation. The largest size (0.8 mm) of Kirschner wires was used to fit the distal intramedullary space of the rat tibiae. Following hemostasis, the layers were closed with interrupted sutures. Per operative antimicrobial prophylaxis, consisting of 50 mg/kg/day Cefazolin-sodium was administered. The animals were randomly divided into 2 groups: 'control' and 'EPO'. The rats in the EPO group were injected subcutaneous with 200 IU/kg/day of rHuEPO-alfa for 7 days, and the ones in the control group with 0.9% sodium chloride. The rats were allowed unrestricted weight bearing after recovery from the anesthesia. These animals were kept in individual cages and allowed free access to tap water and a standard pellet diet. The cages were housed in a temperature of 24°C, humid air (55%) and 12 hours of day/night light controlled room. Three animals in the control group died of unknown reasons during follow-up, and a surgical wound infection did not develop. The remaining 7 control and 10 EPO group rats were sacrificed 3 weeks after surgery. The fractured tibiae and fibulae were removed by careful dissection, followed by resection of the fibulae, and the intramedullary Kirschner wires were pulled out by applying small torsional movements. Radiographs were performed after the animals were killed. All radiographs were randomized and independently scored by 2 orthopedists, who were unaware of the treatment that the animal had received. Common radiological parameters include the following categories: Periosteal reaction, bone union, and remodeling, which can be semi-quantitated base on a scoring system.[1] Median radiographic scores were calculated for each group. Eight tibiae (3 from control group, 5 from EPO group) were prepared for histological examination. The specimens were fixed in 10% neutral buffered formaldehyde for 2 days and than decalcified with 1.5% aqueous hydrochloric acid for 3 days. The tibiae and fibulae were separated and embedded in paraffin that allows obtaining 5-micron sections from each block and stained with hematoxylin-eosin. The degree of fracture healing was determined by light microscopic examination according to a histological scoring system.[1] Common histological parameters include the following categories: callus formation, bone union, marrow changes, and cortex remodeling, which can be semi-quantitated base on a scoring system.[1] The grading was carried out blindly without knowledge of which treatment had been given. Median fracture healing scores were calculated for each group. Nine tibiae (4 from control group, 5 from EPO group) were prepared for the mechanical test. Control and EPO groups were numbered so as to keep the biomechanic measurements blind. The tibiae were kept frozen at –20°C for further analysis. Prior to the tests, the tibiae were placed in a humid medium and kept there for 4 hours until they thawed to room temperature. The distal and proximal parts of the tibiae were cut to obtain a better adjustment to the 3-point bending fixture. The tibiae were placed on the 3-point bending configuration on the Lloyd LS500 material testing machine (Southampton, UK). The 500-N load cell was used for load detection, and the sampling rate was 4.0 Hz. The loading speed was

Table 1 - Results of mean radiological evaluation score, mechanical parameters, and histological evaluation score.

Parameters	Experimental group Mean ± SD	Control group Mean ± SD	Significance level*
Radiological evaluation score (XP-Total)	6.10 ± 1.30	4.1 ± 1.5	$p<0.05$
Mechanical parameter (Max-Load)	342.3 ± 88.40	24.0 ± 3.0	$p<0.05$
Histological evaluation			
Callus formation	2.70 ± 0.48	2.75 ± 0.50	$p>0.05$
Bone union	1.90 ± 0.56	1.25 ± 0.50	$p>0.05$
Marrow changes	3.30 ± 0.67	3.00 ± 0.81	$p>0.05$
Cortex remodeling	0.90 ± 0.31	0.25 ± 0.50	$p<0.05$
*Non-parametric Mann-Whitney U-test			

2 mm/min, and the load was applied at the mid-span in anteroposterior direction, with a span length of 40 mm. Bones were kept in a humid medium during the tests. The load-deflection curves were stored in the computer to be processed later to obtain fracture load values. All values were analyzed using a Sigma stat program (SPSS-10, SPSS Inc., Chicago, IL, USA.). The differences between experimental and control group were compared by using Mann-Whitney U-test and the level of significance was set at $p<0.05$. All results are shown in **Table 1**. There was a statistically significant difference the radiographic scores ($p<0.05$), cortex remodeling values ($p<0.05$), and fracture load values ($p=0.001$) between the control and EPO groups (**Table 1**).

Angiogenesis is considered essential for proper fracture healing. Long-standing clinical evidence has established a strong correlation between impairment of vascular function or development and failure of fracture healing.[2] In addition, studies in animal models have demonstrated that new blood vessels are intimately linked with healing fracture tissues, and that experimental challenges that disrupt angiogenesis, including physical impairment, diseases (for example, diabetes), and antimetabolite or radiation treatments, can slow, but usually not prevent, fracture healing.[2] Yet, despite abundant correlative evidence and the recognition that angiogenesis is a possible therapeutic target to improve fracture healing, we understand very little about its precise role in the healing process, and why disruption of angiogenesis prevents fractures from healing properly. Vascularization is observed at the transition of preosteoblasts to mature osteoblasts during both development and fracture healing. This suggests that bone cells may interact with endothelial cells, probably through the secretion of angiogenic factors.[3] Most osteogenic factors stimulate angiogenesis, if not directly, then indirectly, through production of angiogenic molecules. These include members of the fibroblast growth factor (FGF), transforming growth factor (TGF), bone morphogenetic protein (BMP), insulin-like growth factor (IGF) and platelet derived growth factor (PDGF) families,[4] as well as vascular endothelial growth factor. Intravital microscopy and angiographic analysis in bone chamber models indicate that angiogenesis temporal precedes osteogenesis.[3] Molecular and cellular biology, together with improvements in imaging and histological techniques, have resulted in the identification of molecules with osteogenic and angiogenic activity. The role of these molecules in the intimate conversation between endothelial cells and bone cells is still being elucidated, but their reciprocal relationship and the potential synergism between potent pro-angiogenic factors (such as VEGF) and strong osteoinductive factors (such as BMP-4), suggest that combination therapies might produce optimum results, particularly for individuals at risk for delayed repair or non-unions.[4]

Erythropoietin is the primary hormonal stimulant of red blood cell production. Interactions have been reported to induce a range of cellular responses, including mitogenesis, chemotaxis, mobilization of intracellular calcium, and inhibition of apoptosis.[5] Among its many potential effects, EPO may stimulate angiogenesis, the process by which pre-existing blood vessels give rise to new vessels. During angiogenesis, proteins from vascular endothelial cells degrade the extra cellular matrix, migrate into the perivascular space, proliferate, align themselves into tubular vessels, and form a vascular lumen.[6] Erythropoietin would also stimulate angiogenesis in the first stage of fracture healing, and thus, it would have positive effect in the early period of the fracture. This stimulation was observed in the early stages of fracture healing in our model. We think that fracture healing may be increased in those rats given EPO, because it stimulates angiogenesis in fracture healing.

We believe that even with the advent of new research into EPO, understanding the basic principles of fracture healing may lead to more effective treatments for patients with fractures. In the future, we might determine how to use EPO and other protein messages that are embedded within our bones as seeds for bone regeneration.

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Effects of moderate alcohol consumption on serum marker enzymes of rabbits

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Despite a large number of epidemiological studies, and the biological plausibility of presumed risks or benefits of moderate drinking of alcohol on the cardiovascular system, liver and cerebral blood flow, or other effects such as cancer, psychological imbalance, birth defects and stroke, there is still conflicting evidence regarding a link between these destructive risks or benefits to moderate alcohol drinking. The effects of alcohol on serum marker enzymes have been investigated.[1-5] In most prospective studies, the assessment of alcohol drinking has been based on self-reporting, which may be unreliable. The aim of the present study was to examine the relationship between alcohol drinking both on serum gamma-glutamyl transferase (GGT), and serum tissues damage markers concentrations such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), which are regarded as a biological markers of alcohol drinking. The enzymes kits used in the present study were obtained from Diagnostic Products Corporation, (DPC; USA).

Animals. This investigation was performed on male white New Zealand (Oryctolagus cuniculus huxley) rabbits weighing 2.5-3 kg. The rabbits were provided by the animal house in the Medical School of Yüzüncü Yıl University, and were housed in 5 groups, each group containing 10 rabbits. All animals were fed with wheat-soybean-meal-based diet and water ad libitum in stainless cages, and received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health. The animals were housed at 20 ± 2 °C temperature with daily light/dark cycles.

Ethanol treatment. A dose of 10% ethyl alcohol was administered orally to the rabbits for acute, subacute, and subchronic treatment. For acute treatment, 10 milliliters of 10% ethyl alcohol was administered by catheter a single time to 10 rabbits. Drinking water containing 10% ethyl alcohol was administered orally to 20 rabbits ad libitum for 20 days for subacute, and 40 days for subchronic treatment. Control rabbits received only tap water. Daily water consumption of each rabbit was approximately 50 ± 5 ml during the tests. Consequently, the ethanol intake was approximately 5 ± 0.5 ml per day. At the end of the treatment, blood samples were obtained from the ear by arterial puncture using a syringe for the determination of enzyme levels. Blood samples were drawn immediately into ice-chilled siliconized disposable glass tubes. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 minutes at 4°C, and enzyme levels were measured in these serum samples. Serum enzyme levels were measured by auto analyzer (BM/HITACHI-911), using the kits.

Table 1 - Serum enzyme levels of rabbits (mean ± standard deviation).

Parameters	Control	3rd	6th	20th day	40th day
AST (U/L)	31.1 ± 11.69	40.5 ± 16.01	42.1 ± 15.6	44.8 ± 15.11	45 ± 15.43
ALT (U/L)	47.3 ± 16.89	43.1 ± 14.3	50.2 ± 13.7	64.8 ± 16.16*	68.2 ± 22.50*
ALP (U/L)	235.4 ± 74.4	271.9 ± 107.4	291.9 ± 120.3	369.9 ± 191.3	356.1 ± 112.3*
LDH (U/L)	422.4 ± 81.5	624.2 ± 103.3*	486.1± 136.6	390.2 ± 173.2	555.6 ± 96.9*
GGT (U/L)	12.9 ± 4.51	14.2 ± 3.65	15.1 ± 4.33	15.5 ± 3.95	17.6 ± 3.47*

*p<0.05, AST - aspartate aminotransferase, ALT - alanine aminotransferase, ALP - alkaline phosphatase, LDH - lactate dehydrogenase, GGT - gamma-glutamyl transferase

All data were expressed as mean ± standard deviation (SD). For statistical analysis, the SPSS/PC+ package (SPSS/PC+, Chicago, IL, USA) was applied. For all parameters, means and SD were calculated according to the standard methods. The Mann-Whitney U-test was employed to examine the differences between means of the treatments and the control. The significance level was accepted at p=0.05 for all tests. The results showed that the treatment of rabbits with alcohol produced changes in the levels of serum ALT, AST, ALP, GGT and LDH. According to the results, the exposure to an acute period of alcohol at the 3rd hour induced a significant increase in the level of LDH, but did not significantly change the other enzymes. The 20 day subacute treatment with alcohol caused a significant increase in the level of ALT, but did not significantly change the other enzymes. As for the others enzymes, no significant differences existed between levels of the treatment groups compared to the control group. The 40 day subchronic treatment with alcohol caused a significant increase in the levels of ALT, ALP, GGT and LDH, but did not significantly change the AST level. To find out the significance of increase in different serum enzymes on exposure to alcohol for third and sixth hours, 20th and 40th days, the data obtained was subjected to Mann Whitney-U test. **Table 1** shows mean and SD of serum enzyme levels of the treated and control rabbits.

This study showed that the biological markers of alcohol drinking, such as the level of serum GGT, AST, ALT, ALP and LDH were significantly higher at different experimental periods. According to these results, it can be speculated that serum enzymes provide additional information in alcoholism, such as GGT. We also found that serum enzymes, such as AST, ALT, ALP and LDH are useful for the assessment of destructive risks related to alcohol drinking as biological markers of alcohol drinking, such as serum GGT level. These findings are both in accordance with and in contradiction with some other studies in which they used generally with humans as the study subject. As shown in **Table 1,** the results indicate that the serum enzyme levels in moderate alcohol consumption significantly increased compared with control. To date, the study of serum enzyme in a model state has not been reported in rabbits; so we were not able to compare our results with others. In this study, moderate alcohol consumption caused a significant alteration in serum enzyme levels even at 10% concentration. Alcohol consumption may lead to the release of these enzyme into plasma as a result of autolytic breakdown or cellular necrosis. These enzymes are mainly localized at the cytoplasm. Any damage in the hepatic cells may result in alteration in the serum levels. The increase in the activities of these enzymes in the serum may result only consequent to impairment of the function of tissues with subsequent liberation of the enzymes into the circulation from the damaged tissue. Lactate dehydrogenase and transaminases are intracellular enzymes, which exist in only a small amount of the serum. Alkaline phosphatase is mainly localized at the cell membrane. Any damage in hepatic cells may result in alteration in ALP activity. Although a number of studies on AST, ALT, ALP, LDH and GGT levels on alcohol consumption have been carried out, the results are mostly in contradiction with each other. Nishmura and Teschke,[1] Rikans and Snowden,[2] and Jousilahti et al[5] observed a significant increase in GGT between alcohol drinking and controls, consequently, these results are in accordance with our result. Horie et al[3] and Murt et al[4] indicated that ALT levels were higher in alcoholic patients than those of normal subjects. Consequently, these reports are in accordance with our findings. On the other hand, the same researchers observed an increase in AST in comparing control. Consequently, these results are in contradiction with our findings. In addition, the present study determined that ALP and LDH levels increased significantly at the subchronic period compared with controls. Our

results are in accordance with the previous results. These controversial data are may be due to the use of different animals as study subjects, treatment time, and the manner and setting of the studies. Even though several theories have been proposed to explain these differences, the mechanisms of enzymes were not well understood. The increase in serum GGT concentration was associated with the increased risk of ischemic stroke, as well as of total strokes.[5] Some investigators[1-4] suggested that the increase of enzymes levels in patients with alcohol resulted from the influence of alcohol on the cells membrane permeability, such as liver and muscle tissues. The other possibilities of an increase in alcohol drinking include malnutrition, hepatic anoxia, and infection. However, the enzymes released from other organs may contribute to the change of levels in enzyme. Our results indicate that the serum ALT, ALP, GGT and LDH levels were significantly higher in alcohol drinking rabbits than that of the control group, supporting these possibilities.

In conclusion, the present study supports the hypothesis that moderate alcohol drinking increases the destructive risks, such as in the liver muscle tissues. Self-reporting of alcohol drinking, and suggestions that lower levels of alcohol consumption can reduce stress; promote conviviality and pleasant and carefree feelings; and decrease tension, anxiety, and self-consciousness however, may be unreliable, and often underestimates the true risk. Also, the use of the biological markers of alcohol drinking, such as serum GGT, ALT, ALP and LDH may be a helpful tool for risk assessment. A better understanding of the pathophysiological mechanism between alcohol drinking, biological markers, and the risk of destructive effects on tissues is an important issue for the primary prevention of moderate drinking. In addition, this study indicated that further experiments should be performed to investigate what is responsible for the elevation of enzymes in the muscle and liver. This test may be used in forensic study if more studies confirm our findings, due to the instability of serum enzyme. Such a test could also be valued in population studies, and would be of interest to understand the molecular basis of refractoriness of alcoholism.

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A study on children and adolescents with disabilities in Kahramanmaras, Turkey

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Disability is described as any restriction or lack of ability to perform an activity in a manner or within a range considered normal for human beings.[1] There is a large and growing number of persons with physical, mental, or sensory disabilities in the world today. However, the incidence and causes of disabilities vary throughout the world according to age, level of economic development, access to health care, educational, environmental, and other factors.[2] The estimated ratio of disabled persons ranges between 5.2-18.2% in the world populations. In developing countries, up to 5% of the children are born disabled or became disabled during their childhood.[3] In Kahramanmaras, a developing city located in the south-eastern region of Turkey, the rate of childhood disability (0-14 years) accounts for 8.2% of all disabilities. Disability is related to the population's health quality as well as the individual's health problem. The types and causes of disabilities should be determined in the population for determining the precautions to prevent being disabled and planning approaches to rehabilitation for the disabled. The main objectives of this study are to determine types and causes of disability, and to determine the marriage type, educational, and economical levels of the parents of disabled children and adolescents taking rehabilitation assistance in Kahramanmaras, Turkey.

Table 1 - The distribution of the causes underlying the disabilities.

Reason for defect	n	(%)	Type of marriages n (%)				x^2-test
			Consaguinity		Non-consanguinity		
Prenatal or natal							
Heredity	77	(11.4)	41	(6.1)	36	(5.3)	χ^2=14.6; p<0.01
Genetic defects	58	(8.6)	28	(4.1)	30	(4.4)	χ^2=6.85; p<0.01
Risky pregnancy	5	(0.7)	0	(0.0)	5	(0.7)	χ^2=1.1; p>0.05
Blood incompability	11	(1.6)	2	(0.3)	9	(1.3)	χ^2=0.4; p>0.05
Mother's chronic illness	22	(3.3)	12	(1.8)	10	(1.5)	χ^2=5.2; p<0.05
Accidents during pregnancy	7	(1.0)	0	(0.0)	7	(1.0)	χ^2=1.9; p>0.05
Disease during pregnancy	19	(2.8)	5	(0.7)	14	(2.1)	χ^2=0.2; p>0.05
Lack of oxygen during birth	194	(28.6)	46	(6.8)	148	(21.9)	χ^2=4.3; p<0.05
Personnel faults during birth	10	(1.5)	3	(0.4)	7	(1.0)	χ^2=0.1; p>0.05
Postmaturity	8	(1.2)	2	(0.3)	6	(0.9)	χ^2=0.0; p>0.05
Prematurity	36	(5.3)	9	(1.3)	27	(4.0)	χ^2=4.7; p>0.05
Total	**447**	**(66.0)**	**148**	**(21.9)**	**299**	**(44.1)**	χ^2=0.34; p>0.05
Postnatal							
Disease	156	(23.0)	44	(6.5)	112	(16.5)	χ^2=0.6; p>0.05
Accidents	40	(5.9)	13	(1.9)	27	(4.0)	χ^2=0.02; p>0.05
Wrong treatment	9	(1.3)	1	(0.2)	8	(1.2)	χ^2=0.9; p>0.05
Malnutrition	6	(0.9)	0	(0.0)	6	(0.9)	χ^2=1.5; p>0.05
Total	**211**	**(31.1)**	**58**	**(8.6)**	**153**	**(22.6)**	χ^2=1.20; p>0.05
Unknown	19	(2.8)	7	(1.0)	12	(1.8)	χ^2=0.3; p>0.05
Total	**677**	**(100.0)**	**213**	**(31.5)**	**464**	**(68.5)**	χ^2=0.8; p>0.05

The data from 677 disabled individuals from 8 private rehabilitation centers, aged 1-25 years, were collected by questionnaire administration. Standardized questionnaire forms were filled by the parents of the disabled persons between January – March 2005. The sex, type of disability, time of being disabled (prenatal, natal, postnatal), causes of disability, the marriage type of parents (consanguinity, non-consanguinity), educational (illiterate, literate, primary school, secondary school, high school, university), and economic levels of the parents were the questions included in the questionnaire. Economic conditions of the families have been evaluated according to their monthly incomes; those who receive 0-750 YTL were recorded as poor, 750-1500 YTL as normal, 1500-2250 YTL as good, and over 2250 YTL as very good. The SPSSX (10.0) package was used to analyze the obtained data. Of the disabled persons, 42% were female and 58% were male. Disabilities of 68.8% were present from birth, either prenatal or natal factors, 31.1% were acquired in childhood or adolescence by various causes (**Table 1**). With respect to data, 46.8% of the individuals had intellectual disabilities, 37% had multiple disabilities, 12.1% had physical, 3.5% had sensory disabilities, while 0.6% of the persons had psychiatric disabilities. Mental retardation (MR) (46.8%), motor mental retardation (MMR) (16.4%), and cerebral palsy (CP) (11.3%) were the major disabilities observed in the individuals. Many factors are responsible for the rising numbers of disabled persons.[3,4] In the present study, lack of oxygen in the fetus during birth due to torsion of the umbilical cord, and dystocia (28.6%), various diseases in the postnatal period (23%), heredity (11.4%), and genetic defects (8.6%) were found to be important factors, as in the other studies. However, the causes underlying disabilities of 2.8% of the individuals were not known. In this study, 31.5% of the parents had consanguineous marriages. The types of consanguinity were first cousin (27.3%), second cousin (2.4%), and distant relative marriages (1.8%). The rate of disabled children due to heredity (p<0.01), genetic defects (p<0.01), and mother's chronic illness (p<0.05) were found to be significantly higher in consanguineous marriages than in non-consanguineous marriages. Whereas, the incidence of disabled children due to lack of oxygen during the birth was significantly higher in non-consanguinity (p<0.05) as compared to consanguinity (**Table 1**).

Studies showed that educational level of the parents is also responsible for the rising numbers of disabled persons.[5] According to the results of this

study, most of the mothers consisted of those who were illiterate (17%), literate (2.1%) or had primary school education (66.3%) The rate of mothers with secondary school (5.1%), high school (6.6%) or university education (2.9%) was very low. The majority of the fathers had primary school education (56.3%), while the fathers with a university education was 12.7%, which was higher than the mothers. The frequency of fathers who were illiterate was 1.8%, literate 1.1%, with secondary school 9.9% and high school 18.3% The relationship between disability and poverty has been clearly established by several studies.[5] The risk of disablement is higher for the poor stratum of the population. In the present study, the majority of families (68.7%) were poor, while 21.3% had normal, 8.9% good, and 1% had very good economic conditions.

The results of this study demonstrated that childhood disability in the Kahramanmaras population stems largely from impairments associated with lack of oxygen during the birth, postnatal diseases, and consanguinity. Our data may be a prospector and initiator for taking preventive measures such as counseling with couples on consanguinity, genetic and prenatal care factors, establishing a system for the early detection of impairments, and education of the society on causes, prevention, and treatment of disability for preventing childhood disability in Kahramanmaras.

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Brucella seropositivity in South and Southeast Turkey

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Brucellosis is a public-health problem in many developing countries, including Turkey. Although the prevalence of *Brucella* is not exactly known in Turkey, the seropositivity has been reported to be approximately 2-6%.[1] The seroprevalence of brucellosis in sheep in this country has been reported to be 1.97% and 1.43% in cows. Approximately 15,000 cases of *Brucella* infections are reported annually according to the 2001 data of the Ministry of Health of Turkey. However, it is believed that the actual number of cases is at least 50,000-100,000 per year, if the unreported and subclinical cases are considered. For this reason, brucellosis constitutes an important public health problem in our country.[2] Other than these data there were no detailed researches in our country in the last 15 years. To set the precautions, first we have to realize the importance of *Brucella* in the regions where characteristically had the highest prevalence and the cities, which are inhabited by immigrants of these regions. The purpose of this study was to investigate the seropositivity of brucellosis in different population in different regions of Southern Anatolia (Mersin, Hatay, Kahramanmaras) and Southeastern Anatolia (Erzurum, Malatya, Diyarbakir, Sanliurfa), where livestock is commonly predominant.

The study was carried out with the cooperation of study teams at 7 different regions. The subjects were chosen by a simple random-sampling method, as follows: 1. The sample group, which was used in the research, was determined by the study centers according to different patients without clinical suspicion of brucellosis 2. The sample group, was consist of patients who were referred to the hospitals without infection diseases. Also, included in this group were patients who were referred to the hospitals with complaints of fever, arthralgia, malaise and lose of appetite and were not diagnosed with brucellosis infection. The samples in these groups were first checked with Rose-Bengal slide agglutination test (RB); positive samples are selected and retested with standard serum tube agglutination (STA) and 2-mercaptoethanol tests. A total of 7458 serum samples were collected from April to October 2004 from 7 different regions namely, Mersin (1293), Hatay (1175),

Kahramanmaras (797), Erzurum (1066), Sanliurfa (989), Malatya (1137), and Diyarbakir (1001). Collected sera were transported to the laboratory at 4^0C and stored at -2^OC until the time of examination For the standardization of the tests, all samples were processed in the Microbiology laboratories of Mersin University Faculty of Medicine. All the serum samples were studied by RB and detection of the level of *Brucella* antibodies in the serum was carried out by serial dilutions (from 1:10 to 1:1280) using *Brucella* bacterial antigen (The Ministry of Agriculture, Veterinary Research Institute, Pendik, Istanbul). Each batch of the test included a positive control and a negative saline control. A definite agglutination of the suspension was read as a positive reaction. Agglutination was not seen in negative samples. For positive samples, the lowest positive titer was determined. A 1:40 titer was used as the cutoff point to determine positive reactors.[3] We treated all STA positive serum samples simultaneously with the 2-mercaptoethanol for the detection of antibody classes either IgM or IgG. Data were processed using the Statistical Package for Social Sciences software version 10.0. Statistical significance was tested with chi-square test. The subjects (n=7458) enrolled in the study were aged 6-78 (median 35.1) years *(3145 males, 4313 females)*. The seropositivity of brucellosis was 1.28% (n=96) using the test of STA. No significant difference was found between gender and age groups. One hundred seven (1.43%) of the samples were positive with the RB test. A 0.14% sample was established as false positive according to STA by the RB test, false negative was not detected with the RB test. When compared with STA, the sensitivity of RB was 99%, and its specificity was the same. Positive predictive value was 89.7%, negative predictive value was 100%. The seropositivity of group I was 1.07% (n=46) and *group II* was 1.56% (n=50). The seropositivity of IgM was 12.5% (n=12) and IgG was 87.5% (n=84). The samples of group I, which show the normal seropositivity of the public were compared according to the regions, the highest seropositivity was detected in Sanliurfa with the percentage of 1.5%, 1.1% Kahramanmaras, 1% Erzurum, 0.8% for Mersin, and Diyarbakir, 0.6% Malatya, and 0.5% Hatay. In group II the highest seropositivity was detected in Diyarbakir with the percentage of 2.8%, 2.2% Malatya, 1.8% Erzurum, 1.7% Hatay, 1.5% Kahramanmaras, and 0.5% Mersin. There were significant differences ($p<0.05$) in seropositivity with the cities compared with [Mersin-Diyarbakir (p=0.005), Mersin-Malatya (p=O.02), Hatay-Diyarbakir (p=O.008)]. There was a statistical difference in seropositivity of the 2-mercaptoethanol test between group I and group II (p=0.045). Examining the distribution table of zoonotic and vectors caused diseases, performed by the Health Ministry in 2003, the data of the total number of cases and their morbidity showed that only Mersin, Hatay, Erzurum and Diyarbakir were studied. For these countries the total number of cases and the morbidity velocity (from hundred thousand) were 70, 3.9, 64, 4.9, 119, 12.3, 1475, 101.5; and for Turkey the total number of cases in a year was determined as 14,572, morbidity velocity was 20.3 from hundred thousands.[2] The seropositivity of *Brucella* was assessed in various studies conducted in Turkey. The seropositivity of *Brucella* was reported to be 1.8-6.7% in various occupational groups of different countries.[4] The determination of the true prevalence of brucellosis, particularly in developing or underdeveloped countries may be impossible due to the inadequacy of poorly organized health center, and the failure of these centers to report all of the cases that come to their attention.[5] The first detailed study regarding *Brucella* in our country was carried out in 1987, which was supported by The Scientific and Technological Research Council of Turkey and carried out by Cetin et al.[4] In this study, 70009 samples collected from 9 different regions (Istanbul, Izmir, Ankara, Sivas, Diyarbakir, Bursa, Konya, Erzurum, Antalya) were used and antibodies for *Brucella* were reported as 1.8% in the normal population, 6% in risk groups, and 6.7% in patients with complaints similar to brucellosis. It is reported that IgM specific antibodies are seen in 16.6% of the seropositive sera.[4]

In our study, IgM antibodies were found in 12.5% (n=12) of the seropositive sera. In addition to this, we realized that Diyarbakir was the city with the highest seropositivity, similar to results obtained by Cetin et al.[1] The seropositivity of brucellosis was found to be 3.2% in elderly population in mid-Anatolia, Turkey. Brucellosis will continue to be an endemic problem in this region for a long time due to the socioeconomic difficulties of the region, the lack of effective control measures on entries and exits of animals across the border or of animal movements within the region, unlawful and unregulated slaughtering of animals and selling of meat (the consumption of meat that has not been inspected by state officials), the consumption of raw milk and products made from raw milk without pasteurization, particularly in spring, decreased the public's information or the presence of incorrect information regarding the contagion of this agent, and the inadequacy of the disease reporting system.

The risk of having *Brucella* should be considered in patients who complaints of high temperature, and in those with nonspecific infections at endemic regions,

for early diagnosis. It is also important to take drastic measures to decrease the morbidity and mortality of disease.

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Erratum

In manuscript "Gene expression profiles of the fibroblasts from breast tumors and normal tissue compared with the tumor expression profiles" Saudi Medical Journal 2006; Vol. 27 (4): 463-469, the title on the highlights should have appeared as follows: Gene expression profiles of the fibroblasts from breast tumors and normal tissue compared with the tumor expression profiles