Original Article https://doi.org/10.47748/tjvr.1245255

TJVR 2024; 8 (1): 1-6

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Paraoxonase, haptoglobin, serum amyloid A, tumor necrosis factor and acetylcholinesterase levels in ewes with pregnancy toxemia

Kamber Narin[1] İsmail Aytekin[2]

[1] *Bigadiç District Directorate of Food, Agriculture and Livestock, Balıkesir, Türkiye*

[2] *Department of Wild Animal Diseases and Ecology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye.*

Correspondence: *İsmail Aytekin (aytekin0331@gmail.com)*

Received: 01.02.2023 Accepted: 12.02.2023

ABSTRACT

Objective: In this study were investigated serum paraoxonase, haptoglobin, tumor necrosis factor-α, acetylcholinesterase, serum amyloid A, nonesterifie fatty acids, glucose, beta hydroxybutyric acid, total protein, aspartat aminotransferase, gama glutamil transferase, cholesterol and triglyceride in ewes with pregnancy toxemia

Materials and Methods: This study material consisted 10 control and 10 group with pregnancy toxemia, total 20 merino hybrid ewes at aged between 2-6 years.

Results: The analysis of blood serum samples revealed that serum amyloid A (SAA), haptoglobin (HPT), tumor necrosis factor-α (TNF), paraoxonase (PON1), acetylcholinesterase (ACHE), aspartat aminotransferase (AST), gama glutamil transferase (GGT) and cholesterol did not differ statistically significant between two groups although SAA, HPT, TNF, PON1, ACHE, AST, GGT levels were higher in ewes with pregnancy toxemia when compared to healthy ewes. Beta hydroxybutyric acid (BHBA) and nonesterifie fatty acids (NEFA) ($p<0.001$), triglyceride and total protein ($p<0.01$) increased that glucose ($p<0.001$) levels decreased in sheep with pregnancy toxemia compared with healthy ewes.

Conclusion: Acetylcholinesterase, paraoxonase, haptoglobin, tumor necrosis factor-α, serum amyloid A concentration researched may prove beneficial laboratory findings disease in sheep with pregnancy toxemia.

Keywords: *Acetylcholinesterase, Paraoxonase, Haptoglobin, Serum amyloid A, Tumor necrosis factor-alfa, Sheep*

INTRODUCTION

Pregnancy toxemia is a disease observed in sheep and goats during the final stages of pregnancy due to the disruption of carbohydrate, glucose, and fat metabolisms. The disease is observed during the last stage of pregnancy and milking period in goats (Bastan et al., 2013; Salar et al., 2018).

There are two forms of cholinesterase in mammals: acetylcholinesterase and pseudocholinesterase. Acetylcholinesterase and pseudocholinesterase are mainly produced in the liver (Kaplay, 1976; Stojevic et al., 2005). Cholinesterase has been measured in cattle, camel, goat, and sheep plasma and liver, and the highest cholinesterase levels were determined in goats (Qarawi et al., 2003).

SAA and haptoglobin levels were higher in Saanen goats with pregnancy toxemia when compared to healthy goats (Albay et al., 2014). Higher SAA levels were found in sheep with pregnancy toxemia when compared to healthy non-pregnant (Mahmoud et al., 2016).

Serum paraoxonase enzyme is associated with HDL cholesterol in mammals and protects the body against several harmful chemicals (Mackness et al., 2017). Paraoxonase levels are low in sheep with fatty liver when compared to healthy sheep (Cao et al., 2017). Paraoxonase-1 levels decrease on the first day of the last month of pregnancy in Turkish Saanen goats and increase on the prenatal 15th and 30th days (Salar et al., 2018).

Tumor necrosis factor-alpha (TNFα) is induced by various stimuli such as tumor cells, bacteria, viruses, parasites, cytokines, and inflammation. TNFα is responsible for neoplastic tissue destruction, and certain local and systemic effects induced by tumors (Çömez, 2006). TNF levels are higher in sheep with pregnancy toxemia when compared to healthy sheep. Higher TNF levels were reported in Saanen goats with pregnancy toxemia when compared to healthy pregnant goats (Albay et al., 2014).

The present study investigated the acetylcholinesterase, paraoxonase, tumor necrosis factor-α, serum amyloid A, haptoglobin, glucose, BHBA, NEFA, cholesterol, triglyceride and total protein levels in ewes with pregnancy toxemia.

MATERIALS and METHODS

Animal material

The animal material included 2-5 years old merino hybrid ewes on a farm in Balikesir province in Turkey with two or more lactation counts, 40-60 kg live body weight and were in the last 4 weeks of pregnancy and exhibited loss of appetite, depression, lethargy, muscle dysfunction, lack of coordination, ataxia and were bedridden. The ewes were clinically examined. Ten pregnancy toxemia and 10 healthy, a total of 20 merino hybrid ewes were used in the study. After the clinical examination of the merino hybrid ewes, 10 ewes with blood BHBA levels ofover 1.5 mmol/L were assigned to the pregnancy toxemia group, and 10 healthy non-pregnant ewes with BHBA levels of below 1 mmol/L were assigned to the control group. All ewes with pregnancy toxemia were in the last 4 weeks of pregnancy and were kept under the same feeding, care, and environmental conditions. All healthy non-pregnant ewes were keptunder the same feeding, care, and environmental conditions.

Blood analysis

10 ml blood samples were collected from V. jugularis of the ewes diagnosed with pregnancy toxemia into tubes without anticoagulant after the clinical examination. The blood samples were immediately centrifuged at 5000 rpm for 5 min to obtain the serum. Two samples were transferred into eppendorf tubes and stored at -80ºC until the analysis. After the blood serum samples were collected, they were sent to a private laboratory for analysis.

Biochemical analysis

Paraoxonase, acetylcholinesterase, tumor necrosis factor-α, haptoglobin, serum amyloid A levels were measured with the ELISA (Enzyme-Linked Immunosorbent Assay) method (SunRed ELISA Kit cat. No: E90440, Eastbiopharm, China). Biochemical analyses were conducted with a Randox brand Daytona model device (United Kingdom). Randox brand kits were used to study the biochemical parameters. BHBA (Cat. No: RB1007) was determined with the enzymatic kinetic method, while the principle of total cholesterol (Cat. No: CH3810) analyse based on the enzymatic endpoint method, glucose (Cat. No: GL 3815), NEFA (Cat. No: FA115) were determined with the colorimetric method, total protein (Cat. No: TP38669) was determined with the Biuret Reagent endpoint method, triglyceride (Cat. No: TR3823) was determined with the lipase/GPO-PAP method.

Ethical statement

This study was conformed according to Balikesir University Animal Experiments Local Ethics Committee Presidency instructions and approved with consensus at the meeting (2019/12-7).

RESULTS

Clinical findings

Anorexia, lagging the herd, lethargy, bad breath, shaky gait, teeth grinding, ataxia, difficulty in standing, blindness, confinement to the ground, head resting, muscle tremors, loss of consciousness, and mortality were observed in the ewes with pregnancy toxemia included in the study.

Biochemical findings

The results of the biochemical analyzes regarding to ewes with pregnancy toxemia and healthy ewes are presented in (Tables 1 and 2). The analysis of blood serum samples revealed that serum amyloid A (SAA), haptoglobin (HPT), tumor necrosis factor-α (TNF), paraoxonase (PON1), acetylcholinesterase (ACHE), aspartat aminotransferase (AST), gama glutamil transferase (GGT) and cholesterol did not differ statistically significant between two groups although SAA, HPT, TNF, PON1, ACHE, AST, GGT

levels were higher in ewes with pregnancy toxemia when compared to healthy ewes. Beta hydroxybutyric acid (BHBA), nonesterifie fatty acids (NEFA), triglyceride and total protein increased that glucose levels decreased in sheep with pregnancy toxemia compared with healthy ewes (Tables 1 and 2).

Table 1. SAA, HPT, TNF, PON1, ACHE levels in merino hybrid ewes with pregnancy toxemia and healthy merino hybrid ewes

Parameters	Healthy merino hybrid ewes (n=10)	Merino hybrid ewes with pregnancy toxemia (n=10)	P value
SAA μg/ml	5.10±3.73	8.70±6.48	NS
HPT μg/ml	112.32±63.73	172.81±101.30	NS
TNF μg/ml	45.28±26.02	73.09±50.04	NS
PON 1 μg/ml	67.20±48.74	110.42±75.77	NS
ACHE ng/ml	10.69±7.18	15.03±9.57	NS

* p<0.05, ** p<0.01, *** p<0.001, NS: Not Significant

Table 2. Biochemical parameters in merino hybrid ewes with pregnancy toxemia and healthy merino hybrid ewes

Parameters	Healthy merino hybrid ewes (n=10)	Merino hybrid ewes with pregnancy toxemia (n=10)	p value
BHBA mmol/L	0.47±0.12	2.83±1.34	p<0.001
Glucose mg/dL	43.80±6.72	24±6.46	p<0.001
NEFA mmol/L	0.14±0.16	1.30±0.63	p<0.001
Total Cholesterol mg/dL	44.5±10.90	47.2±13.25	NS
Triglyceride mg/dL	15.80±4.02	22.10±6.53	p<0.01
Total Protein g/dL	4.75±0.99	5.98±1.25	p<0.01
GGT U/L	33.90±8.06	40.30±9.35	NS
AST U/L	78.1±19.93	129.4±109.06	NS

* p<0.05, ** p<0.01, *** p<0.001, NS: Not Significant

DISCUSSION

Pregnancy toxemia is a disease observed in sheep and ewes during the last stages of pregnancy due to the disruption of carbohydrate, glucose, and fat metabolisms (Bastan et al., 2013; Albay, 2014; Salar et al., 2018).

It was reported that lagging behind the herd, loss of appetite, lethargy, shaky gait, bad breath, difficulty in standing, teeth grinding, blindness, head resting, confinement to the ground, loss of consciousness, muscle tremors, and mortality are observed in ewes with pregnancy toxemia (Mahmoud et al., 2016; Salar et al., 2018; Asmaa et al., 2019; Gaadee et al., 2021). In the present study, loss of appetite, lagging behind the herd, teeth grinding, bad breath, shaky gait, ataxia, head resting, blindness, muscle tremors, incoordination, the difficulty of standing, confinement to the ground, loss of consciousness, and mortality were observed in ewes with pregnancy toxemia.

Glucose levels were lower in sheep with pregnancy toxemia when compared to healthy sheep (Kabakçı et al., 2003; Mahmoud et al., 2016; Prasannkumar et al., 2016; Gaadee et al., 2021). It was reported that glucose levels were lower in Awassi sheep with pregnancy toxemia when compared to both healthy non-pregnant and healthy pregnant sheep (Khaled, 2011). The glucose level in Barki sheep with pregnancy toxemia was also decreased when compared to both healthy non-pregnant and healthy pregnant sheep (Mahmoud et al., 2016). In the present study, it was determined that the glucose levels in ewes with pregnancy toxemia were lower when compared to the glucose levels of healthy ewes.

It was reported that total protein levels were lower in sheep with pregnancy toxemia when compared

to healthy non-pregnant and healthy pregnant sheep (Gaadee et al., 2021). Total protein levels were lower in sheep with pregnancy toxemia when compared to healthy pregnant sheep (Asmaa et al., 2019). In the present study, although total protein levels were increased in ewes with pregnancy toxemia when compared to healthy sheeps.

It was found that BHBA levels were higher in sheep with pregnancy toxemia when compared to healthy sheep (Khaled, 2011; Gurdoğan et al., 2014; Mahmoud et al., 2016). Higher BHBA levels were reported in sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep in the last month of pregnancy (Mahmoud et al., 2016). BHBA levels were higher in ivesi sheep with pregnancy toxemia when compared to both healthy and pregnant sheep with subclinical toxemia (Gurdoğan et al., 2014). It was reported that BHBA levels were higher in Awassi sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep (Khaled, 2011). In the present study, it was established that BHBA levels in ewes with pregnancy toxemia were higher when compared to healthy ewes.

The NEFA levels increased more in sheep with clinical pregnancy toxemia when compared to both healthy non-pregnant and healthy pregnant sheep in the last period of pregnancy (Asmaa et al., 2019). The NEFA levels were found to be higher in sheep with pregnancy toxemia when compared to healthy sheep (Sauza et al., 2019). It was found that NEFA levels were higher in sheep with pregnancy toxemia when compared to non-pregnant and healthy sheep in the last month of pregnancy (Mahmoud et al., 2016). In the present study, NEFA levels were higher in ewes with pregnancy toxemia when compared to healthy ewes.

A study conducted on Barki sheep indicated that the cholesterol levels were lower in healthy sheep in the last period of pregnancy when compared to the healthy non-pregnant sheep (Asmaa et al., 2019). It was reported that cholesterol levels decreased in sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep in the last month of pregnancy (Gaadee et al., 2021). It was found that the cholesterol levels were lower in sheep with pregnancy toxemia compared to healthy sheep (Prasannkumar et al., 2016). In the present study, no statistically significant difference was found between the cholesterol levels of the ewes with pregnancy toxemia and healthy ewes.

Triglyceride levels were higher in sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep in the last month of pregnancy (Mahmoud et al., 2016). Higher triglyceride levels were determined in sheep with pregnancy toxemia when compared to healthy sheep (Kabakçı et al., 2003). It was found that the triglyceride levels were higher in Saanen goats with pregnancy toxemia when compared to healthy pregnant goats (Albay et al., 2014). In the present study, triglyceride levels were higher in ewes with pregnancy toxemia when compared to healthy ewes.

Pseudocholinesterase levels were determined in forty Nubian goats, and the topical fenthion administration decreased the pseudocholinesterase levels (Fuentes et al., 2006). Acetylcholinesterase levels were lower in clinical ketosis cows when compared to the control group (Simonov et al., 2015). In a study conducted on the liver parameters of cows with ketosis, it was found that the cholinesterase values were lower when compared to the control group (Sun et al., 2015). Three distinct types of cholinesterase levels were measured in the plasma and liver tissue of the cattle, camels, goats, and sheep, and the highest cholinesterase levels were determined in goats (Qarawi et al., 2003). In the present study, it was found that the acetylcholinesterase levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

SAA levels were higher in Saanen goats with pregnancy toxemia when compared to healthy pregnant goats (Albay et al., 2014). Higher SAA levels were found in sheep with pregnancy toxemia when compared to healthy non-pregnant (Mahmoud et al., 2016). In a study conducted on Barki sheep, SAA levels were found higher in healthy pregnant sheep in the last period of the pregnancy when compared to the healthy non-pregnant sheep (Asmaa et al., 2019). Another study conducted on Ivesi sheep also found that SAA levels increased in sheep with pregnancy toxemia when compared to healthy sheep (Gurdoğan et al., 2014). In the present study, it was found that the SAA levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

It was also determined that the haptoglobin levels increased more in sheep with pregnancy toxemia when compared to healthy non-pregnant sheep (Asmaa et al., 2019). Haptoglobin levels were found to be higher in sheep with pregnancy toxemia when

compared to healthy non-pregnant (Mahmoud et al., 2016). In Ivesi sheep with pregnancy toxemia haptoglobin levels were increased when compared to healthy and pregnant sheep with subclinical pregnancy toxemia (Gurdoğan et al., 2014). In the present study, it was found that the haptoglobin levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

It was reported that the paraoxonase levels in sheep with fatty liver decreased on the 8th and 16th days when compared to healthy sheep (Cao et al., 2017). It was found that the serum paraoxonase levels were lower in postpartum Holstein cows with fatty liver when compared to healthy non-pregnant cows (Farid et al., 2013), however, the authors also argued that there was no standardized method for paraoxonase measurement, commonly used method was paraoxon hydrolysis and different results could be obtained due to methods that measured paraoxonase, thus, concluded that the physiological interpretations of these levels were challenging (Farid et al., 2013; Camps et al., 2009). In a study conducted on Saanen goats in Turkey, it was reported that the serum paraoxonase-1 levels in the last month of pregnancy were lowest on the 0th day, high on the -15th day, and highest on the -30th day (Salar et al., 2018). In the present study, it was found that the paraoxonase levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

It was found that the TNF values were higher in animals with ketosis when compared to healthy animals (El-Deep et al., 2017; Zhanga et al., 2018). The TNF levels in cows with subclinical ketosis were higher when compared to healthy cows (Brodzki et al., 2021). A study conducted on Barki sheep revealed that TNF levels increased in sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep in the last period of pregnancy (Asmaa et al., 2019). TNF levels increased in sheep with mild and severe pregnancy toxemia when compared to healthy sheep (Yarım et al., 2007). In the present study, it was found that the TNF levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

CONCLUSION

In conclusion, acetylcholinesterase, paraoxonase, haptoglobin, tumor necrosis factor-α, serum amyloid A concentration researched may prove beneficial laboratory findings disease in sheep with pregnancy toxemia.

REFERENCES

Abdelaal A, Zaher H, Elgaml SA, Abdallah H. Prognostic value of serum cardiac troponin and nitric oxide as cardiac biomarkers in pregnancy toxemic goats. Global Vet. 2013; 11:817-823.

Albay MK, Karakurum MC, Sahinduran S, Sezer K, Yıldız R, Buyukoglu T. Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. Vet Medicina. 2014; 59(7):336-342.

Asmaa A, Darwish IAElE. The diagnostic value of acute phase proteins in barki ewes with pregnancy toxemia. AJVS. 2019; 62(1):27-37.

Aytekin İ. Gebelik toksemisi. Koyun ve keçi hastalıkları. 2nd ed. Bursa: Stüdyo Star Ajans;. 2020. p.203-205.

Bastan İ, Salar S. Koyun ve keçilerde gebelik toksemisi. Harran Üniv Vet Fak Derg. 2013; 2(1):42-47.

Brodzki P, Marczuk J, Lisiecka U, *et al.* Comparative evaluation of cytokine and acute-phase protein concentrations in sera of dairy cows with subclinical and clinical ketosis as a different view of the causes of the disease. Vet World. 2021; 14:1572-1578.

Camps J, Marsillach J, Joven J. Measurement of serum paraoxonase-1 activity in the evaluation of liver function. World J Gastroenterol. 2009; 15(16):1929-1933.

Cao Y, Zhang J, Yang W, *et al.* Serum paraoxonase as an indicator for fatty liver in sheep. J Vet Res. 2017; 61:97-102.

Çömez Yİ. Prostat adeno kanseri tanısı ile takip edilen hastalarda TNF-alfa-308 A/G polimorfizim sıklığının belirlenmesi ve belirli parametreler ile ilişkisinin belirlenmesi. [PhD Thesis]. İstanbul: Sağlık Bakanlığı Kartal Dr. Lütfi Kırdar Eğitim ve Araştırma Hastanesi; 2006.

El-Deeb WM, El-Bahr SM. Biomarkers of ketosis in dairy cows at postparturient period: acute phase proteins and pro-inflammatory cytokines. Vet Arhiv. 2017; 87(4):431-440.

Farid AS, Honkawa K, Fath EM, Nonaka N, Horii Y. Serum paraoxonase-1 as biomarker for improved diagnosis of fatty liver in dairy cows. Vet Res. 2013; 9:73-89.

Felix HD, Gonzalez HF, Madrid J, *et al.* Acute phase proteins in experimentally induced pregnancy toxemia in goats. J Vet Diagn Invest. 2011; 23:57-62.

Fuentes VO, Fuentes PI. Butyrlcholinesterase levels in nubian and saanen goats in the highlands of Mexico: Effect of organophosphate fenthion after topical application. J. Anim Vet Adv. 2006; 11:278-881.

Gaadee HIM, Gehan MS. Pregnancy toxemia in ovis aries, and role of metabolic disorder causing ıt. Res J Vet Pract. 2021; 9(1):1-8.

Gurdogan F, Balıkçı E, Yıldız A. Some acute phase proteins, oxidative stres biomarkers and antioxidant enzyme activities in ewes with pregnancy toxemia. IJVR. 2014; 15(3):297-299.

Kabakcı N, Yarım G, Yarım M, Duru Ö, Yagcı BB, Kısa Ü. Pathological, clinical and biochemıcal investigation of naturally occuring pregnancy toxemıa of sheep. Acta Vet.2003; 53(2):161-169.

Kaplay SS. Acetylcholinesterase and butyrylcholinesterase of developing human brain. Biol Neonate. 1976; 28:65-73.

Khaled MA. Oxidant and antioxidant profile of hyperketonemic ewes affected by pregnancy toxemia. Vet Clin Pathol. 2011; 40(1):60-65.

Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. Gen Pharmac. 1998; 31:329-336.

Mahmoud A, Ibrahim IE. Clinico-biochemical diagnosis of pregnancy toxemia in ewes with special reference to novel biomarkers. AJVS. 2016; 48(2):96-102.

Petersen HH, Nielsen JP, Heegard PMH. Application of acute phase protein measurements in veterinary clinical chemistry. Vet Res. 2004; 35:163-187.

Prasannkumar R, Vasava RG, Jani HV, Goswami SD, Tandel FB. Studies on clinical signs and biochemical alteration in pregnancy toxemic goats. Vet World. 2016; 9(8):869-874.

Qarawi AA, Ali BH. Variation in the normal activity of esterases in plasma and liver of camels (Camelus dromedarius), Cattle (Bos indicus), Sheep (Ovis aries) and Goats (Capra hircus). J Vet Med A Physiol Pathol Clin Med. 2003; 50:201-203.

Salar S, Baştan İ, Başţan A, Pekcan M, Sel T. Investigation of changes in metabolic parameters and paraoxonase-1 during the transition period in turkish saanen goats. Kafkas Univ Vet Fak Derg. 2018; 24 (1):117-122.

Samimi AS, Aghamiri SM, Nazifi S, Asadi Z, Farhang M. Changes of acute-phase proteins during different physiological conditions in dairy saanen goats. Comp Clin Pathol. 2020; 29:729-732.

Sımonov M, Vlızlo V. Some blood markers of the functional state of liver in dairy cows with clinical ketosıs. Bulg J of Vet Med. 2015; 18(1):74-82.

Souza LM, Mendonça CL, Assıs RN, Filho EFO, Gonçalves DNA, Souto RJC, Soares PC, Afonso JAB. Cardıac bıomarkers troponın and ck-mb ın ewes affected by pregnancy toxemıa. Small Rumin Res. 2019; 177:97-102.

Stojevic Z, Pirsljin J, Milinkovictur S, Zdelartuk M, Ljubic B. Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. Vet Arhiv. 2005; 75:67-73.

Sun Y, Wang B, Shu S, *et al*. Critical thresholds of liver function parameters for ketosis prediction in dairy cows using receiver operating characteristic (ROC) analysis. Vet Quarterly. 2015; 35(3):159-164.

Yarım GF, Karahan S, Nisbet C. Elevated plasma levels of ınterleukin 1β, tumour necrosis factor α and monocyte chemotactic protein 1 are associated with pregnancy toxaemia in ewes. Vet Res Com. 2007; 31:565-573.

Zhanga Y, Lia X, Zhang H, *et al*. Non esterified fatty acids over-activate the tlr2/4-nf-κb signaling pathway to ıncrease ınflammatory cytokine synthesis in neutrophils from ketotic cows. Cel Physiol and Biochem. 2018; 48:827-837.

ACKNOWLEDGMENTS

This study was summarized from first author's master thesis.

Author contributions: İA and KN designed the study. İA and KN participated in drafting and revising the manuscript.

İA: İsmail Aytekin, KN: Kamber Narin

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to İA.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.