REPUBLIC OF TÜRKİYE
ERCIYES UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF VETERINARY INTERNAL MEDICINE

SERUM ALPHA-1-ACID GLYCOPROTEIN CONCENTRATIONS IN COMMON INFECTIOUS DISEASES OF CATS IN DIFFERENT AGE GROUPS

Prepared By
Humaira Ahmad KHAN

Supervisor
Prof. Dr. Vehbi GÜNEŞ

Master's Thesis

January, 2023
KAYSERİ

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The present study was supported by Erciyes University Scientific Research Projects Unit (BAP) with project number 11510.

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KAYSERİ

COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that all the information in the present study was obtained in accordance with academic and ethical rules. I also state that I have fully cited and referenced all materials and results that are not inherent in the present study, as these rules and behavior require.

Humaira Ahmad KHAN

Signature ……

SUITABILITY FOR GUIDE

The MSc thesis entitled "**Serum Alpha-1-Acid Glycoprotein Concentrations in Common Infectious Diseases of Cats In Different Age Groups**" has been prepared in accordance with Erciyes University Institute of Health Sciences thesis preparation and writing guide.

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ACCEPTANCE AND APPROVAL PAGE

The present study entitled "**Serum Alpha-1-Acid Glycoprotein Concentrations in Common Infectious Diseases of Cats in Different Age Groups**" prepared by **Humaira Ahmad KHAN** under the supervision of **Prof. Dr. Vehbi GÜNEŞ** was accepted by the jury as MSc. Thesis in Department of Veterinary Internal Medicine, Erciyes University Institute of Health Sciences.

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Juror: Prof. Dr. İlker ÇAMKERTEN

Juror: Prof. Dr. İhsan KELEŞ

APPROVAL:

That the acceptance of this thesis has been approved by the decision of the Institute's Board of Directors with the ……...…….. date and ……. numbered decision.

……. /……../ ……

Prof. Dr.Bilal AKYÜZ

Institute Director

DEDICATION

Dedicated

To

My beloved parents, teachers and

My supporting & loving husband **Dr. Muhammad Imran LEGHARI**

ACKNOWLEDGMENT

This master's thesis was carried out in the Erciyes University Veterinary Training, Research and Practice Hospital. The thesis study was conducted between 01/12/2021 and 01/12 /2022 as a part of **Erciyes University Scientific Research Projects Unit (BAP)** with project number TYL-2021-11510.

In the name of Allah, the Most Gracious and the Most Merciful. All praises to Allah for the strengths and His blessings in completing this thesis. There are many people whom I want to acknowledge for their support, help and encouragement during the journey of preparing this thesis.

Being in a foreign country and working in an active veterinary hospital has been a period of intense learning for me, not only in the scientific arena, but also on a personal level. Writing this thesis has had a big impact on me. I would like to reflect on the people who have supported and helped me so much throughout this period.

I would particularly like to single out my supervisor **Prof. Dr. Vehbi GÜNEŞ**, Department of Veterinary Internal Medicine, Erciyes University, Kayseri. I want to thank you for your excellent cooperation and for all of the opportunities I was given to conduct my research. It is whole-heartedly expressed that your advices for my research proved to be a landmark effort towards the success of my research. Words cannot express my gratitude to my professor, a fatherly figure for me, whose valuable patience and feedback helped me successfully surpass any hurdles and obstacles.

I would like to pay special thankfulness, warmth and appreciation to the persons below who made my research successful and assisted me at every point to cherish my goal. Major member of the supervisory committee, **Prof. Dr. İhsan KELEŞ**, Department of Veterinary Internal Medicine, Erciyes University, Kayseri, whose help and sympathetic attitude at every point during my research helped me to work on time.

I appreciate the sincerity, help and encouragement of all my teachers **Prof. Dr. Öznür ASLAN, Prof. Dr. Mehmet ÇİTİL, Prof. Dr. Ali Cesur ONMAZ, Dr. İlknur KARACA BEKDİK,** for their never-ending support and trust in me. Special thanks to **Dr. Gencay EKİNCİ** and **Arş. Gör Emre TÜFEKCİ**, for their guidance at every step of making this project possible.

Thanks to my parents, especially my father **Dr. Hussain Ahmed KHAN**, for always encouraging me and letting me know nothing is impossible to achieve for a woman if she struggles to make it possible. Words cannot express my gratitude for my mother **Mrs. Nabeela Sabahat KHAN** whose prayers made it all possible for me.

Thanks to my husband **Dr. Muhammad Imran LEGHARI** for putting up with my craziness, letting me fulfill my dreams, for always being so encouraging, supportive and for extending his support morally and emotionally. This endeavor would not have been possible if he was not there for me.

I would be remiss in not mentioning my siblings **Hadia Ahmed KHAN** and **Muhammad Abdul Muqsit KHAN**, who were there to cheer me up whenever I was at my lowest.

I would also like to thank my friend **Alfatih Mohammad Ahmed ABOZAID** for his constant support and help. Special thanks to all my friends and colleagues who worked with me in the Erciyes University Veterinary Training, Research and Practice Hospital. Thanks to laboratory assistant **Hayal TAŞKIN** for her love and support.

Humaira Ahmad KHAN

January, 2023

Kayseri

FARKLI YAŞ GRUPLARINDAKİ KEDİLERİN YAYGIN ENFEKSİYÖZ HASTALIKLARINDA SERUM ALFA-1-ASİT GLİKOPROTEİN KONSANTRASYONLARI

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ÖZET

Tez çalışmasının amacı alfa 1-asit glikoprotein (AGP)'in kedilerde hastalık teşhisi için potansiyel bir biyobelirteç olarak yeterliliğini araştırmak ve kedilerin çeşitli yaygın enfeksiyonlarında serum AGP konsantrasyonlarını belirlemektir. Fiziksel klinik muayene, hematoloji, biyokimya analizi, idrar analizi, ultrasonografi ve radyoloji gibi önemli parametrelerin analizleri de yapılmıştır. Bu tez çalışmasına çeşitli yaygın enfeksiyonlara sahip toplam 45 kedi dahil edilmiş ve 5 hastalık grubuna ayrılmıştır (FIP= 13, non-spesifik gastroenterit= 13, akut hepatit= 4, solunum yolu enfeksiyonu= 7 ve alt üriner sistem enfeksiyonu= 8), ve 10 sağlıklı kediden oluşan bir kontrol grubu da kullanılmıştır. Çalışmada yer alınan kediler çeşitli yaş gruplarına ve ırklara aitti. Hasta kedilerde ortalama serum AGP konsantrasyonları FIP için 260.29 ± 11.57 mg/L, non-spesifik gastroenterit için 253.18 ± 26.56 mg/L, akut hepatit için 326.37 ± 20.32 mg/L, solunum yolu enfeksiyonları için 185.46 ± 23.60 mg/L ve alt üriner sistem enfeksiyonları için 197.36 ± 40.27 mg/L olarak hesaplanmıştır. Kontrol grubundaki kediler için ortalama serum AGP konsantrasyonu 200.28 ± 19.38 (mg/L) olarak hesaplanmıştır. Hepatitli kedilerin ortalama serum AGP düzeylerinin diğer hastalık grupları ve kontrol grubuna göre oldukça yüksek olduğu belirlenmiştir. Hastalık grubunun ortalama serum AGP konsantrasyonlarının kontrol grubuna göre istatistiksel olarak anlamlı oranda yüksek olduğu bulunmuştur (P= 0.032). Serum AGP konsantrasyonlarının hesaplanmasına ek olarak, sonuçları karşılaştırmak ve akut faz proteinlerinden daha iyi bir tanısal biyobelirteç olduğunu değerlendirmek için serum Amiloid A (SAA) konsantrasyonları da hesaplanmıştır. Hastalık grubunun ortalama serum SAA konsantrasyonları kontrol grubundan yüksekti, ancak serum AGP konsantrasyonlarının sonuçları gibi istatistiksel açıdan önemli değildi (P= 0.451).

Serum AGP analiz sonuçları, tez çalışmasında güçlü bir biyobelirteç özelliği sergilemiş ve elde edilen sonuçların gelecekte serum AGP konsantrasyonlarının yaygın kedi bulaşıcı hastalıklarının hastalık teşhisi için güvenilir bir biyobelirteç görevi görebileceği sonucuna varılmıştır.

Anahtar Kelimeler: Alfa-1-Asit Glikoprotein; kedi enfeksiyöz hastalıkları; yarışmalı ELISA.

SERUM ALPHA-1-ACID GLYCOPROTEIN CONCENTRATIONS IN COMMON INFECTIOUS DISEASES OF CATS IN DIFFERENT AGE GROUPS

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Master Thesis, January 2023

Supervisor: Prof. Dr. Vehbi GÜNEŞ

ABSTRACT

The purpose of the present study is to calculate the serum alpha-1-acid glycoprotein (AGP) concentrations in various common infections of cats to investigate the competency and sensitivity of AGP as a potential biomarker for diagnosis of diseases. Analyzes of important parameters such as physical clinical examination, hematology, biochemistry analysis, urinalysis, ultrasonography and radiology were also performed. In this thesis study a total for 45 cats with various common infections were included, and divided into 5 disease groups (FIP= 13, non-specific gastroenteritis= 13, acute hepatitis= 4, respiratory tract infection= 7, and lower urinary tract infection= 8) and a control group containing 10 healthy cats. Cats recruited in the study belonged to various age groups and breeds. Mean serum AGP concentrations in patient cats were calculated as for FIP= 260.29 ± 11.57 (mg/L), non-specific gastroenteritis= 253.18 ± 26.56 (mg/L), acute hepatitis= 326.37 ± 20.32 (mg/L), respiratory tract infections= 185.46 ± 23.60 (mg/L), and lower urinary tract infections= 197.36 ± 40.27 (mg/L). Mean serum AGP concentration for cats in the control group was calculated as 200.28 ± 19.38 (mg/L). It was determined that the mean serum AGP levels of cats with hepatitis were quite high compared to the other disease groups and the control group. The mean serum AGP concentrations of the disease group were found to be significantly higher than the control group (P= 0.032). In addition to serum AGP concentrations, serum Amyloid A (SAA) concentrations were also determined to compare results and evaluate whether it is a better diagnostic biomarker than other acute phase proteins. The mean serum SAA concentrations of the disease group were higher than the control group, but were not significant (P= 0.451), as were the results of serum AGP concentrations. Serum AGP analysis results showed a strong biomarker feature in the present study and it was concluded that serum AGP concentrations could serve as a reliable biomarker for the diagnosis of common feline infectious diseases in the future.

Keywords: Alpha-1-Acid Glycoprotein (AGP); Feline infectious diseases; competitive ELISA.

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ABBREVIATION AND SYMBOLS

AGP	: Alpha-1-acid Glycoprotein
ALP	: Alkaline phosphatase
APP	: Acute Phase protein
APR	: Acute Phase reaction
AST	: Aspartate Aminotransferase
CBC	: Complete Blood Count
CG	: Control Group
DG	: Disease Group
DNA	: Deoxyribonucleic acid
ELISA	: Enzyme-linked immunoassay
FCoV	: Feline Coronavirus
FCV	: Feline Calicivirus
FeLV	: Feline leukemia virus
FHV	: Feline Herpes Virus
FIP	: Feline infectious peritonitis
FIV	: Feline Immune-deficiency virus
FPV	: Feline Panleukopenia virus
MCHC	: Mean Corpuscular Hemoglobin Concentration
MCV	: Mean Corpus Volume
MPV	: Mean Platelet Volume
OD	: Optical Density
PCR	: Polymerase chain reaction
RBC	: Red Blood cells
SAA	: Serum Amyloid A
Temp.	: Temperature
WBC	: White Blood cells

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1. INTRODUCTION AND AIM

Inflammation is an important component of the pathogenesis of the immune system, metabolic and cardiovascular diseases against infections. Early detection of organ dysfunction using biomarkers of inflammation in cats is essential, as the risk of progression of irreversible organ damage is high in a variety of commonly known infections. AGP is an acute phase protein and has been noted to be a marker of inflammation and neoplasia in serum samples from humans and dogs. AGP concentration increases two to five-fold during acute phase response such as sepsis and inflammation. However, AGP concentrations and its potential effects on various common infections have not been evaluated in cats. The basic aim of this thesis study is to examine differences in AGP concentration in clinically healthy cat population and to compare AGP concentrations in cats with various diseases.

Inflammatory biomarkers such as AGP are recognized as prognostic tools to predict risk, control response to therapy, and adjust nutritional biomarkers for accurate interpretation. AGP is a major plasma protein with a variety of physiological roles, including immune modulation, binding and transport of basic or neutral compounds including drugs, capillary barrier function maintenance, and metabolic regulation. AGP is a positive acute phase protein whose concentration in the blood increases in various conditions such as infections, cancers, injuries and obesity. AGP has been reported to exhibit immunomodulatory functions in various cell types, including leukocytes, platelets, and endothelial cells. The diagnostic potential of AGP is not only limited for the diagnosis of viral disease it can also be used for the diagnosis of clinical situations

such as gastroenteritis, cystitis, urolithiasis, pancreatitis, hepatopathy, injection site sarcoma, renal failure, etc. For this reason, in this thesis study cats with different ages and having various infections were recruited. It was also aimed to investigate the comparative analysis of AGP with SAA levels, another acute phase protein whose effectiveness as a biomarker has also been well demonstrated in various studies.

2. GENERAL INFORMATION

2.1.Domestication of cats

Wildcats are a solitary Old-World species; whereas five subspecies are found in the regions of Europe, sub-Saharan Africa, China, Central Asia and the Near East. Scientists managed to gather genetic material from modern-day domestic and wild cats, from three different continents. Their analysis shows that about 130,000 years ago the ancestor of all domestic felines lived in the Near East. The O'Brien wildcats that lived in the Fertile Crescent extended from the eastern Mediterranean to the regions of Turkey and Mesopotamia. Upon gathering information regarding five mitochondrial DNA lineages in modern felines, the team of researchers believe that the domestication of cats actually happened in the Middle East. Cats have been opportunistic creatures that learned to take advantage from the civilizations, and also learned to interact with humans. With the passage of time the people not only became lenient towards the cats but also started to welcome the cats in their homes and provided them with a comfortable and cozy place to take rest. Ancient Egyptians preserved both the animals and the people, so they could live for eternity. The process is called mummification (Armitage and Clutton-Brock 1981).

To understand the social, cultural and environmental history of ancient Egypt, animal mummies have proven to be a rich source of information. In old times, four different types of animal mummies were preserved. Some of them are the pet animal mummies, some are the animal offerings for humans that die, and some are the sacrificial animal mummies. Pet animal mummies were made of those animals that were loved by their owners. These pets were carefully and respectfully mummified and then buried either within the owner’s graves or alongside the graves of the owner or the family. It has also

been noticed that most of the beloved pet animal mummies have their own coffins. The animal mummies made for the human offerings were mummified with the belief that the humans would not starve in their eternal life.

The Cairo Museum has the world's largest number of animal mummies on display. In the Cairo Museum, the last study made of the animal mummies was carried out in 1905; since then, for the purpose of showcasing the mummies for a wider audience, the mummies have been transferred to other museums in Egypt (Armitage and Clutton-Brock, 1981).

In ancient Egypt, the meat of various animals including poultry were buried with the deceased either in individual coffins of their own or were wrapped and put in huge baskets alongside the owner's graves. Sacred animals, including cats, were respected and worshiped all through their lives and were also luxuriously treated upon their demise. Adored and loved creatures or the animal mummies, known as Votive mummies in ancient Egypt, were devoted as contributions to the holy places of specific gods, to whom these creatures were sacred. It was a process similar to humans lighting votive candles in the churches (Salima Ikram 1995).

During 664-332 BC, a huge number of cats were mummified by the ancient Egyptians. There has been a confusion regarding the morphology and sizes of wildcats and domesticated cats in the identification of mummified cats. Therefore, the long bones and mandible from mummified cats were genetically analyzed to support the mummy identification. In the University of California, the DNA extracted from mummies was extracted in a specialized and developed DNA laboratory. The process also included the direct sequencing of the mtDNA from each long bone and mandible. The direct sequencing was done from 246 and 402 bp of the mtDNA. The data was compared to the previously published papers and experimentations made, and the DNA evidence showed that all the three mummies, from which the sample bones were taken for experimentations, represent a common domestic cat mitotype. The data was compared with the cat specimens from the wild cats belonging to *Felis silvestris silvestris*, *Felis chaus* and *Felis silvestris tristrami.* The conclusion was made that the common ancestor of the cats is common in modern-day Egypt and the Middle East. Estimation was made that the date of origin of the mummies' mitotypes date back to 7.5 thousand years

before they were mummified. This possibly dates back to the pre-dynastic and Early Dynastic Periods of Egypt. This data is very important and is the very first evidence to support the fact that the domestic cat *Felis silvestris cactus* was used by Egyptians in old time for the purpose of making votive mummies. It also supports the fact that the Egyptians started to domesticate the cats long before they started to mummify the animal. Ancient Egyptian culture is famous for its veneration and cat mummification (Ginsburg et al. 1991).



Figure 2.1. Photograph of mummified cat by Richard Barnes, National Geographic

Genetic studies on mitotypes of the domesticated cats show that the domestication of cats actually happened in the Near Eastern regions (Lipinski et al. 2008). The occurrence of cats in Egypt may be the result of migrations or trade to Egypt from the Levant region (Kurushima et al. 2012). Domestic cats are loved around the world, being one of the most admired and desired pets. After the sampling and data analysis from cats in Quanhucun, a village in China, it was found that around 5300 years ago, cats hunted grain-eating animals. This also shows the relationship between humans and cats, because cats may have been fed by humans (Hu et al. 2014).

Cats are well-known in history for being pest control agents and as a pet, but the domestication of cats is not well known enough. DNA analysis of the domestic cats shows that the *Felis silvestris lybica*, from Egypt and Near East, is the ancestor and the

first type of cat being domesticated. During the Neolithic period, the spread of cats is seen to have occurred geographically. When the Egyptians started to migrate and spread around the globe in the old world, the spread of cats is also seen to have accelerated during that period of time, known as the Classical period. The pattern of the geographical spread of the cats is seen along the sea and land trade routes. A change in the coat color of cats, resulted after the direct breeding, most probably after the Middle Ages. Therefore, with trade, and migrations, cats can be found in almost all remote areas of the world, except Antarctica. The cats are the most loved pets and they also served as the barn, country, and ship cats, for the purpose of pest control. Rodents are the particular reason for keeping cats as companion animals to prevent both economic losses and the spread of diseases (Ottoni et al. 2017).

Five ancestors of the domestic cats are the *Felis silvestris silvestris, Felis silvestris lybica, Felis silvestris ornata*, *Felis silvestris cafra,* and *Felis silvestris bieti.* These wildcats are known as European wildcat, Near Eastern wildcat, Central Asian wildcat, Southern African wildcat, and Chinese desert cat respectively. All of these cats spread around the world with the help of humans, either via human migration or trade (Driscoll et al. 2007).

Generally, Egypt and Cyprus have been believed to have domesticated the cats as the first civilizations in the world, but later it was found that the cats are not native to these regions, and may have been brought from the region of Levantine. Therefore, the credit for domesticating the cats equally goes to the Middle East (Driscoll and Kitchener 2009). An ancient 9500 years old grave in Cyprus, has been found to have accommodated a human and a cat. A brief timeline of domestication of cats from a wild to a domestic animal is given below in Table 2.1.

Table 2.1. Timeline of taming of cats from wild to domestic

TIMELINE	TAMING FROM WILD
According to the researchers	The Middle Eastern wildcat took thousands of years to become a pet.
10,500-9500 years ago,	Mice were found in the grain storage units in Israel. Cats found an opportunity to get close to the humans for preying on these mice.
9500 years ago,	The earliest evidence of human and cat relationship was observed, when a human and cat were found buried in the same grave, on the Mediterranean island of Cyprus.
3700 years ago,	In Israel, an ivory cat figurine was found, which suggests the presence of cats in the Fertile Crescent.
3600 years ago,	Earliest evidence of a fully domesticated cat was found in Thebes, ancient Egypt. The artists depicted the domestication of cats.
2300 years ago,	Export of cats outside Egypt was banned by the ruler Ptolemy, because cats were worshiped there.
2000 years ago,	Common domestication of cats in Europe was observed after the reveal of cat remains preserved at the German Tufting site in Schleswig. Cats can also be found in European arts and literature.
1800's	Harrison Weir, a British history artist, states the development of modern cat breeds in the British Isles.
1871	A cat show was organized in the Crystal Palace, London. The show included man-made cat breeds.
2001	Birth of first cloned kitten called “CC”, at School of Veterinary Medicine & Biomedical Sciences, Texas.

But are today's house cats really domesticated? Yes, they definitely are. Although they meet the criteria for tolerance of humans, most domestic cats are feral and do not rely on humans to feed them. And while other domestic animals such as dogs look quite

different from their wild ancestors, the average domestic cat retains much of its wild body plan. It shows a few morphological differences, such as slightly shorter legs, a smaller brain. Charles Darwin noted that a longer gut in cats may be an adaptation for cleaning kitchen scraps. The house cat did not stop developing. With artificial insemination and in vitro fertilization technology, cat breeders today are pushing domestic cat genetics into unexplored areas; they cross breed house cats with other cat breeds to create exotic new breeds. For example, the Bengal and Roach resulted from the domestic cat mating with the Asian leopard cat and caracal, respectively. The domestic cat may therefore be on the verge of an unprecedented and radical evolution into a multi-species combination future can only be imagined (Driscoll and Kitchener 2009).

2.2.Importance of Cat ownership

Cats are pets that are warmly welcomed in our home, given pet names and considered just like a family member. The exact date or time when the cat-human relationship began is still not clear. Initially it was thought that the first domestication of cats was done in Egypt. Fossils records from Cyprus suggest that the cat-human relationship dates back to around 7000 years ago (Driscoll et al. 2018). Cats are one of the most popular pets in western countries. In 1990's, the number of domesticated cats was estimated to be 35 million in European countries and 59 million in the United States of America (Serpell 1996).

In 1992, Bradshaw discussed the cat-human relationship in detail and suggested that the interaction of humans and cats is totally unique and different from any other human-animal relationship. He suggested that there is a pattern in the behavior of cats. The behavioral pattern shows similarities with the social behavior among cats themselves (Bradshaw 2016). Turner suggested that cats tend to control the relationship with humans and hence the interactions can be opportunistic sometimes. Turner observed that cat-initiated interactions tend to be longer that the human-initiated relationships, especially the ones initiated by the active owners who want to interact more often (Turner and Rieger 2001).

Mertens suggests that domestic cats prefer to interact more with adult humans, especially with adult female owners (Mertens 1991). Domestic cats tend to interact

more with female human owners. The interaction with female human owners is more intense which is due to the fact that females mostly are the members of households and feed their pets (Bradshaw 2016). Cats are one of the most popular pets with an average of 1.7 million households in the world having either one or more cats as their pets. It has been proven that a pet helps to relieve stress, provides companionship and stability in the life of the owners. It has also been observed that a pet can help in the improvement of health status of the owners (Khalifa et al. 2021).

Budiansky (2003), suggests that cats are highly adaptive to different habitats. Cats have also been observed to show signs of neoteny, which is retaining behaviors and features similar to juvenile periods of life. Neoteny features of cats help them to domesticate more easily; becoming more playful, less afraid of the environment and very adaptable to new situations. It has also been observed that many cats coming from shelters or as stray cats, find it difficult to adjust indoors with close contact with their human owners. This shows that domestication of cats and their socialization with humans is still incomplete. Cats are no doubt known as solitary animals, but socialization with other stray or domesticated cats can happen, which totally depends upon the early age of socialization (Clark 2007).

If early socialization is not done, cats tend to remain alone for the rest of their lives. It has been observed that 2 to 8 weeks of age of a cat is the most critical time for the socialization of domesticated cats with either humans or other animals (Turner and Rieger 2001). A study carried out in Austria in 2003, states that the cats are found to have emotional equivalence the same as a romantic partner. Studies show that a cat does not forget the kindness and will return the favor later. As a result of the study, it is clear that affection of a cat is no less than a dog, and it is just stereotypical to judge the love of a cat for its owner. Owners of cats are psychologically healthier as compared to those who do not own cats or any other pets. In research, the cat owners have claimed to be happier, more confident, more focused, not nervous, having better sleep hours and facing the problems in life more efficiently because of sound mindedness. Owners of cats are considered to be having a lower level of psychiatric illness, making them psychologically healthier than the ones owing no pet at all (Straede and Gates 1993).

2.3.Importance of routine health care of cats

Nowadays cats live longer because of special care and good focus on their routine health check. As veterinarians, it is our duty to make sure cats live a long quality life. When a cat reaches the age of 7 to 10 years, it starts to show aging signs. Some cats do not show the aging signs as late as 12 years of age. Some cats do not show any age-related changes at all, which makes the owners assume there are no concerned health issues. After a certain age, there is a prominent decrease in general health, body scoring and in activities of domesticated cats. If special care and attention is given to senior cats, they can lead healthier, longer and more comfortable lives. A geriatric health screening process is normally performed by veterinarians for the cats. In older cats, unfortunately, the scientific information of clinical laboratory abnormalities and normal blood pressure units and measurements is quite insufficient. In the past few years, the life expectancy of cats has increased in European countries and in the United States. The increase in life expectancy of domesticated cats has increased the number of senior and geriatric cats. Most veterinary clinics offer a package of geriatric health care that includes a detailed and thorough history taken by the owner, general physical examination, measurement of blood pressure, and some other detailed body examinations including eye examination. Due to the fact that the information of clinical and laboratory abnormalities in older cats is insufficient, therefore, it becomes difficult to interpret the laboratory test results (Paepe et al. 2013)

Every year millions of domesticated cats are abandoned at shelters for euthanasia. The most common reason associated with abandonment of domesticated cats is unfavorable prognosis and urological symptoms. Recently, it has been reported that 23% to 67% of cats have oral health issues. In addition to that, the prevalence range of obesity in domesticated cats has also increased from 1.8% to 40%, which is increasing gradually. Hyperthyroidism is also increasing frequently in cats (Buffington 2002).

2.4.Common infections of cats

To diagnose infectious diseases in a cat various options are available. For many years now, the presence of microorganisms in cats have been detected with the help of various cytological and histopathological techniques, and also microbiological cultures. These techniques have limitations, as it is required to detect an organism large enough and in a

sufficient number to be seen under a microscope. As an infection progresses, the immune system of the host's response also increases, which decreases the visualization sensitivity of the microorganisms, making it difficult to detect efficiently. Special knowledge for preservation and handling is necessary to process a culture microbiologically. The process takes way longer to progress and is not efficient clinically. A diagnostic test is required to be highly sensitive, specific, reliable, practically acceptable and cost efficient. Therefore, performing molecular assays in the diagnosis of disease in cats is recently in demand for the diagnosis of organisms that are relatively difficult to detect in a short time (Veir, Lappin, and Dow 2006).

It is believed that cats are resistant to lower urinary tract bacterial infections, but reports show that there are 1% of healthy cats having positive cultures and 1-3% of cats having lower urinary tract diseases presenting obvious clinical signs. According to research on senior cats, with co-morbidities due to chronic kidney disease, diabetes mellitus and other hormonal problems such as hyperthyroidism, have been reported to have significant urinary tract bacterial infections (White et al. 2013).

Cats are reported to have various naturally occurring infections, along with some viral infections such as leukemia virus and immunodeficiency virus. In cats, diversity and variation can be seen in clinical signs and symptoms presented by various infections including, gastrointestinal tract infections, respiratory tract diseases, diseases of central nervous system, hepatological diseases, dermatologic and ophthalmic infections, urinary tract infections, hormonal system diseases, hematological system diseases, musculoskeletal system diseases and other infections like retroviral infections. Clinical signs and symptoms are directly credited to the viral infections that are primary, but immunodeficient cats can also get various secondary bacterial and viral infections and show a variety of clinical signs (Veir, Lappin, and Dow 2006).

In cats, not only bacteria can cause lower respiratory tract infections, but parasites, fungi and many viruses can also be the reason for these infections. Due to the wide range of etiological possibilities of lower respiratory tract infections in cats, it becomes quite difficult to clinically make a tentative diagnosis. There can be an uncertainty and non-specificity in the consistency of clinical signs and symptoms presented historically, clinically, hematological and radiographically (Foster and Martin 2011).

A huge population of cat owners believe that it is necessary for domesticated cats to roam outside the house premises for a healthy and active life. It is mostly ignored that roaming outside comes with a lot of risks of disease transmission for the cats. It has been noticed that the domesticated cats that roam outside are not familiar with other street cats and can get into fights more often. There is a high risk of traffic accidents and contracting serious viral illnesses like Feline immunodeficiency viral infection. There is also a high possibility for your pet to get lost outside and suffer (Jongman 2007).

Diseases that can be seen mostly in the senior cats include epilepsy, cardiac arrest, hepatitis, diabetes mellitus, eclampsia, hypothyroidism and other metabolic, nervous system and cardiovascular system disorders. Cats at the age of 1 to 1.5 years, can be detected having diseases like cardiomyopathy and mitral valve regurgitation. Reports suggest that cardiovascular system disorders in cats increase as the age increases. To efficiently evaluate diseases in cats, the age factor plays a vital role (Braley J, 1994). In kittens, under the age of 6 months, the parasite infestation in the gastrointestinal tract is one of the most common problems. The main reason is mother-feeding in the form of a group and regularly being in contact with other kittens. There is a high probability for a kitten to get various secondary bacterial and viral infections due to immunodeficiency, if the underlying gastrointestinal tract issue is not treated in time (Sykes and Hartmann 2013).

Nematodes like *Toxocara cati* are known to transmit from cat mother to kittens via transplacental and colostral routes. Hence, it is extremely important to vaccinate the mother cat against viral and parasitic infections. It is also recommended to prevent and treat any infections occurring during pregnancy. There is also a huge probability for kittens to contract parasites from the mother cat, because of the fact that ascaris larvae can easily colonize in the mother cat's somatic tissues. This capability of ascaris larvae decreases the potential effectiveness of antiparasitic medication, and hence, the parasites are easily transmitted to the kittens during the pregnancy period (Paepe et al. 2013). Even though the incidence of cardiovascular diseases in cats increases with age, there is also a genetic tendency of various breeds of cats to have proneness to these diseases (Khalifa et al. 2021).

2.4.1. Feline infections included in the thesis study

Feline infectious peritonitis virus (FIP) is an immune mediated viral infection of feline breeds infecting both wild and domesticated cats. In 1963, a condition causing vasculitis and pyogranulomatous in cats was identified. The infection was thought to be immune mediated, causing inflammatory reactions. Later, in 1978, a virus was recognized, which was marked as feline infectious peritonitis virus in the year 1978 (Hartmann 2005). The exact transmission of FIP viral infection is under research, but the oral route has been considered to be mostly involved in FIP virus transmission from cat to cat (Takano et al. 2019). Feline coronavirus (FCoV) triggers the FIP viral infection in cats. In domesticated cats that live as a single cat, the feline coronavirus (FCoV) seroprevalence has been calculated as 25%, while of those domesticated cats that stay in the form of group, the seroprevalence is very high and is calculated to be 75-90%. Most of the time, feline corona viral infections cause mild enteritis, or tend to stay asymptomatic. There is 5-12% probability of the developing FIP viral infection (Healey et al. 2022).

Feline corona virus can be found infecting respiratory and gastrointestinal systems of many animals. The virus has two biotypes i.e, enteric and infectious peritonitis (André, Miller, and Whittaker 2020). FIP viral infection is estimated to infect 1 out of every 200 cats brought for treatment at American veterinary teaching hospitals. FIP has also been reported as one of the major factors of deaths in kittens in the United Kingdom. Feline coronavirus (FCoV) belongs to a group of positive stranded enveloped RNA viruses, known as corona-viridae. FIP viral infection is not only a frequently occurring infection in cats, but also a very fatal one. The fact that makes it more deadly is the absence of an effective and persistent management, also lacks an authentic and reliable diagnosis. If there is an availability of a tentative diagnostic tool, it would lessen the case rate that are subjectify to euthanasia. The non-specificity of clinical signs and symptoms makes it even more difficult for the veterinarians to diagnose FIP viral infection more efficiently. The pathological, hematological and biochemical abnormalities are also scarce. The specificity of commonly used tests to detect the FIP virus is also quite low (Hartmann 2005).

Ocular discharge, keratitis and uveitis are the most common clinical signs shown by the cats suffering from the non-effusive form of FIP viral infection. A veterinarian suspecting a cat to have acquired FIP, always prefers to carry an ophthalmic examination. History and physical examination play a vital role in the diagnosis of FIP. The treatment protocol for the cats suffering from FIP includes anti-inflammatory drugs and supportive medications (Andrew 2000). Good predictive values are found when the fluids of cats are subjected to diagnostic assays. Definite diagnosis of FIP viral infection could not be made while considering the variables found in the serum alone. The definitive diagnosis of a deadly disease like FIP is important and can only be achieved by histopathology or by detecting the Feline coronavirus antigen intracellularly by the immunofluorescence method. It has also been reported that feline coronavirus can be detected with the help of staining performed immunohistochemically. If effusion is present in the cat, staining of macrophages is reported to detect FIP 100% (Sykes and Hartmann 2013).

For about 40 years, Feline calicivirus infection has been considered one of the most important pathogens of cats. Feline calicivirus (FCV) is a highly pathogenic virus that affects cats under the age of 12 months. FCV belongs to the family Caliciviridae and genus Vesivirus. FCV can be both asymptomatic and symptomatic, displaying clinical signs and symptoms of the upper respiratory system and buccal cavity. It has been reported that FCV can scarcely cause pneumonia, abortions and death in infected cats. Reports suggest that in the United States and European countries, FCV can cause severe damages to the cats' health and may lead to death. Lately, a term virulent systemic disease (VSD) has been used fo the Feline calicivirus infection in these countries. Mortality rate of cats suffering from VSD in the United States and Europe has been reported to be as high as 50% (Caringella et al. 2019).

Clinical signs of FCV can range from causing ulcers of oral cavity and nasal cavities, to severe subcutaneous edema and ulcers on pinna and paws of cats. Reports suggest that FCV vaccines can reduce the intensity of the infection but cannot completely terminate it or protect the cats from being carriers. FCV is most endemic in cats found in shelters and pet stores, with a concentrated population of cats in close contact with each other. A single cat carrying FCV can easily transmit the virus to a healthy cat population. Potent

virulent variants can be exposed after the immune response of the infected cat, progressing to systemic infections. For the efficient diagnosis of the FCV, no specific laboratory or clinical marker has been developed until now. Highest mutability of the FCV at genetic levels makes it difficult to point out a specific pathogenicity marker. Best diagnosis of FCV can be made with the availability of history of infected companion cats (Caringella et al. 2019).

Endoparasites are one of the major reasons for mortality in kittens. Ascarids become pathologically notable when larvae migrate to the gastrointestinal system of the kittens (Miller 2020). Most important ascarids are *Toxocara canis* and *Toxocara cati*, posing serious public health and animal health distress (Jenkins 2020). Severe diarrhea and gastroenteritis are caused by zoonotic protozoans including *Giardia intestinalis* and Cryptosporidium spp. being zoonotic, these protozoans are transmissible to humans via contaminated water, food, or contact with the infected animals. Fecal-oral route being the primary route of transmission is responsible for water-borne diseases globally (Önder et al. 2021). Cystoisospora species infecting cats are C. felis and C. rivolta. Coccidiosis is most seen in newly-weaned kitten (Dubey 2018).

One of the most common infections of pets is urinary tract infection. It has been reported that about 12% of cats present clinical signs and symptoms of urinary tract infections. Female and older cats are the most affected ones. Clinical signs include bacteriuria, pyuria, urolithiasis or urinary neoplasia (Johnstone 2019).

Upper respiratory tract diseases are common in cats either living in shelters or in shared housing environments. Bacteria is mostly shed via ocular, nasal and oral routes. Common clinical signs and symptoms include corneal ulcers, conjunctivitis, oral ulcers, hyperemia, and sometimes blepharospasm (Nguyen et al. 2019).

Hepatitis is one of the major problems seen in cats. Most of the viral hepatic conditions are seen due to hepatitis E virus (Capozza et al. 2021). Two types of hepatitis are clinically seen in cats i.e, acute and chronic hepatitis. Cats affected with acute hepatitis present clinical signs such as jaundice, appetite loss, weight loss, vomiting, yellow discoloration of gums, and dehydration (Fragkou et al. 2016).

2.5. Acute phase proteins and their importance in cats

A natural immune reaction, acute phase response (APR), occurs due to the changes in hepatic metabolism, after the body is subjected to an immunological stress, such as infections, trauma and neoplastic growths. Cytokines are produced following the injury or an infection, along with the activation of inflammatory cells. With the help of laboratory techniques, changes such as numbering of leukocytes in blood, decrease in blood plasma levels, hormonal imbalance, altered concentrations of various minerals and changes in acute phase proteins can be measured. Positive acute phase proteins are synthesized by hepatic metabolism, including, C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp), α1-acid glycoprotein (AGP) etc. Orosomucoid was the former name given to α1-acid glycoprotein (AGP) (Gruys et al. 2005).

Acute phase proteins are considered important markers representing an activated immune response as well as inflammation process (Ceron et al. 2018). In various diseases of companion animals, the ability of APP to act as markers is very useful in clinical diagnosis. Practically, APP has been observed to reduce viral infectivity. Potency of antiviral treatment protocols and vaccines can be determined by measuring the levels of APP. Acute phase proteins hold a significant place as therapeutic agents (Perez 2019).

Generally, the changes in the levels of APP are corresponding to the severity and complications of an infection. The ability of acute phase response to be initiated even before the immune reaction, makes it most eligible to be used as a marker for diagnosis of disease. Veterinary studies and research are quite inadequate, due to high priced assay kits and very few papers being published. Few research papers published on the role of acute phase proteins in animals, support the fact that APP are useful for the diagnosis of disease, management and prognosis, in important diseases such as feline infectious peritonitis, canine inflammatory bowel disease, leishmaniasis, canine pyometra, bovine mastitis etc. In the research performed on healthy and sick cats, acute phase proteins (positive) were found to have higher levels in diseased cats as compared to the healthy ones. It was also found that levels of serum amyloid amylase (SAA) were comparatively higher in senior cats, female cats and diseased cats, while the concentration levels of α1-acid glycoprotein (AGP) and haptoglobin (Hp) were found to

be higher in older cats. Acute phase proteins have the capability to act as markers in diseased cats (Kann et al. 2012).

Positive Feline APPs include serum amyloid A (SAA) and alpha-1-acid glycoprotein (AGP). In cats, the concentrations of acute phase proteins are seen to increase during an acute inflammatory response. Studies have revealed an increased concentration of APPs in cats suffering from diseases like cystitis, renal failure, and other neoplastic and inflammatory diseases. Feline serum amyloid A (SAA) is an APP that is considered to respond primarily towards inflammation or infections. Most importantly the serum concentrations of SAA have been seen to increase during cats' exposure to diseases such as kidney diseases and urinary tract diseases (Dinallo et al. 2022).

In cats, acute phase proteins along with clinical signs, laboratory tests and other diagnostic techniques, can act as biomarkers for inflammation and to help assess prognosis of the diseases. Serum amyloid amylase (SAA) is considered to respond maximum towards inflammation, later comes AGP and Hp. C-reactive protein (CRP) is not very well known to respond actively towards inflammation. For cats, the most applicable acute phase proteins include SAA and AGP. Blood concentration levels of SAA can increase by 10 to 50 folds, in diseases such as feline infectious peritonitis (FIP), leukemia, feline calicivirus infection, chlamydiosis, leukemia, diabetes mellitus, cancer and infectious immunodeficiency infection. In European countries, AGP is used as an indicator for FIP. In cats infected with Feline immunodeficiency virus (FIV), AGP is found to be useful in early interferon treatment. Concentrations of AGP and Hp are seen to elevate in cats with anemia, or those cats suffering from pyothorax, abscesses, lymphoma, sarcomas, carcinomas, round cell tumors and fat necrosis. During inflammation, Haptoglobin (Hp) concentrations have been found to elevate 2 to 10 folds, such as in FIP (Rosa and Mestrinho 2019).

2.5.1. Feline Alpha 1-acid glycoprotein (AGP)

An acute phase glycoprotein synthesized by the liver, known as alpha-1-acid glycoprotein (AGP), has a prominent potential to act as a biomarker for diagnosis of disease and prognosis. Alpha-1-acid glycoprotein has been reported to regulate immune responses, such as pro-inflammation and anti-inflammatory reactions. It has also been reported that AGP carries ligands such as steroid hormones, serotonin, melatonin and

histamine, as well as various neutral and basic lipophilic drugs. It cannot be said that only AGP can act as a diagnostic tool for a disease; rather it can act as a biomarker for some diseases along with clinical findings and laboratory tests results (Keser et al. 2021).

Basis of every immune system response to a pathological change in the body is inflammation. Alpha-1-acid glycoprotein is not only a biomarker for diagnosis of disease but also helps in disease prognosis, tracking disease progress and response towards treatment, and also as a nutritional biomarker. While serum is considered the source of biomarkers, urine and saliva have also been extensively used by researchers to achieve similar goals (Gannon et al. 2019).

One role of AGP is to inhibit the tumor producing enzymes. This unique character of AGP helps to decrease the tumor invasion in surrounding tissues. It has also been studied that AGP plays an important role in the platelet aggregation, lymphocyte proliferation, phagocytosis and chemotaxis. Alpha-1-acid glycoprotein may also have a function of working as a defense system against microbial infections in various animals. Serum AGP concentrations have been seen to increase in both malignant and non-malignant tumors. Concentrations of AGP have been studied in different diseases in various species including humans and other animals such as dogs, cats, pigs, cows and chickens. The studies on AGP concentrations are made on diseases such as viral diseases and other inflammatory disease conditions. There are few studies on AGP concentrations in tumorous conditions in animals. A recent study was made on lymphoma in dogs and it was found that concentrations of serum AGP were elevated in dogs with lymphoma as compared to healthy dogs (Selting et al. 2000a).

In humans, pediatric nephrologists extracted AGP from the urinary samples of obese children and have found that AGP acts as a biomarker for the detection of early glomerular injury. It has also been found that AGP acts as a more potent biomarker as compared to albuminuria (Medy´nska et al. 2021). In women suffering from polycystic ovary syndrome (PCOs), AGP has been used as a biomarker. The AGP concentration levels were measured and compared its concentration levels with hormonal and metabolic abnormalities in women suffering from PCO alpha-1 acid glycoprotein

concentrations were found to be high in the women with PCOs (Freitas De Medeiros et al. 2021).

A study performed on breast cancer suggests that AGP concentrations increase in the patient's plasma. It has been found that the levels of AGP in the plasma of breast cancer patients progress with the disease progression. It can therefore be called as a biomarker for disease prognosis in breast cancer patients. Some reports suggest increased levels of AGP in patients with lung cancer (Qiong and Yin 2022). Being the first protection mechanism against an infection, AGP concentrations increase during sepsis. It has also been found that AGP concentrations decrease in plasma when antibiotic treatment protocol is followed. It may therefore act as a good biomarker for prognosis of disease, but further inquiry is required due to lack of literature on this topic (Hsiao et al. 2019).

AGP concentrations elevate in infections or injury. In cats, the elevation of AGP in the case of Feline infectious peritonitis (FIP) was reported in 1997. Recently, it has been found that the concentrations of AGP can increase in cats after the recovery from FIP or even in cats who did not suffer from the infection at all. In the effusive form of FIP, there is an elevation in the concentrations of AGP, but the levels tend to stay normal if the cat is suffering from no effusive form of FIP. During the diagnosis of disease, if the concentrations of AGP are normal, FIP is ruled out. Predicting disease outcome and prognosis, is also possible with the concentrations of AGP, such as used in humans with sepsis (Addie et al. 2022).

In cats suffering from feline chronic gingivostomatitis, AGP concentrations in serum were found elevated (Mestrinho L et al, 2020). A study made with cats suffering from obstructive feline lower urinary tract disease, it was found that serum AGP concentrations increased to 1.9-fold, whereas the SAA concentrations increased to 13 times. The study suggests that the two major positive feline APPs i.e, SAA and AGP, can help monitor inflammation in cats suffering from FLUTD (Dinallo et al. 2022).

2.5.2. Feline serum amyloid A (SAA)

All species have their own specific major and moderate acute phase proteins. In cats, serum amyloid A (SAA) has been known as the major specific APP, while others such as AGP and Hp are known as the moderate ones. Some researchers report AGP as the

major feline APP, but there is a conflict between researchers as the literature on this subject is scarce. It is also debatable that APPs may not be specific at all, and may react differently towards different diseases. SAA is found to be higher in senior and female cats. SAA is reported to have increased in feline diseases including upper respiratory tract disorders, pneumonia, pyometra, and in infectious diseases like FIP. Researchers also found that there were no notable serum concentrations of feline SAA, in cases such as cardiomyopathy, hyperthyroidism, and diabetes mellitus. It was also found that serum concentrations of SAA increase when the diseases are in their initial stages. Therefore, SAA has been found useful to act as a clinical biomarker for diagnosis of disease and prognosis. SAA serum concentrations are seen to tremendously increase in cases such as infectious diseases, trauma, surgery, tumors, and in immune mediated diseases (Cray 2012).

In the study on the serum concentrations of feline SAA in various feline diseases, it was found that SAA increases in conditions such as respiratory tract infections, gingivitis, stomatitis, gastroenteritis, pancreatitis, hepatic diseases, urinary tract and renal diseases, pyometra, FIP, trauma, tumor and in ketoacidosis condition (Yuki et al. 2020). Serum SAA concentration in lions is also reported to increase in cases such as lingual bone fracture, gastritis, hemorrhagic enteritis, pancreatic problems and hairball obstruction. The SAA levels in above mentioned conditions remained high throughout the study, showing poor prognosis (Kimura 2022).

SAA serum concentrations in healthy animals are as low as 1μg/mL, but can tremendously increase up to 1000 folds during inflammation, and decrease after treatment and recovery. Among all APPs, SAA serum concentrations have been vastly studied in the field of animal theriogenology, especially in animal reproduction, in various animals including cats, dogs, horses, and ruminants. The point of investigation is to find whether SAA can act as a trustworthy clinical biomarker of infections and inflammatory conditions. Serum SAA concentrations in female dogs suffering from pyometra are increased, because of the presence of endometrial inflammation. After the condition was surgically treated with the help of ovariohysterectomy procedure, the serum SAA concentrations were seen to decrease after post-operative treatment. SAA was found capable of being a potential biomarker in conditions such as sepsis induced

by pyometra in female dogs. An increase in the serum SAA concentrations in female cats suffering with pyometra has also been reported. Serum SAA concentrations in both canine and feline increases in conditions such as sepsis, post-operative inflammatory conditions, endometritis, endometrial hyperplasia, and in pyometra. The increase in serum SAA concentrations in the reproductive system of pets enables SAA as a clinical diagnostic and prognostic tool. However, there are some limitations in the regular clinical usage of SAA kits as a biomarker, such as, unavailability of portable testing device, analysis test is tardy and also costly, and the unavailability of reference values of SAA in laboratory practices (Bazzano et al. 2022).

3. MATERIAL AND METHODS

The research and experimentations were carried out at the Department of Internal Medicine, Faculty of Veterinary Medicine at Erciyes University, Kayseri, Turkey. The study was officially approved by the university's commission for ethics, Erciyes University Animal Experiments Local Ethics Board (ERÜ HADYEK), under the protocol no. 21/205. The owners of the cats that were recruited in the research were well informed and guided about all the procedures and protocols to be conducted during the study, and a signed copy of the consent form for their willingness of the pet owner to the procedure was obtained.

The diagnosis of disease, laboratory tests and treatment were carried out on the cats brought to Erciyes University Veterinary Training, Research and Practice Hospital. A total of 55 cats were recruited for the study, regardless of breed, age, gender or weight. The animals were divided into two major working groups: the control group (CG), composed of 10 healthy cats, both neutered and unneutered, and the Diseased group (DG), composed of 45 cats, both neutered and unneutered, with a diagnosis of various common infectious diseases including feline infectious peritonitis, feline calicivirus infection, respiratory system diseases, renal and urinary tract disorders, gastritis, giardiasis, hepatitis, pyelonephritis and ileus. All cats included in the CG and EG were subjected to extensive clinical examinations, medical histories, laboratory testing, ultrasonography and x-ray imaging (if needed), to enable accurate results. In clinical examination the evaluations included were: heart rate (bpm), respiratory rate, body temperature (°C), body hydration status, and mucosa examination.

Initially it was decided that a total of 72 cats shall participate in the study, consisting of 18 cats in the control group (CG) and 54 cats in the Disease group (DG). Due to tremendously increased economic inflation in Turkey, there has been a huge decline in

the number of cases brought for treatment at the Erciyes University Veterinary Training, Research and Practice Hospital. The pandemic lockdown scenario and later the post-Covid 19 stress also greatly affected the number of cases in the hospital. To avoid evaluation of old samples and to ensure efficient results, the samples that were collected more than 10 months ago were discarded. The above-mentioned factors greatly affected the total number of cases.

Table 3.1. Division of working groups

Animal Specie	**Working Groups**	**Number of Animals**
Cats	Group 1: Control Group (CG)	10
	Group 2: Disease Group (DG)	45
	Total Animals	**55**

3.1. Inclusion criteria

To ensure a proper record and data maintenance of the clinical cases recruited for the study, the general information of the subjects, previous treatment history, clinical physical examination record, appropriate ancillary laboratory test records, ultrasonography records, radiographic records and record of the environment the subjects are living in were attached in individual file folders of each subject. The general information of the subjects recruited for the study includes age, gender, breed, coat color, microchip details (if available), type of housing environment (i.e, either alone or shared housing with other cats), type of food provided (i.e, either commercially prepared cat food or homemade food), previous disease history, previous treatment record, previous surgical records, vaccination and deworming status, and the status of being neutered or unneutered.

The clinical examination protocols included an extensive physical examination before appropriate ancillary laboratory testing or other procedures like sonography and radiography. Physical examination of each cat recruited for the study included general body appearance confirming the body symmetry, body condition score confirming the

nutrition status, mentation and the level of consciousness towards the environment, posture and gait of the cat, hydration status (adequate, marginal or inadequate), vital signs analysis including body temperature (via rectal thermometer), heart rate (bmp), respiratory rate, perfusion or mucous membrane examination, general ophthalmic and nasal examination, buccal cavity examination, general skin examination, auscultation, percussion and abdominal palpation. Clinical signs and symptoms were recorded.

After the successful clinical examination and record keeping, the laboratory testing was done to accurately diagnose the disease, especially the evaluation of the body system involved, evaluation of the disease type (viral, bacterial or parasitic) and to evaluate the stage of the specific disease. Laboratory tests include complete blood count analysis, blood biochemistry analysis, fecal sample examination, Giemsa microscopic slide staining and urine analysis (if needed).

3.2. Sample collection

Sample collection from all cats was performed at Erciyes University Veterinary Training, Research and Practice hospital. According to the disease type and condition, three types of samples were collected from the cats, i.e, blood samples, fecal samples, and urine samples. Blood sampling was performed after drawing blood from jugular, cephalic or femoral veins. Vacutainer blood collection tubes were used to collect the blood. For complete blood count analysis, the anticoagulant EDTA based tube was used. For the blood chemistry, the gel-based serum separator tubes without an anticoagulant were used. Fresh fecal sampling was performed with the help of stool collection spatula, directly from rectum to avoid any contamination of stool with bacteria, parasitic eggs or larvae. The urine samples were collected from cats suffering from suspected urinary tract or renal diseases. Urine sampling was performed either by cystocentesis or catheterization.

3.3. Laboratory testing

After fresh sampling, the samples were immediately transferred to the laboratory at Department of Internal Medicine, Faculty of Veterinary Medicine at Erciyes University. Laboratory analysis included complete blood count analysis, blood biochemistry analysis, fecal sample microscopic examination, Giemsa staining and urine analysis.

3.3.1. Complete Blood Count Analysis

Blood samples from all cats participating in the study were collected via cephalic, jugular or femoral veins. Vacuumed EDTA anticoagulant based tubes (BD, K3 EDTA, 3.8 ml) were used for blood samples collected for complete blood count analysis (Hematological analysis). The blood samples were analyzed within half an hour of the blood collection from live animals. After the blood collection from the animals, the collection tubes were immediately transferred to the laboratory where the tubes were placed in a rotator (Hemolab) for about 3 minutes before the analysis was performed for each cat. The purpose was to ensure thorough mixing and to prevent any blood clot from forming in the samples. Hematological analysis was performed with the help of Mindray BC2800 complete blood count device. The results were attached in the individual file records of each cat. The parameters included in the results were leukocytes count, erythrocytes count, hematocrit levels, hemoglobin levels, and platelets levels, to ensure if the cat is healthy or is infected.

3.3.2. Blood Biochemistry Analysis

Blood samples from all cats were collected via cephalic, jugular or femoral veins. Vacuumed gel-based collection tubes (BD, Vacutainer™ SST™ II, Advance Tubes, 5 ml) were used to collect the blood for blood biochemistry analysis. The blood samples were analyzed in the laboratory the same day as of the collection. In the laboratory, the blood samples were held for 15 minutes before being transferred to the centrifuge machine (Rotofix 32 A, Hettich, Zentrifugen). The samples were centrifuged at 3000 x rpm for 15 min to extract the serum from the blood samples. Serum sample (500 µl) from each cat was stored in two micro centrifuge tubes (Eppendorf, 1,5 ml tubes), and stored at 80° centigrade, until the ELISA analysis for AGP and SAA was performed. Biochemistry analyzer (Randox, RX Monaco) was used to perform the automatic biochemistry analysis. The parameters that were analyzed included, urea, creatinine, total protein, albumin, alkaline phosphatase (ALP), and aspartate aminotransferase (AST).

3.3.3. Fecal Sample Analysis

Fecal sampling and analysis were performed in cats that were suffering from digestive tract disorders. The fecal samples were collected with the help of stool-collection

spatula. The stool was immediately transferred to clean and dry screw-top containers. The stool collection containers were only opened at the time of microscopic analysis. A non-invasive test was performed using a binocular microscope (Zeiss, Primo Star microscope) in the laboratory to indicate the presence of any intestinal parasites and bacteria. Fecal samples helped to analyze if the cat is suffering from giardiasis, coccidiosis, worms and also increased number of bacteria in stool.

3.3.4. Urine Analysis

Urine sampling and analysis was performed in cats that were suffering from suspected urinary tract disorders. Cystocentesis or catheterization were used to perform urine collection. Cystocentesis was performed with the help of sterile needles and syringes. The urine sample was immediately transferred to urine collection containers. Catheterization was performed with the help of sterile catheters, to avoid contamination and transference of bacteria from urethra to urinary bladder. To reduce pain and irritation, a lidocaine-based ointment was applied locally. Urine was collected directly into urine collection containers. The containers were only opened at the time of urine analysis. Urine analysis was performed using a dipstick analyzer (Hasvet, VUC, urine analyzer). The dipstick test result provides us with the information regarding important parameters such as, leukocyte count, PH level, protein count, glucose, ketones, bilirubin, and presence of blood, creatinine and calcium. Urine sedimentation was performed after centrifuging the urine sample in a centrifuge machine at 3000 x rpm for 15 min. Sedimentation helps to identify the presence of cells, casts, crystals and microbes in urine.

3.3.5. ELISA Analysis

Enzyme-linked Immunosorbent Assay (ELISA) test kits were used to determine the serum AGP and SAA concentrations in both control (CG) and Disease groups (DG).

3.3.5.1. Feline SAA ELISA Test

Feline SAA serum concentrations were determined in both control (CG) and Disease groups (DG), using the Cat Amyloid A (SAA) ELISA Kit (SunRed Biotechnology Company, China), according to the instructions of the manufacturer. Feline Serum Amyloid A (SAA) kit works on the principle of using a double-antibody sandwich

enzyme-linked immunosorbent essay (ELISA). The plate provided in the kit is pre-coated with Feline SAA monoclonal antibodies.

Preparation of feline SAA standard dilution was performed with the help of already provided standard reagent in the kit, which was diluted according to the instructions given in the Table 3.2.

Table 3.2. Feline SAA standard dilution preparation

400 ng/ml	Standard No. 5	120 µ 1 Original Standard + 120 µ 1 Standard diluents
200 ng/ml	Standard No. 4	120 µ 1 Standard No. 5 + 120 µ 1 Standard diluents
100 ng/ml	Standard No. 3	120 µ 1 Standard No. 4 + 120 µ 1 Standard diluent
50 ng/ml	Standard No. 2	120 µ 1 Standard No. 3 + 120 µ 1 Standard diluent
25 ng/ml	Standard No. 1	120 µ 1 Standard No. 2 + 120 µ 1 Standard diluent

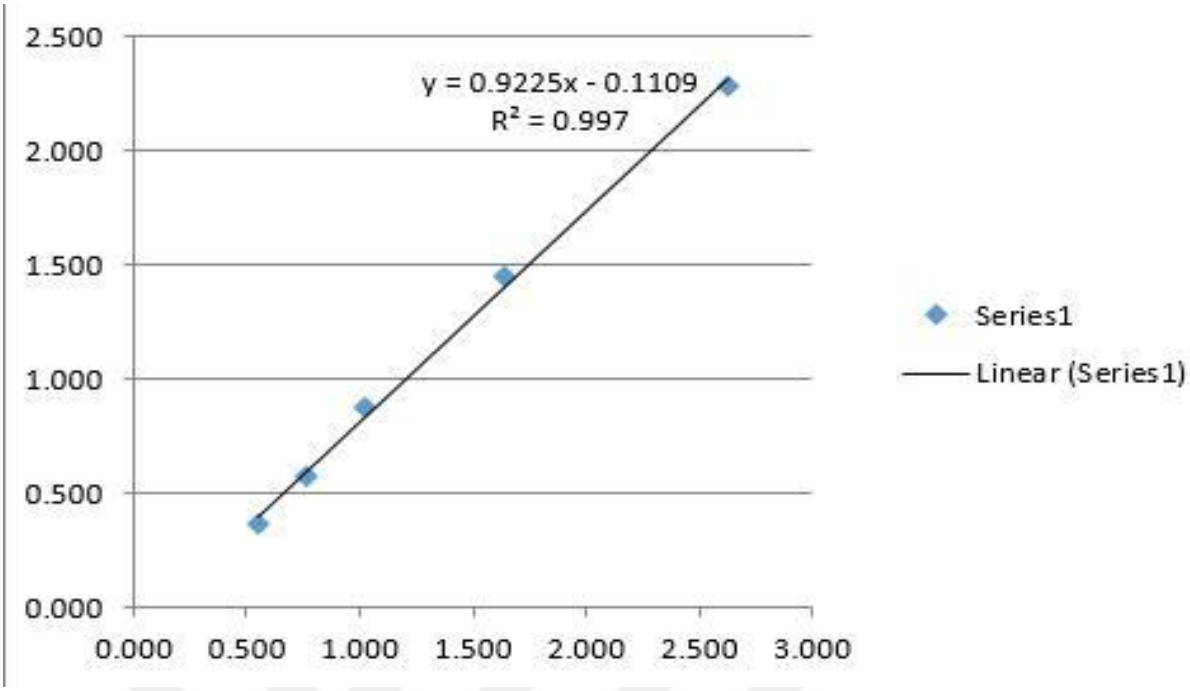


Figure 3.1. Feline SAA Standard curve

3.3.5.1.1. Feline SAA Analysis

1. Blank wells: Two blank wells are separated and marked. No sample was introduced in these two wells.
2. Standard wells: Standard 50 µ 1 and Streptavidin-HRP 50 µ 1 were added.
3. To be tested wells: Sample 40 µ 1 was added, followed by both SAA-antibody 10 µ 1 and Streptavidin-HRP 50 µ 1. Sealing membrane was used to seal and a gentle shake was done. The plate was incubated at 37°C for 60 minutes.
4. Washing: Washing concentrate was used to dilute five times with distilled water, membrane was carefully removed and liquid was drained well with the help of shaking.
5. Chromogen solution A 50 µ 1 was added, followed by the chromogen solution B 50 µ 1 into each well, was mixed gently and incubated at 37°C for 10 minutes away from any source of light.
6. Stop solution 50 µ 1 was added into each well to stop the reaction. Stop solution changed the color from blue to yellow immediately.

7. Final measurement was performed by considering the blank well as zero, and the optical density was measured within the 15 minutes after the stop solution was introduced in the wells. The optical density (OD) was kept under 450 nm wavelength.
8. Biotek ELx800 ELISA device was used to obtain a wavelength of 450nm.
9. Standard curve linear regression equation was calculated according to the concentration of the standard and the OD values. Corresponding sample's concentration was calculated by applying the OD values of the sample on the regression equation.
10. For calculation of results a software was used.

3.3.5.2. Feline AGP ELISA Test

To determine the serum AGP concentrations in all the cats recruited in the study (both CG and EG), the Cat Alpha-Acid Glycoprotein ELISA kit (SunRed Biotechnology Company, China) was used. The procedure was performed according to the manufacturer instructions. Feline AGP ELISA kit works on the principle of using a double-antibody sandwich enzyme-linked immunosorbent essay (ELISA). The wells in the kit are pre-coated with Feline SAA monoclonal antibodies. The plate provided within the kit is pre-coated with Cat A-AGP antibody. All reagents provided within the kit were first brought to room temperature. The standard diluent is important because it serves as the zero standard (0 mg/L). Standard reagent provided in the kit was diluted according to the instructions given in the Table 3.3 and 3.4.

Table 3.3. Feline AGP standard dilution preparation

1600 mg/L	Standard No. 5	120 µ 1 Original Standard + 120 µ 1 Standard diluents
800 mg/L	Standard No. 4	120 µ 1 Standard No. 5 + 120 µ 1 Standard diluents
400 mg/L	Standard No. 3	120 µ 1 Standard No. 4 + 120 µ 1 Standard diluent
200 mg/L	Standard No. 2	120 µ 1 Standard No. 3 + 120 µ 1 Standard diluent
100 mg/L	Standard No. 1	120 µ 1 Standard No. 2 + 120 µ 1 Standard diluent

Table 3.4. Feline AGP suggested dilution of standard solution

Standard Concentration	Standard No. 5	Standard No. 4	Standard No. 3	Standard No. 2	Standard No. 1
3200 mg/L	1600 mg/L	800 mg/L	400 mg/L	200 mg/L	100 mg/L

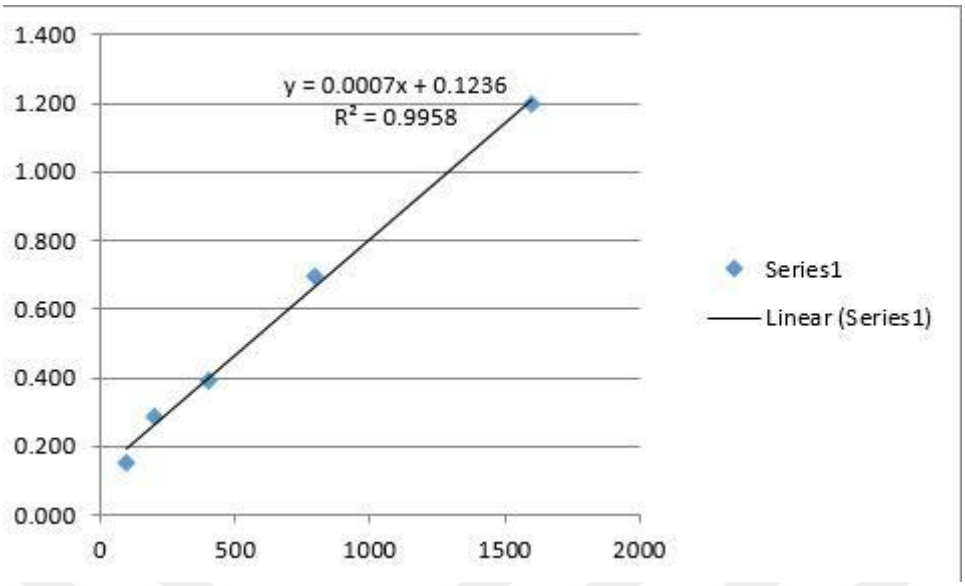


Figure 3.2. Feline AGP Standard Curve

3.3.5.2.1. Feline AGP Analysis

1. As instructed, the reagents, standard solutions and samples were prepared before the analysis. The water buffer was also prepared as instructed.
2. Number of the strips required for the assay was determined and were inserted in the frame for use.
3. Blank wells: Two blank wells are separated and marked. No sample was introduced in these two wells.
4. Standard wells: In the standard wells, 50 µ1 standard was added.
5. Sample wells: In the sample wells, 40 µ1 sample was added followed by 10 µ1 of anti-A-AGP antibody.
6. Streptavidin-HRP (50 µ1) was added in both the standard and sample wells. It was not added in the blank control well.
7. The plate was sealed carefully, shake a little and incubated at 37°C for 60 minutes.
8. Washing: The seal was removed and the plate was washed five times using a wash buffer. All wells were soaked with the wash buffer for 30 seconds for each wash.

9. Substrate solutions: 50 µ1 of the substrate solution A was added into each well followed by the addition of 50 µ1 of the substrate solution B. Plate was sealed again and was allowed to incubate at 37°C for 10 minutes in the dark.
10. Stop solution: 50 µ1 of the stop solution was added in each well that turned the blue color into yellow.
11. Optical density (OD value) was determined with the help of a microplate reader and the wavelength was set at 450 nm within 10 minutes after the addition of stop solution.
12. For calculation of results a software was used.

3.4.Statistical Analysis

Statistical analysis of the study was performed by using a software program, IBM-SPSS for Windows release 25.0 (SPSS Inc, Chicago, IL, USA), and probability value of ($P < 0.05$) was considered significant. The data was analyzed statistically by using One Way Analysis of Variation (ANOVA). To determine whether the data obtained from the study was suitable for normal distribution or not; ROC (Receiver operating characteristic) and Pearson correlation coefficient were used. To express the qualitative data % (n) was used. To express the normally distributed data, mean and standard error were used.

4.FINDINGS

Major clinical signs and symptoms for the cats suffering from respiratory tract disorders included bronchitis, sternutation (sneezing), labored breathing, coughing, anorexia, lethargy, pale mucosa, keratoconjunctivitis, laryngitis, tracheitis, pyrexia/fever and rhinitis. Some cats were presented with secondary infections along with the respiratory tract infections, such as giardiasis, coccidiosis, and fungal infections. A few cats suffering from acute hepatic disorders participated in the study. Major clinical signs and symptoms included jaundice, anorexia, and yellow discoloration of gums, excessive and dark colored urination, coma, nystagmus, incoordination, fever, vomiting, dehydration and excessive thirst. Cats suffering from FIP presented multiple sets of clinical signs and symptoms. In the initial stages, cold-like symptoms were presented, while in advanced stages ocular and neurological signs were presented. Major clinical signs and symptoms recorded in cats suffering from FIP included, abdominal distention, fever in some cases, ascites, vomiting, diarrhea, yellowish discoloration of gums and eyes, lethargy, pica, ataxia, seizures, ocular lesions, uveitis, anisocoria, iritis, keratic precipitates, muscle weakness, dysphagia, urinary incontinence, paralysis and shortness of breath.

The cats suffering from nonspecific gastroenteritis were presented with multiple sets of clinical signs and symptoms, depending upon the etiology of the problem. The signs and symptoms recorded from the subjects suffering from viral infections included bloody diarrhea, vomiting, lethargy, anorexia, severe dehydration, depression, and in some cases lesions inside the buccal cavity of cats. Parasitic infections such as giardiasis and coccidiosis led to clinical signs such as intermittent greenish diarrhea (with or without blood and mucous), distended abdomen, severe dehydration and persistent vomiting. Cats suffering from bacterial gastroenteritis were presented with clinical signs and

symptoms such as fever, prostration, bloody diarrhea, severe dehydration and vomitinging. In some cases, respiratory symptoms were also recorded due to secondary infections.

In the cats suffering from renal and lower urinary tract disorders, the clinical signs and symptoms included dysuria, bloody urine, polyuria, dehydration, excessive thirst, decreased appetite, lethargy, depression, vomitings, and obstructive urine. A single case of calicivirus was also included in the study, the clinical signs and symptoms included fever, ulcers on tongue, nasal congestion and excessive drooling. Some cases included in the gastroenteritis category were also presented with secondary signs and symptoms resembling calicivirus infection, but as the infection was not tentatively diagnosed in these cases, the cases were not included in the calicivirus infected cats list.

A case of pyometra (sepsis) was also included in the study, and later was shifted to the Department of Gynecology and Obstetrics for further treatment. Clinical signs and symptoms included lethargy, anorexia, purulent vaginal discharge and depression. Another case included in the study was diagnosed with ileus, and was shifted to the Department of Surgery for further treatment. Clinical signs and symptoms included diarrhea, vomiting, severe abdominal pain and swelling, refusing to eat and lethargy.

4.1.Physiological Parameters Findings

Physiological parameter findings of cats recruited for the study are given in Table 4.1. There was no significant difference found in the cats presented with diseases like calicivirus infection, pyometra and ileus because only one case was presented for each of these diseases.

There was no significant difference between the groups in terms of temperature and heart rate variables (respectively; P= 0.092, P= 0.953). Mean respiratory rate levels of cats with respiratory tract infections were found to be significantly lower as compared to the cats with non-specific gastroenteritis, FIP and UTI (respectively; P= 0.011, P= 0.003, & P= 0.004) Mean respiratory rate levels of cats with acute hepatitis were found to be significantly lower than cats with FIP (P= 0.005).

Table 4.1. Physiological Parameters Findings

Physical Parameter	Disease Group (DG)					Control Group (n= 10)	P - Value
	Feline Infectious Peritonitis (n= 13)	Non-specific Gastroenteritis (n= 13)	Acute Hepatitis (n= 4)	Respiratory Tract Infection (n= 7)	Lower Urinary Tract Infection (n= 8)		
Temp. °C	38.32 ± 0.26	38.70 ± 0.24	39.0 ± 0.64	39.54 ± 0.28	38.33 ± 0.71	38.24 ± 0.15	0.092
Resp. Rate Per minute	27.9 ± 1.89 A	26.53 ± 1.38 AC	20.0 ± 3.78 BC	19.28 ± 1.64 B	28.62 ± 2.29 AC	24.2 ± 1.34 ABC	0.024
Heart Rate bpm	141.36 ± 3.49	151.38 ± 12.63	139.33 ± 9.52	138.0 ± 4.02	143.87 ± 4.12	137.90 ± 3.40	0.953

ABC; Different letters in the same row indicate statistical differences between disease groups.

RR: Respiratory rate, HR: Heart rate, T: Temperature. Normally distributed data were expressed as m0ean ± standard error.

4.2.Blood Biochemistry Analysis Findings

Biochemistry analysis findings of cats recruited for the study are given in Table 4.2. There was no significant difference found in the cats presented with diseases like calicivirus infection, pyometra and ileus because only one case was presented for each of these diseases.

Mean urea levels of cats with FIP were found to be significant compared to the cats in the control group (P= 0.050). Mean total protein levels of cats with FIP were found to be significantly lower than cats with non-specific gastroenteritis (P= 0.032). Mean total protein levels of cats with non-specific gastroenteritis were found to be significantly lower than cats with urinary tract infection (P= 0.048). Mean total protein levels of cats with FIP, acute hepatitis, respiratory tract infection and lower urinary tract infection were found to be significantly lower than cats in the control group (respectively; P= 0.006, P= 0.022, P= 0.037 & P= 0.011). Mean AST levels of cats with acute hepatitis were found to be significantly lower than cats with FIP, non-specific gastroenteritis, respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.000, P= 0.000, P= 0.000, P= 0.000 & P= 0.000). Mean ALP levels of cats with acute hepatitis were found to be significantly lower than cats with FIP, non-specific gastroenteritis, respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.000, P= 0.000, P= 0.000, P= 0.000 & P= 0.000). Mean albumin levels of cats with FIP were found to be significantly lower than cats with non-specific gastroenteritis and respiratory tract infection (respectively; P= 0.004 & P= 0.000). Mean albumin levels of cats with non-specific gastroenteritis were found to be significantly lower than cats with lower urinary tract infection and cats in the control group (respectively; P= 0.006 & P= 0.002). Mean albumin levels of cats with respiratory tract infection were found to be significantly lower than cats with lower urinary tract infection and cats in the control group (respectively; P= 0.001 & P= 0.000). Mean Globulin level was calculated as 5.89±0.39 g/ dL in cats with FIP. In the same group, mean Albumin Globulin ratio (A/G) was calculated as 0.52±0.64. In control group, mean A/G was calculated as 0.81±0.19. There was no significant difference between the groups in terms of creatinine levels.

Table 4.2. Blood Biochemistry Parameters Findings

Blood Biochemistry Parameter	Disease Group (DG)					Control Group (n= 10)	P - Value
	Feline Infectious Peritonitis (n= 13)	Non-specific Gastroenteritis (n= 13)	Acute Hepatitis (n= 4)	Respiratory Tract Infection (n= 7)	Lower Urinary Tract Infection (n= 8)		
Urea mg/dL	70.17 ± 24.60 A	50.02 ± 24.58 AB	21.66 ± 4.66 AB	21.72 ± 2.10 AB	68.81 ± 21.33 AB	14.13 ± 1.96 B	0.561
Total Protein g/dL	8.99 ± 0.64 A	7.28 ± 0.42 B	9.57 ± 1.59 AB	8.63 ± 0.25 AB	9.00 ± 1.07 AD	6.64 ± 0.28 C	0.045
Creatinine mg/dL	2.37 ± 0.66	2.45 ± 1.48	0.95 ± 0.52	0.86 ± 0.47	1.65 ± 0.37	0.79 ± 0.06	0.913
AST IU/L	49.31 ± 15.82 A	34.99 ± 5.62 A	131.4 ± 29.84 B	45.42 ± 6.20 A	44.21 ± 9.05 A	25.74 ± 3.13 A	0.002
ALP IU/L	35.41 ± 9.65 A	46.94 ± 4.94 A	401.0 ± 360.5 B	36.48 ± 4.21 A	28.00 ± 5.98 A	30.39 ± 4.10 A	0.014
Albumin g/dL	3.10 ± 0.25 AC	3.95 ± 0.15 B	3.43 ± 0.40 AC	4.37 ± 0.27 B	3.06 ± 0.33 C	2.98 ± 0.09 AC	0.001

ABCD; Different letters in the same row indicate statistical differences between disease groups.

AST: Aspartate aminotransferase, ALP: Alkaline phosphatase. Normally distributed data were expressed as mean ± standard error.

4.3.Complete Blood Count Analysis Findings

Complete blood count (CBC) findings of cats recruited for the study are given in Table 4.3A and 4.3B. There was no significant difference found in the cats presented with diseases like calicivirus infection, pyometra and ileus because only one case was presented for each of these diseases. There were some significant differences found in the p-value between different disease groups.

Mean WBC levels of cats with FIP were found to be significantly lower than cats with non-specific gastroenteritis and acute hepatitis (respectively; P= 0.030 & P= 0.041). Mean WBC levels of cats with non-specific gastroenteritis were found to be significantly lower than cats with lower urinary tract infection (P= 0.037). Mean Lymph levels of cats with FIP, non-specific gastroenteritis, acute hepatitis, respiratory tract infection and lower urinary tract infection were significantly lower than cats in the control group (respectively; P= 0.000, P= 0.000, P= 0.004, P= 0.042, & P= 0.000).

Mean Lymph levels of cats with FIP were found to be significantly lower than cats with respiratory tract infection (P= 0.014). Mean Lymph levels of cats with non-specific gastroenteritis were found to be significantly lower than cats with respiratory tract infection (P= 0.013). Mean granulocyte levels of cats with FIP were found to be significantly lower than cats with acute hepatitis (P= 0.023). Mean granulocyte levels of cats with non-specific gastroenteritis were significantly lower than cats with respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.029, P= 0.036, & P= 0.010).

Mean lymph percentage levels of cats with FIP were significantly lower than cats with respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.027, P= 0.021, & P= 0.015). Mean lymph percentage levels of cats with nonspecific gastroenteritis were significantly lower than cats with respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.002, P= 0.001, & P= 0.001). Mean lymph percentage levels of cats with acute hepatitis were significantly lower than cats with respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.011, P= 0.010, & P= 0.008).

Mean granulocyte percentage levels of cats with FIP were found to be significantly lower than cats in the control group (P= 0.000). Mean granulocyte percentage levels of cats with non-specific gastroenteritis were significantly lower than cats with respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.004, P= 0.004, & P= 0.000). Mean granulocyte percentage levels of cats with acute hepatitis were found to be significantly lower than cats in the control group (P= 0.002). Mean granulocyte percentage levels of cats with respiratory tract infection were found to be significantly lower than cats in the control group (P= 0.025). Mean granulocyte percentage levels of cats with lower urinary tract infection were found to be significantly lower than cats in the control group (P= 0.016). Mean RBC levels of cats with FIP were found to be significantly lower than cats with respiratory tract infection (P= 0.026).

Mean MCV levels of cats with FIP and non-specific gastroenteritis were found to be significantly lower than cats with lower urinary tract infection (respectively; P= 0.038 & P= 0.029). Mean MCHC levels of cats with FIP were found to be significantly lower than cats in the control group (P= 0.025). Mean RDW levels of cats with non-specific gastroenteritis and respiratory tract infection were found to be significantly lower than cats in the control group (respectively; P= 0.035 & P= 0.011). Mean platelets levels of cats with FIP and non-specific gastroenteritis were found to be significantly lower than cats with acute hepatitis (respectively; P= 0.000 & P= 0.000). Mean platelets levels of cats with acute hepatitis were significantly lower than cats with respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.022, P= 0.003, & P= 0.019).

Mean MPV levels of cats with FIP were found to be significantly lower than cats with acute hepatitis and cats in the control group (respectively; P= 0.002 & P= 0.004). Mean MPV levels of cats with non-specific gastroenteritis were found to be significantly lower than cats with acute hepatitis and cats in the control group (respectively; P= 0.013 & P= 0.000). Mean MPV levels of cats with acute hepatitis were significantly lower than cats with respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.002, P= 0.012, & P= 0.000). Mean MPV levels of cats with respiratory tract infection were found to be significantly lower than cats in the

control group (P= 0.030). Mean MPV levels of cats with lower urinary tract infection were found to be significantly lower than cats in the control group (P= 0.001).

There was no significant difference between the groups in terms of monocytes, monocytes percentage, HCB, HCT and MCH levels.

Table 4.3A. Leukogram parameter findings

Leukogram Parameter	Disease Group (DG)					Control Group (n= 10)	P - Value
	Feline Infectious Peritonitis (n= 13)	Non-specific Gastroenteritis (n= 13)	Acute Hepatitis (n= 4)	Respiratory Tract Infection (n= 7)	Lower Urinary Tract Infection (n= 8)		
WBC 10^9/L	7.88 ± 1.68 A	15.69 ± 3.94 BC	19.6 ± 4.58 B	9.0 ± 1.72 AC	7.43 ± 2.33 A	9.15 ± 0.80 AB	0.063
Lymphocytes 10^9/L	1.16 ± 0.13 A	1.19 ± 0.18 AD	1.5 ± 0.32 AB	2.70 ± 0.90 B	1.57 ± 0.41 ADB	3.98 ± 0.46 C	0.000
Monocytes 10^9/L	0.69 ± 0.13	0.77 ± 0.17	1.03 ± 0.46	0.60 ± 0.11	0.57 ± 0.20	0.5 ± 0.74	0.090
Granulocytes 10^9/L	5.98 ± 1.46 A	9.92 ± 3.78 A	18.13 ± 6.11 BC	5.71 ± 0.91 A	3.60 ± 0.89 A	6.83 ± 1.22 A	0.092
Lymphocyte %	17.8 ± 2.85 A	13.2 ± 3.05 AC	10.0 ± 4.61 AD	29.98 ± 4.97 B	30.0 ± 4.28 B	30.0 ± 3.36 B	0.001
Monocyte %	7.50 ± 1.30	5.40 ± 1.23	7.46 ± 5.20	6.32 ± 0.59	7.50 ± 1.14	4.84 ± 0.61	0.721
Granulocyte %	71.19 ± 3.48 A	78.44 ± 4.04 B	72.96 ± 8.25 A	60.05 ± 5.46 A	60.72 ± 5.46 A	45.22 ± 2.69 C	0.000

ABCD; Different letters in the same row indicate statistical differences between disease groups.

WBC: White blood cells, normally distributed data were expressed as mean ± standard error

Table 4.3B. Erythrogram Parameters Findings

Erythrogram Parameter	Disease Group (DG)					Control Group n= 10	P - Value
	Feline Infectious Peritonitis n= 13	Non-specific Gastroenteritis n= 13	Acute Hepatitis n= 4	Respiratory Tract Infection n= 7	Lower Urinary Tract Infection n= 8		
RBC 10^2/L	6.24 ± 0.78 A	7.62 ± 0.58 AB	7.69 ± 0.49 AB	8.79 ± 0.47 B	7.38 ± 1.22 AB	7.67 ± 0.51 AB	0.561
HGB g/dL	10.51 ± 0.76	12.95 ± 0.93	11.53 ± 1.53	13.27 ± 0.55	11.86 ± 1.89	10.84 ± 0.67	0.512
HCT %	26.90 ± 3.35	31.86 ± 2.71	34.53 ± 4.38	38.21 ± 1.79	34.86 ± 5.86	35.16 ± 2.03	0.488
MCV fL	42.73 ± 1.39 A	42.63 ± 0.89 A	42.30 ± 4.35 AB	42.72 ± 1.05 AB	46.86 ± 1.97 B	43.38 ± 0.93 AB	0.389
MCH pg	18.43 ± 1.74	16.83 ± 0.99	15.36 ± 0.92	14.77 ± 0.30	17.75 ± 1.70	15.11 ± 0.51	0.547
MCHC g/dL	42.95 ± 4.35 A	39.72 ± 2.05 AB	34.36 ± 0.49 AB	34.77 ± 0.43 AB	38.31 ± 4.18 AB	33.80 ± 0.45 B	0.419
RDW fL	30.94 ± 0.97 AB	28.61 ± 2.07 A	31.0 ± 0.70 AB	26.54 ± 1.80 A	32.13 ± 1.50 AB	33.56 ± 1.82 B	0.091
Platelets 10^9/L	187.0 ± 43.08 A	195.30 ± 19.32 A	629.66 ± 304.3 B	335.14 ± 94.61 A	250.25 ± 58.35 A	340.40 ± 34.76 A	0.019
MPV fL	7.84 ± 0.47 A	7.22 ± 0.18 A	4.80 ± 1.73 B	8.15 ± 0.43 A	7.37 ± 0.69 A	9.76 ± 0.40 C	0.001

ABC; Different letters in the same row indicate statistical differences between disease groups.

RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cell distribution width, MPV: Mean platelet volume. Normally distributed data were expressed as mean ± standard error.

4.4.Feline SAA Analysis Findings

Feline SAA analysis findings of cats recruited for the study are given in Table 4.4. There was no significant difference found in the cats presented with diseases like calicivirus infection, pyometra and ileus because only one case was presented for each of these diseases. There were no significant differences found in the p-value between different disease groups.

Table 4.4. Cat SAA ELISA analysis findings

Cat SAA ELISA Kit	Disease Group (DG)					Control Group n= 10	P - Value
	Feline Infectious Peritonitis n= 13	Non-specific Gastroenteritis n= 13	Acute Hepatitis n= 4	Respiratory Tract Infection n= 7	Lower Urinary Tract Infection n= 8		
SAA (ng/ml)	108.16 ± 40.15	58.03 ± 10.37	76.21 ± 19.40	85.73 ± 11.35	86.06 ± 29.57	61.15 ± 7.08	0.451

Serum Amyloid Amylase. Normally distributed data were expressed as mean ± standard error.

4.5.Feline Serum AGP Analysis Findings

Feline serum AGP analysis findings of cats recruited for the study are given in Table 4.5. There was no significant difference found in the cats presented with diseases like calicivirus infection, pyometra and ileus because only one case was presented for each of these diseases.

There were some significant differences found in the p-value between different disease groups. Mean AGP levels of cats with acute hepatitis were significantly lower than cats with respiratory tract infection, lower urinary tract infection and cats in the control group (respectively; P= 0.007, P= 0.015, & P= 0.012).

Table 4.5. Cat AGP ELISA analysis findings

Cat AGP ELISA Kit	Disease Group (DG)					Control Group n= 10	P - Value
	Feline Infectious Peritonitis n= 13	Non-specific Gastroenteritis n= 13	Acute Hepatitis n= 4	Respiratory Tract Infection n= 7	Lower Urinary Tract Infection n= 8		
AGP (mg/L)	260.29 ± 11.57 A	253.18 ± 26.56 A	326.37 ± 20.32 B	185.46 ± 23.60 AC	197.36 ± 40.27 AC	200.28 ± 19.38 AC	0.032

ABC; Different letters in the same row indicate statistical differences between disease groups.

AGP: Alpha-glycoprotein. Normally distributed data were expressed as mean ± standard error.

5. DISCUSSION

The present study is the first to evaluate concentrations of AGP in cats of various age groups, suffering from various diseases and their comparison with not only the healthy cats but also the intra-group comparison of the serum AGP concentration of the cats in the disease group. The major focus was to cover all possible clinical diseases, including all body systems of cats, brought to the Erciyes University Faculty of Veterinary Medicine Training, Research and Practice Hospital. Diseases that were included in the present study are feline infectious peritonitis, gastroenteritis, hepatitis, respiratory tract infections and urinary tract infections.

According to last three years (01.01.2019-31.12.2021) patient record of Erciyes University, Faculty of Veterinary Medicine, Training, Research and Practice Hospital, the rate of various infectious diseases among cats that were brought to the Department of Internal Medicine was reported as 2019: 2708 cases, 2020: 3083 cases, 2021: 5355 cases, making a total of 11.146 patients. The rate of most common infectious diseases brought was reported as, feline infectious peritonitis (FIP) (8%), feline immunodeficiency virus infection (FIV) (0.6%), feline leukemia (FeLV) (0.3%), feline infectious anemia (10.2%), feline panleukopenia (14.3%), feline herpes virus (FHV) (9.7%), feline calicivirus (FCV) (5.9%), pneumonia (18%), gastroenteritis (8%), enteritis-diarrhea (12.9%) and other diseases (12.1%). This is an unpublished data. Such a high number of cases motivated us to consider studying serum AGP concentrations in blood, as a potential biomarker for the early diagnosis of disease in cats. We did not only consider studying serum AGP from the APPs, but also compared results of various diseases in terms of serum SAA concentrations in the blood of the subjects. A total of 55 cats participated in the study and were divided into five disease groups (FIP= 13,

non-specific gastroenteritis= 13, acute hepatitis= 4, respiratory tract infection= 7, and lower urinary tract infection= 8) and a control group containing 10 healthy cats. The cats that were added to the control group were either brought for regular vaccination or deworming to the hospital. The cats were given a careful and detailed physical check-up, followed by a series of laboratory tests to ensure the health status of cats.

Some samples were collected from the diseases like pyometra, feline calicivirus infection and ileus, however, due to the low number of samples collected from these diseases, they were not added into the disease groups before performing the statistical analysis.

Diagnosis of the diseases and the grouping was made on the basis of physical clinical parameters, such as body temperature, respiratory rate and heart rate, also the laboratory analysis, such as complete blood count (hematology), blood biochemistry, urine analysis and fecal analysis. Ultrasonography and radiology were also considered for some clinical diseases for more tentative diagnosis and group forming. After the successful diagnosis of disease and group forming, the serum was stored until a desired number of cases of a specific disease were collected. ELISA kits were used to perform the double-antibody sandwich enzyme-linked immunosorbent assay.

Cats brought for check-up at the hospital were carefully assessed. Before the assessment, the cats were properly handled and restrained. In 2006, in the Journal of Veterinary Behavior, Erika et al, published the guidelines for careful handling of anxious and fearful cats. All the guidelines given were found helpful in handling depressed and fearful cats. Guidelines were followed throughout the process of animal check-up and sample collection at the hospital.

Before the tentative diagnosis of disease was made, all cats were initially assessed on the basis of medical history provided by the owner, clinical signs and symptoms, and later the laboratory tests were performed for differential diagnostic purposes. Clinical signs and symptoms were carefully evaluated during physical examination and with the help of history taken in accordance with the guidelines mentioned in a detailed study of clinical assessment and management in cats, published by Gunn-Moore (2011).

For the cases of FIP, the Feline coronavirus (FCoV) antibody rapid test kits were used for tentative diagnosis of the disease and to address the problem with better treatment

protocols. For tentative diagnosis and management of FIP proper methods should be adopted. One of the most frequently used tests is the FCoV antibody rapid test kit. Antibody testing is considered reliable by many veterinarians worldwide. There are many factors that affect the reliability of these kits available commercially. The most important factor is the choice of correct laboratory or brand (commercially prepared) for best results. The methodologies to interpret the results may also vary with various commercially prepared kits and with the protocol of each laboratory. Hartmann in 2005 reported that a sample collected from a cat, suspected to have acquired FIP, was sent to five different laboratories for antibody titer test, and all of the laboratories presented different results. Therefore, we cannot completely trust or rely on these tests. There is a huge possibility of false positive and false negative results because the presence of antibodies in cats is not the indication for presence of FIP infection, likewise, the absence of antibodies is not the indication for the absence of FIP infection. It has been reported that 10% of rapid antibody tests give negative results when performed in cats with clinical signs ruling out FIP. It has also been reported that the cats with the effusive form of FIP show negative results because of low antibody levels that are lost during effusion (Hartmann 2005).

In this thesis study, out of 13 cats with clinical signs of FIP, only 6 samples showed positive antibody rapid test results. Therefore, these rapid test kits cannot be trusted alone and a series of other tests like clinical examinations, hematology, biochemistry analysis and ELISA analysis should also be performed. Our results are found to be in accordance with previous studies (Niels C. Pedersen 2009).

FIP affected cats mostly present clinical signs and symptoms related to respiratory and gastrointestinal systems (André, Miller, and Whittaker 2020). Major clinical signs and symptoms present in the FIP affected cats are ocular discharge, keratitis, uveitis, ascites (abdominal fluid with yellow color and sticky consistency), enteric infections, pleural effusion, abdominal distention, fever, dyspnea, anorexia and lethargy (Andrew 2000). Clinical signs and symptoms recorded in the present study included the fever in some cases, ocular discharge, keratitis, anorexia, abdominal distention, dyspnea, enteric infections, uveitis, lethargy, pleural effusion and ascites that are found in accordance and compatible with other published reports (Tekelioglu et al. 2015).

In addition to these clinical signs and symptoms cats suffering from FIP presented cold-like symptoms initially, followed by ocular and neurological signs in advanced stages (Moyadee et al. 2019). In this thesis study, major clinical signs and symptoms presented by cats suffering from FIP included, abdominal distention, fever in some cases, ascites, vomiting, diarrhea, yellowish discoloration of gums and eyes, lethargy, pica, ataxia, seizures, ocular lesions, uveitis, anisocoria, iritis, keratic precipitates, muscle weakness, dysphagia, urinary incontinence, paralysis and shortness of breath (Gökçe and Cengiz 2019). Clinical findings were found to be consistent with the reported clinical findings for cats with FIP, in previous published studies (Healey et al. 2022).

Cats affected with nonspecific gastroenteritis mostly present clinical signs and symptoms such as bloody or watery diarrhea, vomiting, lethargy, dehydration, anorexia, fever, abdominal pain, hematochezia and depression (El-Metwaly, Hassan, and El Sayed Abo Hashem 2021). Clinical signs and symptoms recorded in the present study included the fever in some cases, bloody diarrhea, watery diarrhea, vomiting, lethargy, anorexia, severe dehydration, depression, that are found in accordance and compatible with other published reports (Di Martino et al. 2019).

In addition to these clinical signs and symptoms cats suffering from parasitic infections such as giardiasis and coccidiosis led to clinical signs such as intermittent greenish diarrhea (with or without blood and mucous), distended abdomen, severe dehydration and persistent vomiting (Sauda et al. 2019). Out of all cases of gastroenteritis brought to the teaching hospital, 80% of the cases accounted for bacterial gastroenteritis. Other types of gastroenteritis ruled on the basis of fecal examination included giardiasis and coccidiosis. Therefore, the group that was created for the cats with gastroenteritis has been given the name nonspecific gastroenteritis. These results are in accordance with previous studies where up to 80% cases of gastroenteritis account for bacterial gastroenteritis (Oh et al. 2021).

Cats affected with acute hepatitis mostly presented clinical signs and symptoms such as jaundice, appetite loss, weight loss, vomiting, yellow discoloration of gums, and dehydration, that are found in accordance and compatible with other published reports (Fragkou et al. 2016). In this thesis study in addition to these clinical signs and

symptoms cats with acute hepatitis had coma, nystagmus, incoordination, and excessive thirst (Mochizuki et al. 2006).

Cats with respiratory tract infections presented clinical signs and symptoms such as nasal discharge, coughing, dyspnea (Abbott et al. 2020), high body temperature, bronchopneumonia, ulcerative lesions of skin and lethargy that are found in accordance and compatible with other published reports (Frymus et al. 2021).

Cats with lower urinary tract infections presented clinical signs and symptoms such as dysuria, abdominal pain, bloody urine (Gannon et al. 2019), polyuria, dehydration, excessive thirst, decreased appetite, lethargy, depression, vomiting, and obstructive urine, that are found in accordance and compatible with other published reports (Johnstone 2020).

Cats with respiratory tract infections presented significantly lower respiratory rate as compared to nonspecific gastroenteritis, FIP and urinary tract infection. Normal range of a cat's respiratory rate varies from 15 to 30 breaths per minutes. In this thesis study, the mean respiratory rate of cats with respiratory tract infections was found to be 19.28 ± 1.64 breaths per minutes. Though the mean respiratory rates in cats with respiratory tract infections was found to be lower than other diseases, the range was not lower than the normal ranges. None of the group presented mean respiratory rates more than 30 breaths per minutes. Some cats with respiratory tract infections were found to have high breath rates, but the mean was in the normal ranges. The variation in the respiratory rates of cats is directly influenced by some important factors like travel from comfort zone (home) to the veterinarian, ambient temperatures and use of some medications such as relaxants, that are commonly being used these days by the cat owners to relax the cats on veterinarian visit (Dijkstra, Teske, and Szatmári 2018).

In cats with acute hepatitis, the mean respiratory rate was found to be significantly (P= 0.050) lower as compared to cats with FIP. The range was not lower than the normal ranges, i.e, 20 breaths per minute. The lower ranges of some cases with hepatitis could be due to liver incompetency and decreased muscle strength that leads to dyspnea (Kaltsakas et al. 2013).

Norris et al, in 2005 reported lymphopenia and anemia as the major hematological test findings for cats with FIP (Me, Malik, and Norris 2019). Moyadee et al. (2019) reported

low levels of mean corpus volume (MCV) and lymphopenia in cats with FIP. In the present study, complete blood count (hematology) test results in cats with FIP, included neutropenia, (lymphopenia and granulocytopenia), low mean corpus volume (MCV), and low mean platelet volume (MPV). Hematological findings were found to be consistent with the reported hematology findings for cats with FIP, in previous published studies (Moyadee et al. 2019).

The possible reason for anemia in cats with FIP is attributed to chronic inflammation. Almost 60% of the cats with FIP are found to have anemia, which is in accordance with findings. The possible reason for changes seen in the levels of leukocytes, including lymphopenia is high levels of stress and low immunity due to chronic infections (White et al. 2013). The hematological analysis results are not specific for FIP and can change according to acute and chronic inflammatory responses of the body. However, Addie (2011) describes lymphopenia as a consistently seen feature in cats with FIP (Moyadee et al. 2019).

In cats affected with nonspecific gastroenteritis, neutropenia, (lymphopenia and granulocytopenia), low mean corpus volume (MCV), and low platelet count was recorded in the present study, which are found in accordance and compatible with other published reports The decrease in the hemoglobin levels is due to blood loss through intestinal hemorrhages leading to bloody diarrhea. Neutropenia observed in the hematological analysis might be due to any secondary infections that may have occurred due to opportunistic bacteria damaging the intestinal epithelium (Kataria et al. 2020).

In cats affected with acute hepatitis, neutropenia, (lymphopenia and granulocytopenia), low platelet count and low mean platelet volume (MPV) was recorded, which are found in accordance and compatible with other published reports (Piewbang et al. 2022). Decrease in the levels of neutrophils is due to the lowered number of white blood cells in response to a weak immune system of the body that may be due to an underlying infection. Functional and structural damage of the liver can also contribute to changed levels of hematological parameters (Lanave et al. 2019). Presence of an infection in the liver alters normal liver function leading to liver failure, representing changes in hematological parameters such as neutropenia and anemia (Piewbang et al. 2022).

In cats affected with respiratory tract infections, neutropenia, (lymphopenia and granulocytopenia), low platelet count, low red cell distribution width (RDW) and low mean platelet volume (MPV) was recorded, which are found in accordance and compatible with other published reports (Stepanović et al. 2020). The reason for changes in hematological parameters in respiratory tract infections is due to lower immunity, stress, the opportunistic bacterial and fungal infections. These secondary infections can cause fluctuation and changes in parameters (Meena et al. 2019).

In cats affected with lower urinary tract infections, low mean platelet volume (MPV) was recorded, which are found in accordance and compatible with other published reports (Chatzis et al. 2020). The low MPV values are due to infections and inflammations in the body. Sometimes, the low MPV are also associated with aplastic anemia and secondary problems such as autoimmune disorders (I. R. Lee et al. 2015).

According to Norris et al, (2005), cats with FIP were found to have high levels of AST, ALP and total protein. In one case the total protein levels were found low in the present study. In the study performed by Moyadee et al. (2019), it was found that cats with FIP had lower urea and albumin levels. Tekelioglu et al. (2015) also reported low levels of albumin and urea. Similarly, in the present study, low urea, total protein and albumin levels were determined in cats with FIP. Furthermore, the levels of AST were found higher as compared to cats with hepatitis. The low levels of total protein in cats with FIP were found only in one case by Norris et al. (2005), while in our study mean total protein was statistically low in all cases. In our thesis study, the protein levels were lower along with low levels of urea and albumin. Glomerulonephritis may cause decreased levels of albumin. Lower levels of urea are often associated with renal diseases or liver problems. Due to anorexia, cats have low food intake and undernutrition leads to weight loss and weakness that also contributes to lower urea levels in the body (Moyadee et al. 2019). One of the most important findings in cats with FIP was low A/G level (0.52±0.64) as compared to control group level (0.81±0.19). It is stated that the optimal cut off value of A/G ratio was determined as 0.8. If the positive predictive value of A/G is lower than 0.8, there is a higher possibility rate for FIP. Whereas if it is higher than 0.8, there is lower possibility rate for FIP

(Addie et al, 2022; Levy and Huttsel, 2022). These findings show high probability of FIP suspected cats in present study were positive with FIP.

In cats affected with non-specific gastritis, low levels of total protein and albumin were recorded in the present study. Decreased levels of protein and albumin in blood biochemistry might be due to tissue perfusion. Low albumin levels might be the result of kidney disease, liver disease, inflammation or infections. High albumin levels are usually the result of dehydration or severe diarrhea (Kataria et al. 2020). The low levels of proteins and albumins are due to anorexia and intestinal malabsorption. Diet intake is declined in gastroenteritis leading to enteropathy that loses proteins from the body. The increase in the bowel permeability due to enteritis also causes the loss of body fluids, proteins and cells (Bhat et al. 2013).

In cats affected with acute hepatitis, decrease in the levels of urea, while increase in total protein, AST and ALP were recorded in the present study which are found in accordance and compatible with other published reports (Piewbang et al. 2022). The increase in the AST and ALP levels might be due to the liver injury. There is an increase in the liver enzymes due to the alterations in the liver parenchyma. This mechanism is activated due to underlying hepatic infections and secondary systemic diseases. Functional and structural damage of the liver can also contribute to changed levels of biochemistry parameters (Lanave et al. 2019).

In cats affected with respiratory tract infections, decrease in the levels of albumin was recorded in the present study, which are found in accordance and compatible with other published reports (Gomez-Lucia et al. 2020). In respiratory distress, cats are seen to have high levels of anxiety and depression leading to lower food intake. Hypoalbuminemia might occur due to various factors such are underlying infections in body such as secondary enteritis, and also due to the use of corticosteroids and bronchodilators used to ease the respiratory tract obstruction (Chen et al. 2015). The important protein albumin is synthesized by the liver, due to liver damage because of factors like prolonged medication and old age, which can cause decline in the synthesis of albumin. Malnutrition and inflammatory conditions also contribute to hypoalbuminemia (Soeters, Wolfe, and Shenkin 2019).

In cats affected with lower urinary tract infections, a mean of all cats with lower urinary tract infections showed an increase in the levels of total protein, which are found in accordance and compatible with other published reports (Kovarikova et al. 2020). In some cases, there was hypoproteinemia that might be due to urinary retention during urinary tract disorders. Hypoproteinemia is correlated with fluid retention in the body. Therefore, low levels of proteins might have been recorded in the biochemistry examination of the lower urinary tract infections (Wenqian et al. 2019).

Serum SAA concentrations were also studied in addition to the serum AGP concentrations. The present study does not only compare the levels of AGP and SAA with healthy cats but also compares the concentrations in between various common infections seen regularly at the Teaching Hospital.

A study performed in puppies and adult dogs was found to be quite similar to our study, where the serum AGP values were evaluated in puppies and adult dogs, belonging to various breeds and genders in various clinical diseases, and the concentrations were compared with the healthy dogs (Yuki, Itoh, and Takase 2010). Although the study was performed in various diseases of dogs and comparison was made with healthy dogs, there was a comparison made between age and gender of dogs as well. It was concluded that the tremendous increase in serum AGP levels was useful in the presence of both viral and bacterial infectious agents and in neoplastic diseases. In addition, the study also included the use of AGP to distinguish between acute and chronic processes in filariasis and hepatopathy. In our thesis study, no comparison was made on the basis of age groups, and gender of cats. Another difference between the two studies is that in our study serum AGP concentrations alone were not measure. In addition, serum SAA concentration using ELISA technique in all diseased groups and the healthy cats were also determined.

In various studies increased values of APPs were determined in cats with not only inflammatory conditions (Addie et al. 2022), but also non-inflammatory conditions such as neoplasia (Giori et al. 2011). There are studies where only serum AGP concentrations were analyzed in cats suffering from FIP, whereas, in a study, not only the serum AGP concentration values but also serum Hp and serum SAA concentration values were determined (Hazuchova, Held, and Neiger 2017). However, above studies also differ

from ours with the fact that the comparison of serum AGP, SAA and Hp values were made only between FIP positive and healthy group, whereas we also considered the intra-group comparison of the serum AGP and SAA concentrations in between different disease groups.

It has been reported that in cats the increase in serum AGP and SAA concentrations are greater than 10-fold, which is a very good concentration increase as a potential biomarker. It has also been studied that serum SAA concentrations are seen to increase 10-fold in horse, cat, dog, cattle, sheep, goat, rabbit, and humans. It was reported that serum AGP reached the peak levels on the 5th day and stayed higher in concentrations until the 7th day in experimentally induced parvovirus infection in dogs. A similar increase in CRP and SAA serum concentrations were reported (Sarp Sevgisunar and Şahinduran 2014). In our thesis study, there was not such a high increase in the AGP concentrations that we could calculate the increased fold, but statistically evaluated and found to be significant.

It has been reported that, during an inflammatory response, the serum concentrations of some APPs, such as CRP and SAA, increase way more than the serum concentrations of AGP (Selting et al. 2000a). This data in dogs is very valuable and it constitutes a definitive reference for cats. There is a dire need of studying AGPs in cats, because as compared to other APPs, the studies on serum concentrations of AGP in cats are very scarce in terms of investigating the sensitivity and specificity of AGP in various diseases in cats. The studies determining AGP in other species are up to date (Dinallo et al. 2022). Some recent studies have investigated the serum AGP concentrations in various diseases of cats; healthy and tumor-bearing cats (Selting et al. 2000b), cats suffering from chronic gingivostomatitis (Mestrinho et al. 2020), cats with obstructive lower urinary tract disease (Dinallo et al. 2022), cats with infectious peritonitis (Addie et al. 2022), and cats with hyperthyroidism (Glück et al. 2022). Though the previous studies have been conducted in various diseases of cats, but only one disease at a time was considered in those studies. Our thesis study compares the serum concentrations of both SAA and AGP in cats belonging to different disease groups, and later compared those concentrations with the control group. We also focused on comparing the results of serum AGP concentrations in between the five disease groups. Our study contributes

in evaluating the specific ELISA kits and the manufacturing company, in the efficiency to measure the acceptable or predicted ranges of serum AGP and SAA levels. The evaluation of ELISA kits helps us to develop more efficient disease diagnostic techniques.

According to the magnitude of the response, CRP and SAA for dogs and SAA and AGP for cats are stated to be major APPs (Tecles et al. 2015) Though in cats, serum SAA levels increase up to 10-fold (Sarp Sevgisunar and Şahinduran 2014), measuring serum AGP has been emerging as an alternative analysis technique or a biomarker rather than SAA, for diagnosis of disease. Measuring the serum AGP concentration has been more focused and researched mostly in feline infectious peritonitis (FIP) cases, but there are no adequate studies in other common feline infections seen on a daily basis (Kajikawa et al. 1999). Therefore, it was considered a dire need to measure the levels of not only serum AGP but also serum SAA levels in various common infections of cats.

There is a wide range of APPs that have been studied and reportedly used for clinical diagnosis and prognosis of small animal diseases, to improve the efficiency of veterinarians in diagnosing various common infections in veterinary clinical medicine. One of the most widely used APP in various species is reportedly the CRP, however, no significant increase, or a very slight increase, in the serum concentrations of CRP during the inflammatory response determined in cats. Increased serum concentration of SAA is considered to be a better indicator, and a biomarker, in terms of cats as compared to other species. Sasaki et al. (2003) reported up to 55-fold increases in SAA in different infectious and inflammatory diseases of cats (Sasaki et al. 2003). Duthie et al. (1997), on the other hand, found increases in AGP concentrations up to 10 times higher than normal in cats with FIP (Duthie et al. 1997). Therefore, for this thesis, investigating SAA and AGP were considered to be evaluated and CRP was not considered, as it has already been widely used.

Some studies have shown a significant increase in the serum concentrations of SAA, AGP and HP, in cats 1-day post-surgical procedures. There are reports suggesting an increase in the serum concentrations of AGP in dogs suffering from lymphoma, different types of carcinomas (except for mammary carcinomas), and sarcomas (Kajikawa et al. 1999). Reports have suggested that the serum concentrations of AGP

have proven to be of clinical importance in monitoring prognosis of dogs suffering from lymphoma that have been treated with doxorubicin (Selting et al. 2000b). In our thesis study, prognosis was not monitored and the sampling was made only once from all the cases in all groups. The main reason for not considering prognosis is the response of owners who cannot afford a long-term treatment and mostly come to the Teaching Hospital only for diagnostic purposes. After the diagnosis has been made, most of the cat owners prefered to reach out to nearby private veterinarians with whom their cats are friendly and do not get much stressed.

Another study reveals that the diagnosis and follow-up of sepsis in cats can be analyzed with the help of measuring the serum AGP. A study conducted to determine the increase of serum concentrations of AGP and messenger RNA levels of APPs reported that serum AGP levels may be useful for accurate diagnosis and prognosis assessment in the cases of sepsis in rats. AGP concentrations in serum are associated with the inflammatory response of the body, the pathophysiological mechanism of AGP in sepsis is not completely understood (Nielsen et al, 2006). In humans, for the early diagnostic and prognostic purposes of sepsis, commercially available ELISA kits are widely used (Hsiao et al. 2019). In our thesis study, one case of pyometra (sepsis) was included. As this thesis study belongs to the Department of Internal Medicine, there is a very low patient ratio coming for the diagnosis of pyometra or other related problems. One case that was included in our thesis study was brought with the complaint of severe dehydration and vomiting. Later, during the clinical examination, vaginal discharge was noticed in the cat. For further evaluation and treatment, the cat was directed to the Department of Gynecology.

The key benefit of conducting the present study is to develop an alternative and sensitive biomarker analysis system for cats in not only diagnosis of disease but also in the follow-up of infections, septic conditions and tumoral diseases. The biomarker is expected to improve both diagnosis of disease and prognosis. Furthermore, even though there is an availability of CRP and SAA kits in small animal medicine, the use of these kits is very scarce in Turkey. The available kits may have low sensitivity for different species of animals, resulting in false or negative results initially. In addition, the kits are relatively expensive and the procedure is quite time-consuming to perform in the

laboratory conditions, such as ELISA, SRID and other tests like immuno-chromatography (Tagata et al. 1996). As most of the kits are imported from other countries, the use on a regular basis is very less because they are quite expensive. We were able to use the ELISA kits in the project to determine the serum AGP and SAA values in cats, but it cannot be used on a daily basis by the veterinarians for the diagnosis of common infections due to high prices and time-consuming procedures.

The high increase in serum AGP concentrations can support the tentative early diagnosis of FIP in cats (Saverio et al. 2007). However, in addition to the increase of the serum AGP concentrations in FIP, its concentrations are also reported to elevate in neoplastic disorders (Correa, Mauldin, and Mooney 2001), tumor-bearing cats (Selting et al. 2000a), other asymptomatic inflammatory disorders and in FCoV-positive cats (Giori et al. 2011). Various studies have demonstrated the importance of AGP as an important biomarker, therefore, the relative parameter is considered an important APP in cats for the diagnosis and prognosis of various non-specific conditions along with FIP affected cats. Nowadays, the definitive and tentative diagnosis of FIP disease in cats is very troublesome for the researchers and veterinarians around the globe. Our thesis study can prove to be fruitful and can lead the researchers around the globe in a positive direction for further studies.

It has been reported in a recent study by Addie et al. (2022), that there is a continuous decrease in the elevated serum AGP levels following the treatment of FIP in cats, which has been found as the fastest and most convenient indicator to distinguish recovery from remission. It has been suggested in this study that monitoring the serum AGP concentrations can be a useful model for recovery in cats suffering from chronic coronavirus infections. The diagnosis of FIP, compatible with the profile of FIP, is based on histopathology, immunohistochemistry, and algorithms of medical history, clinical signs and symptoms, clinicopathological abnormalities, high titer results of FCoV antibodies, high levels of serum AGP concentrations, lymphopenia, anemia and hyperglobulinemia. There is a need to create a standard for helping veterinarians to treat cats suffering from FIP and to know when to stop the treatment protocols (Addie et al. 2022).

The high serum AGP levels are not specific only for the FIP affected cats, but may also elevate in other infectious diseases. AGP being a crucial parameter for diagnosis can help to strengthen diagnostic efficacy of other suspected infectious agents in cats.

In 2020, Yuki et al, reported significantly higher levels of serum SAA concentrations in cats with FIP as compared to cats in the control group. Similar results were published in studies by Hazuchova et al. (2017) and Tecles et al. (2015), with a significant high increase in serum SAA concentrations in cats with FIP (Hazuchova, Held, and Neiger 2017). In the present study, although the levels of serum SAA were calculated quite high in cats with FIP, the mean SAA statistically was not significant and hence not declared higher as compared to other groups. Serum SAA findings were not found to be consistent with the reported serum SAA findings for cats with FIP, in previous published studies. Similarly, the levels of SAA for cats with gastroenteritis, hepatitis, respiratory tract infections and urinary tract infections were not significant statistically and hence not declared higher as compared to the control group in the present study.

One of the reasons for the difference in findings from other published studies can be the sensitivity difference and efficacy of the commercially manufactured cat SAA ELISA kit that was used during the present study. The other reason can be the tremendous decrease of APPs once an anti-inflammatory medicine is introduced in the body system. The anti-inflammatory medication stops the inflammatory response of the body, and hence the serum APP concentrations also decrease (Hsiao et al. 2019). There was a decline in the concentrations of APP once the antimicrobial treatment was introduced in the patients. Cats that were brought to Erciyes University Veterinary Education, Research and Practice Hospital, mostly came for a tentative diagnosis after being treated at a private veterinary clinic. The concentrations of APPs must have been high, according to previous research, but due to the antimicrobial treatment, the cats' plasma concentration of SAA was not as high as expected. Another reason is the acute inflammatory response that as compared to chronic inflammatory response ends earlier. On the other hand, some serum APP concentrations were also not measured high during the acute inflammatory reactions (Jain, Gautam, and Naseem 2011).

In the present study, although the levels of serum AGP were very high for cats with FIP, but when the comparison was made with the other disease group and the control group,

the concentration of AGP in cats with FIP was not found significant. Similarly, although the levels of serum AGP were calculated quite high in cats with nonspecific gastroenteritis the mean AGP was not significant statistically and hence not declared higher as compared to other groups. The serum AGP concentrations in cats with respiratory tract infections and urinary tract infections were not recorded high as compared to the healthy group.

The only disease group that gave favorable results was cats with acute hepatitis. Serum AGP concentrations in all cases of acute hepatitis were found significantly high and considered highly valuable. The results are compatible with studies on hepatic diseases in which there is a high increase of serum AGP levels in humans (Lee et al. 2006).

In one case of ileus and in pyometra, the concentration of AGP was also very high as compared to any other disease group, which are compatible with previous reports (Addie et al. 2022), but statistical analysis could not be made.

Although AGP has been seen to play an important role as a biomarker for diagnosis of disease, because the concentrations immediately increase during inflammation after the introduction of infections; Park et al. (2022) speculated that AGP does not always increase significantly. In a study performed on bovine paratuberculosis, compared fetuin and AGP as biomarkers for the disease, it was found that AGP is a poor diagnostic biomarker. In deep discussion, Park H et al, claimed that previous studies indicate AGP as a biomarker for diagnosis of bovine paratuberculosis diagnosis, but pointed out age difference between calves. Therefore, it was speculated that the difference between sources of samples may create a huge difference between final results (Park et al. 2022).

AGP is an acidic protein that is highly soluble not only in water but also in various polar organic solvents. Studies show that AGP has hydrophobic characteristics making it a favorable for binding organic compounds (Bteich 2019). Each AGP molecule has availability of only one drug binding site. In addition to that, the concentration of AGP in plasma is very low. Therefore, whenever a drug is bound to AGP, the binding is quite saturable, leading to toxicological consequences (Huang and Ung 2013). It has also been studied that some neutral and basic drugs such as diazepam, disopyramide and chlorpromazine can easily bind with AGP (Deb et al. 2018). These published studies have been investigated in human medicines, however there is no potential study on this

subject in animal medicine as yet. Hence, it can be concluded in the light of these studies that though the concentrations of serum AGP have been studied to increase during inflammatory response of body of cats (Addie et al. 2022), the introduction of drugs in the body could potentially alter and cause a drop in the concentrations of AGP. The arguments support our thesis study results.

Çoşkun et al. (2021) reported that acute phase proteins increase right after an inflammatory response begins, elevate higher in the first 24 hours and stay elevated until the inflammatory stimulus continues. Therefore, it should be noted that cases brought to Erciyes University Veterinary Hospital showing normal ranges of serum AGP in serious diseases like FIP were mostly brought after a basic treatment using anti-inflammatory medication, that was already done at private veterinary clinics or at home. The effect of anti-inflammatory medication suppresses the inflammatory stimulus in the body and tries to decrease pain of the patient, which in turn decreases the concentration of AGP, or any other APPs in the serum.

Hsiao et al. (2019) found the concentrations of AGP tremendously increasing in the patients' plasma who were suffering from sepsis, but there was a decline in the concentrations of AGP once the antimicrobial treatment was introduced to the patients. Technically, it helped to predict the disease outcome and prognosis but, in the case of cats that were brought to Erciyes University Veterinary Education, Research and Practice Hospital, mostly came for a tentative diagnosis after being treated at a private veterinary clinic. The concentrations of AGP must have been high, according to previous research, but due to the antimicrobial treatment, the cats' plasma concentration of AGP was not as high as expected.

Addie et al. (2022) performed experiments on various cats suffering from FIP, both effusive and non-effusive forms. There was a rise in the AGP levels seen in all the cats suffering from effusive form of FIP, except for two cats, from whom the blood was drawn after the treatment had already started. The AGP concentrations in the blood of cats with non-effusive FIP were normal. Therefore, the argument supports the results of the present study.

Selting et al. (2000) mentioned in an article regarding serum AGP concentrations in dogs with tumors, that significant increase in the serum AGP concentrations in dogs

with tumors was not always recorded. It was also noted that dogs with both benign and malignant tumors did not have any different serum AGP concentrations as compared to the dogs from healthy or control groups. Therefore, there is no potential study made yet in which all dogs with tumors show higher concentrations of serum AGP. Reports (Furukawa T et al, 1986; De Rick AF et al, 1987) also suggested that serum AGP concentrations are insensitive and non-specific to underlying diseases. In a study, AGP concentrations in cats with clinically diagnosed Feline infectious peritonitis (FIP) were elevated. It was also reported in the same study that there was an increase in AGP concentrations in cats suffering from feline immunodeficiency viral infection. Hence, AGP is not specific to any disease. It increases initially in all inflammatory conditions and is also seen to decrease after clinical treatment has been started (Selting et al. 2000a).

According to Sevgisunar and Şahinduran 2014, more extensive and detailed studies are needed to first understand the function of acute phase proteins and then the clinical uses of these proteins. It has also been suggested that the measurement methods adopted for APP values are still in need of improvement and species-specific ELISA kits have not yet been indicated. It has also been suggested that there is no availability of other trustable parameters that can help veterinarians in subclinical determination of the underlying disease state.

There were some limitations in this thesis study. One of the major limitations might be low number of clinical cases in the acute hepatitis diseased group. The low number of cases might have given high levels of serum AGP concentrations when statistically analyzed. There is room for more research and a chance that the levels might change with an increased number of cases. Other major limitation of the present study might be the specificity of the ELISA kits used, that were imported from China. For future study prospects, it is suggested that studying a comparison of results obtained from different ELISA kits may give better results. Cases brought to the Practice Hospital were either treated at home with an anti-inflammatory medication or brought very late for the diagnosis of disease and treatment. This could have altered the serum AGP concentrations. For future study prospects it is suggested to study the serum AGP

concentrations in the cats that were not treated with any medication before and to study the prognosis of the diseases while keeping the cats under veterinary surveillance.

6. CONCLUSION AND SUGGESTIONS

- In the present study, serum AGP concentration in cats with various common infections, including viral, bacterial and parasitic infections was calculated with the help of cat serum AGP ELISA kit. The aim of the study was to declare AGP as a useful biomarker for diagnosis of disease and to save as many cats from mortality as possible.
- Serum AGP concentration of cats with FIP were found high and in accordance with the previous studies. It can be concluded that serum AGP can be used as a potential biomarker for a tentative diagnosis of FIP in cats.
- Most important and favorable results of the present study were the highly elevated serum AGP concentrations of cats with acute hepatitis. AGP can be used in cats with acute hepatitis, for not only the tentative diagnosis but also follow-up of treatment. There is a room for extensive study with a high number of cases.
- In cases such as sepsis and ileus, the serum AGP concentrations were very high but we cannot declare it as a potential biomarker. More samples with the concern of these diseases should be performed to decide such speculation.
- Though, serum AGP concentrations are determined high in the cats with FIP, there is still room for more extensive study and research work on the subject. Serum AGP should be used in combination with other inflammatory biomarkers as well in the tentative diagnosis of FIP.
- There is a dire need of extensive study in the field of serum AGP as a potential biomarker to help veterinarians in developing a diagnostic tool in order to decrease the mortality of cats suffering from important clinical cases such as FIP, hepatitis, sepsis and ileus. In accordance with the present study, AGP is one

the most important APPs that can be trusted in future for the tentative diagnosis of important diseases such as hepatitis, sepsis and ileus.

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YÜKSEK LİSANS TEZİ

ORİJİNALLİK RAPORU

%12	%8	%7	%4
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	Submitted to Mansoura University Öğrenci Ödevi	%2
2	www.utsakcongress.com İnternet Kaynağı	%1
3	www.science.gov İnternet Kaynağı	%1
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5	José Joaquín Cerón,, Peter David Eckersall,, Silvia Martínez-Subiela. "Acute phase proteins in dogs and cats: current knowledge and future perspectives", Veterinary Clinical Pathology, 2005 Yayın	<%1
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RESUME

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THESIS -

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