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Posted Date: 27 August 2024

doi: 10.20944/preprints202408.1908.v1

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Possibility of Total Cholesterol/High-Density Lipoprotein Ratio as a Prognostic Indicator through Correlation Analysis with Hyperlipidemia Related Diseases in Dogs

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Abstract: In veterinary medicine, the significance of High-Density Lipoprotein (HDL) measurements is not well-documented than it is in humans. The HDL level can be measured in dogs, and through referring to the normal range, it is possible to find out what this means for various endocrine diseases and hyperlipidemia diseases. Aim of this study is to measure the HDL levels in dogs with various conditions and to evaluate whether the Total Cholesterol (TC), HDL ratio is effective as a prognostic indicator in various hyperlipidemia and endocrine diseases. Through prospective clinical trial, sixteen client-owned dogs and cats visiting a local private practice were divided into three groups; five dogs without hyperlipidemia or metabolic disease (Group 1); eight animals with Cushing's, hypothyroidism, and gallbladder sludge (Group 2) and three diabetics, urinary disorders, and healthy cats (Group 3). In two dogs, the TC/HDL values were between 2 and 3; in two dogs, the values were between 3 and 4; in two dogs, the values were between 4 and 5. In three dogs, the TC/HDL values were between 5 and 6 and in three dogs, the ratio values were between 6 and 7. The other value was higher than 8. Except two dogs which showed lower value than 3, all dogs had concurrent endocrine disease including Cushing's disease, hypothyroidism, gall bladder mucocele, hyperlipidemia. There was a difference in each group in terms of the TC/HDL value compared to the group with underlying disease. This means that TC/HDL values can be an excellent indicator of endocrine disease in dogs as well. In cats, although this is a very small sample, a high TC/HDL value of 9 points are shown in the cat which had diabetes mellitus. However, for more statistically significant results, a larger sample group for further investigation is needed.

Keywords: Hyperlipidemia; High-density lipoprotein; Total cholesterol; Cushing's disease; Hypothyroidism; Total cholesterol/High-density lipoprotein ratio

1. Introduction

The importance of hyperlipidemia in human medicine has been emphasized over the years. The complications of hyperlipidemia include endocrine diseases such as diabetes and pancreatitis, but fatal cardiovascular diseases such as stroke and myocardial infarction are also closely related to this condition [1] [2]. Therefore, researchers have conducted studies aiming to prevent such complications through efforts to improve hyperlipidemia, and in the process, drugs to improve hyperlipidemia such as Statins and Fenofibrate[3] have been developed [4]. Lipid which is absorbed into the body through lacteal in small intestine after digestion must travel through blood vessels, but since lipid is fat-soluble, not water-soluble, it cannot dissolve in the blood vessels and move on its own. Therefore, it forms a lipoprotein complex and moves throughout the body's organs, changing into various forms including chylomicron, Very Low-Density Lipoprotein (VLDL), Low-Density Lipoprotein (LDL),

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HDL accordingly (Figure 1). When chylomicrons are absorbed from lacteal lymphatic vessel [5] in the small intestines, they pass through the liver to become VLDLs, then to adipose tissue to become LDLs, and then to muscles and blood vessels and other organs to condense into HDLs, and the low-density, high-volume lipids are removed.

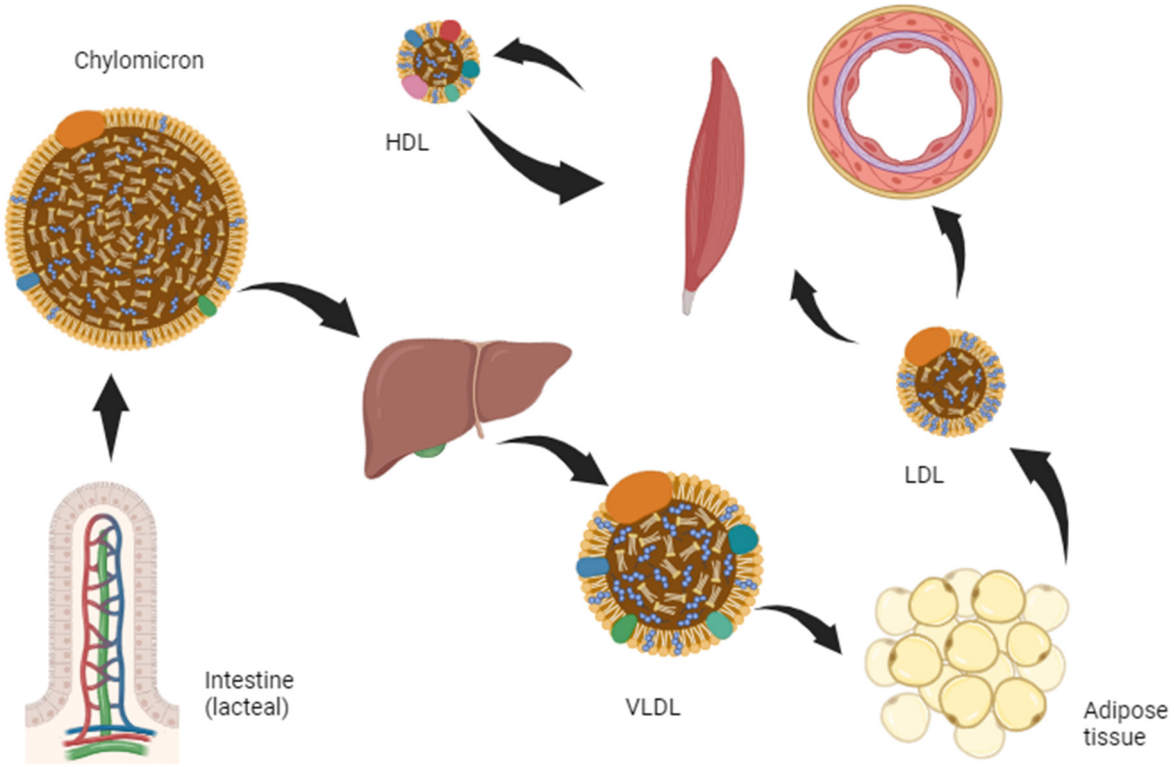

Figure 1. When chylomicrons are absorbed from the intestines, they pass through the liver to become VLDLs, then to adipose tissue to become LDLs, and then to muscles and blood vessels and other organs to condense into HDLs, and the low-density, high-volume lipids are removed.

HDL has been found to play a role in removing lipids from blood vessels, which gives rise to its image as good cholesterol. As HDL levels rise, triglyceride (TG) and LDL levels decrease in relative terms, which can prevent cardiovascular diseases such as stroke and myocardial infarction. However, there is growing awareness recently that higher HDL does not always guarantee healthier condition and that it is better to evaluate this biomarker in comprehensive aspect of health condition [6].

In the veterinary field, there are relatively few studies on the role of HDL and its relationship with hyperlipidemia complications. Additionally, the frequency and importance of cardiovascular diseases such as stroke and myocardial infarction are lower than expected. One study explored the normal range of HDL in dogs [7], but HDL is not yet routinely measured in the clinical field. The findings may provide clues as to why strokes and myocardial infarctions occur less frequently in dogs than in humans. In fact, due to shorter life span of dog than human and other critical disease such as myxomatous mitral valve disease and lymphoma, the risk of atherosclerosis is relatively low than in human. Nevertheless, arteriosclerosis and myocardial infarction due to hyperlipidemia in dogs have been reported recently [8].

In this study, we measured HDL in dogs and cats who visited primary veterinary hospitals and examined its relationship with endocrine diseases such as hyperlipidemia, diabetes, Cushing's, and hypothyroidism. Since the number of patients visiting primary hospitals is limited, it is important to collect as much data as possible. There is no choice but to be sampled due to various species and various underlying diseases. This is valuable data for follow-up research with a uniform population in referral hospitals or in university. Also, there has been few research on HDL levels in cats. These

 doi:10.20944/preprints202408.1908.v1

samples of cats could provide supplementary data for follow-up research. It also can be a useful resource for follow-up research with amino acid sequence comparison.

2. Materials and Methods

Blood samples were collected from sixteen client-owned dogs and cats visiting Bundang New York animal hospital located in South Korea. All blood samples were collected from the cephalic vein in a fasted state without anesthesia or sedation and centrifuged at 14500RPM for 1 minute, and the sera were directly analyzed using DRI-CHEM NX500 (Fujifilm), a dry chemistry analyzer, immediately. Only when measuring dog HDL, the serum was diluted 5 times with sterilized WFI, and the other samples were measured directly without dilution at room temperature. JASP was used for data analysis and graph creation (https://jasp-stats.org), and amino acid sequence analysis was performed using BioEdit (https://bioedit.software.informer.com/7.2).

3. Results

Upon analyzing the correlation with the TC/HDL ratio by dividing the presence or absence of hyperlipidemia-related endocrine diseases such as hypothyroidism, Cushing's, gallbladder mucocele, and liver cancer into 1 and 0, concurrent disease and TC/HDL value showed strong positive correlation significantly (Data 1). Interestingly, the dogs which had lower TC/HDL value than 3 had no disease but other dogs had various diseases which had higher TC/HDL value than 3. This data provides that TC/HDL cut-off value as a healthy condition biomarker can be 3 which is similar in human medicine [9].

In general, it is known that, the higher the TC/HDL ratio in humans, the higher the likelihood of various cardiovascular diseases. Therefore, this ratio has been used as a prognostic indicator for cardiovascular diseases such as stroke and arteriosclerosis. The lower the ratio is than 5, the better the prognosis; less than 3.5 is considered good, and if it is more than 6, the risk of cardiovascular disease is high [10]. Also, TC/HDL ratio has been excellent indicator for endocrine disease such as insulin resistance diabetes [11].

The originality of this study was to investigate whether the TC/HDL ratio is effective prognostic biomarker for cardiovascular disease and endocrine disease in client-owned dogs and cats through actual measurements and it was first trial at general practice. There was clear limitation to reveal correlation between cardiovascular disease and TC/HDL value because there was no concurrent heart disease in the samples. Nevertheless, in aspect of endocrine disease related to hyperlipidemia such as Cushing's disease, hypothyroidism, gallbladder mucocele, these data showed excellent correlation between TC/HDL and concurrent diseases. In fact, in human patients with Cushing's, a decrease in the TC/HDL ratio before and after remission can be observed, and thus, this can be used as a prognostic evaluation factor [12]. In addition, studies have shown that the incidence of gallbladder polyps is strongly related to the non-HDL/HDL ratio [13]. Also, in human hypothyroidism, the TC/HDL ratio is lowered after appropriate treatment [14]. In addition, there are research results showing that TC/HDL can be an important prognostic indicator in the case of non-alcoholic fatty liver disease, although not for liver cancer, as in our case [15]. Additionally, the TC/TG ratio was also measured to examine its potential as a prognostic evaluation factor.

Additionally, TC/TG value can be indicator for small, dense LDL, negative correlation in human medicine [16]. In human, small, dense LDL is an emerging biomarker for cardiovascular disease [17],[18]. It is also related to metabolic disease including diabetes mellitus [19]. Therefore TC/TG value can also be negatively correlated to small, dense LDL in veterinary medicine. Pleasurably, lowest TC/TG value was shown in hyperlipidemia, hepatocellular carcinoma dog, #9 (Table 1).There are some study related to LDL and hepatocellular carcinoma in human medicine [20] [21] but this result needs more investigation because of small sample and difference between human and dog.

Interestingly, low HDL values were shown in dogs which had lipid metabolism related disorder (Cushing, hypothyroidism) #6,11,12 (Table 1). Also, low HDL value was also shown in diabetic cat #2 (Table 2). Low HDL level is strongly related to non-insulin dependent diabetes mellitus (type 2) in human medicine [22] and in cats most of type of diabetes mellitus are type2 [23] so it is plausible data.

 doi:10.20944/preprints202408.1908.v1

Table 1. Signalment and concurrent disease of 16 dogs and cats used in correlation and comparison study.

Dog #	Body weight (kg)	BCS (1 to 9)	Breed	Sex	Age	HDL (60~140)	TC (111~312)	TG (30~133)	TC/HDL	TC/TG	Visit purpose	Concurrent disease	Heart Murmur (0 to 6)
1	3	5	Pomeranian	Spayed female	9y	140	450	78	3.21	5.77	Annual checkup	None	0
2	23	4	Jindo	Male castrated	9y	56	352	78	6.28	4.51	Annual checkup	None	0
3	2.2	4	Poodle	Female	9m	64	172	218	2.68	0.79	Achilles tendon repair surgery	None	0
4	3.65	5	Maltese	Spayed female	3y7m	80	223	19	2.78	11.7	Right patella luxation surgery	None	0
5	4.2	5.5	Poodle	Spayed female	7y7m	56	369	104	6.6	3.54	Regular checkup	Gallbladder mucocele	0
6	3.7	4	Poodle	Male castrated	13y	46	233	225	5.06	1.03	Regular checkup	Cushing/GERD	1
7	2.85	5	Chiwawa	Spayed female	11y2m	83	450	170	5.42	2.64	Regular checkup	Cushing	0
8	5.15	5.5	Maltese	Male castrated	12y	87	290	247	3.33	1.17	Cognitive disorder treatment	Cognitive disorder/Cushing	0
9	15.3	4	Shetland Sheepdog	Male castrated	13y5m	65	315	500	4.84	0.63	Cancer treatment	Liver cancer (HCC)	2
10	2.95	4	Maltese	Spayed female	14y9m	92	450	500	4.89	0.9	Cognitive disorder treatment	Gallbladder mucocele/ Cognitive disorder	0
11	8.8	4.5	Jindo	Spayed female	8y7m	42	243	36	5.78	6.75	Regular checkup	Hypothyroidism	0
12	3.68	6	Pomeranian	Male castrated	4y9m	42	360	402	8.57	0.89	Regular checkup	Hypothyroidism	0
13	3.5	6	Pomeranian	Male castrated	5y5m	26	160	170	6.15	0.94	Dental scaling	None	0

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Table 2. Signalment and concurrent disease of 3 cats for supplementary data.

Cat #	Body weight (kg)	BCS (1 to 9)	Breed	Sex	Age	HDL (60~140)	TC (85~176)	TG (17~104)	TC/HDL	TC/TG	Visit purpose	Concurrent disease	Heart Murmur (0 to 6)
1	8.1	9	American Shorthair	Spayed female	5y	75	85	21	1.13	4.04	Dysuria	Dysuria	0
2	3.9	4	Korean Shorthair	Male castrated	6y 6m	21	202	117	9.53	1.72	Insulin treatment	Diabetes	0
3	3	4	Korean Shorthair	Spayed female	10m	93	138	36	1.48	3.83	OHE surgery	None	0

4. Discussion

This study showed TC/HDL value can be excellent biomarker of lipid metabolism related endocrine disease and its cut off value can be 3. the role of HDL in the veterinary field has not yet been fully elucidated and it requires attention in additional research because of small number of samples in this study. This study is significant as the first attempt to measure HDL in actual patients using general biochemical equipment in a primary general practice rather than a laboratory. Also, comparing the amino acid (Figure 2) related to HDL (Cdkal1) [24], which is identical between humans, dogs, cats, and pigs, shows that the role of HDL in cardiovascular disease may be similar among species. The reason why strokes and myocardial infarctions in dogs occur less frequently than in humans could be multifactorial[25] rather than solely genetic, and involve the environment, food, and stress[26]. Additionally, if we compare the incidence of stroke and arteriosclerosis in Schnauzers, which genetically tend to have hyperlipidemia more than other species [27], and also measure the normal range of HDL in Schnauzers, we will be able to gain a deeper understanding of the role of HDL in cardiovascular disease. Cats are more likely to develop blood clots [28] than humans, and dogs [29]. The amino acid sequence of feline ApoA-1, the related protein, is slightly different to that in other species (Figure 2). This may be related to HDL, which plays a role in transporting lipids from blood vessels to other organs [30]. To date, there has been no research on the correlation and mechanism between HDL levels and thromboembolism in cats. Further study may reveal the role of HDL in cardiovascular disease and thromboembolism in felines.

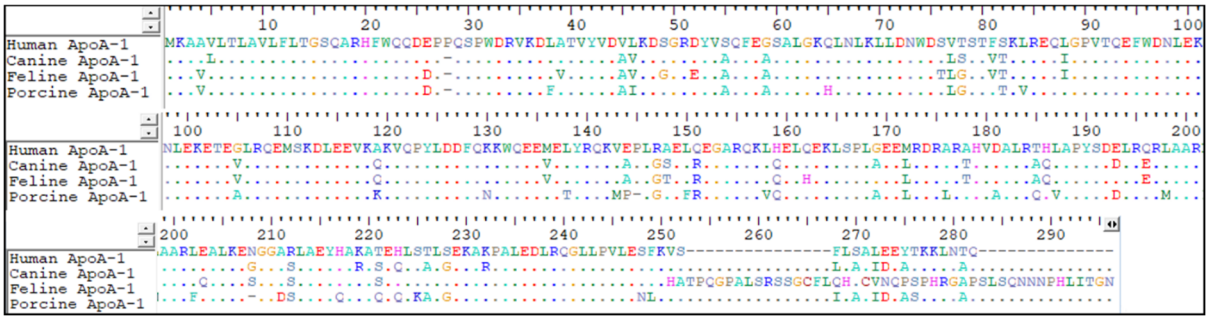

Figure 2. Cats are more likely to develop blood clots than humans, pigs, and dogs. They also have lower HDL levels than dogs. The amino acid sequence of feline ApoA-1, the related protein, is slightly different to that in other species. This may be related to HDL, which plays a role in transporting lipids from blood vessels to other organs. Concurrent disease including hypothyroidism, gall bladder mucocele, Cushing's disease, Hepatocellular carcinoma, hyperlipidemia are shown 1 and no concurrent disease are shown 0. Concurrent disease and TC/HDL value show strong positive

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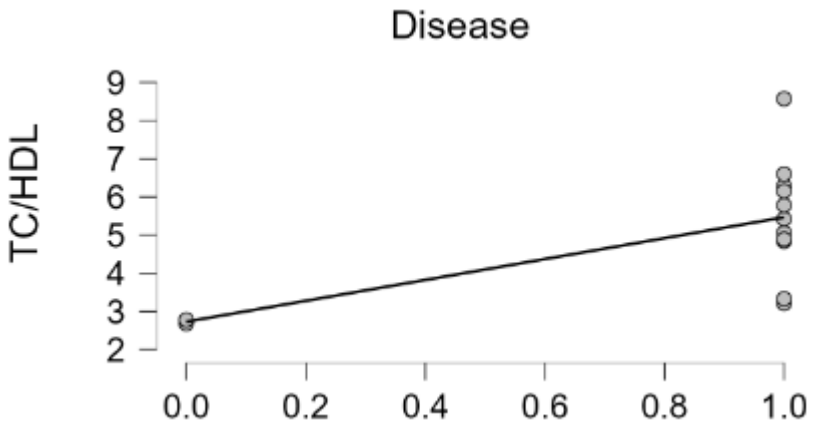
correlation significantly and the dog which have lower TC/HDL value than 3 have no disease (Pearson's r 0.599, p-value 0.031).

Data 1



(Pearson's r 0.599, p-value 0.031)

Author contributions: K.Choi designed the idea, collected, analyzed data and ,wrote the manuscript. All authors reviewed the manuscript

Data availability statement: Data supporting this study are included within the article and/or supporting materials

Ethical statement: Informed consent was waived (or exempted) from IRB due to the retrospective design. Prior to collecting samples, all pet-owners signed an informed consent.

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