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Effect of serum lipid subfractions on nuclear magnetic resonance spectroscopy's ability to detect coronary atherosclerosis early

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Abstract
Nuclear magnetic resonance spectroscopy, also known as NMRS, is a cutting-edge method based on nuclear magnetic induction theory and quantum optics that has found extensive usage in the domains of chemical analysis, life science, and other disciplines. The method is quantitative and repeatable, making it particularly effective for detecting and analyzing tiny compounds in complicated solutions like blood and urine. With a high degree of standardization and automation, it can assess 114 hemo lipoprotein subfractions and particles in human serum/plasma in a targeted way (exact quantification) and produce precise and dependable detection findings. All processes may be tracked back to fulfill our requirements for non-targeted analysis, which is suited for biological fluid specimens and water-soluble specimens from tissue or cell extracts. The 114 lipoprotein subfractions and particles can be targeted using nuclear magnetic resonance spectroscopy (NMRS), which can detect the serum lipid profile in its entirety. The analysis of the serum lipid profile, the correlation between the levels of lipid subfractions and lipid particles and the degree of coronary stenosis, and the development of a coronary stenosis early warning system can therefore improve the current risk model on the inadequacy of the risk stratification of the patients. These studies are based on a cohort of individuals with varying degrees of coronary atherosclerosis. The development of a coronary artery stenosis early warning system might enhance the patient risk classification models already in use. To analyze the correlation of lipid subfractions and particle levels with cardiovascular events and therapeutic efficacy, to explore more effective biomarkers for lipid-lowering monitoring, and to evaluate the efficacy of classical Chinese medicinal preparations.

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1. Introduction

Cardiovascular disease (CVD) is a development of abnormal body health conditions from abdominal obesity to hypertension to faulty lipid and glucose metabolism to insulin resistance to metabolic syndrome to illnesses like coronary heart disease [1]. Atherosclerotic cardiovascular disease (ASCVD), such as coronary atherosclerotic heart disease (CHD), stroke, and others, are frequently associated by more prominent atherosclerosis in those with cardiovascular metabolic syndrome [2]. The prevalence of cardiovascular metabolic disorders is rising as living standards rise and lifestyles change. There is no disputing the fact that atherosclerosis is considered to be primarily a lipid-driven disease due to the strong correlation between lipid accumulation and the onset of the disease. As the four primary lipid screening indices, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) have received widespread recognition as

early indicators of cardiovascular disease. LDL-C has served as the primary indicator in ASCVD early warning, medication counseling, and lipid-lowering monitoring in recent years despite increased emphasis to the therapeutic relevance of alternative lipids such ApoA1, apolipoprotein B, and lipoprotein a.The search for better ASCVD risk predictors and therapeutic interventions to control risk factors has become crucial to the prevention and treatment of cardiovascular disease, though, as many patients' residual cardiovascular risk remains uncontrolled despite the achievement of guideline-based lipid control. For the detection and study of tiny molecules in complicated solutions including plasma, serum, and urine, NMR spectroscopy is a particularly useful measure of lipid metabolism that may identify 114 subtractions and particles of hemolipoproteins in human serum/plasma. Therefore, it is anticipated that the measurement of serum lipoprotein subtractions by NMR spectroscopy would help develop a coronary artery stenosis early warning system, as well as investigate more potent pharmacogenetics biomarkers and direct the usage of medications.

2. Coronary atherosclerosis

Coronary atherosclerotic lesions are a frequent condition that have a significant death rate [3]. According to the 2020 China Healthy Lifestyle Guidelines for the Prevention of Cardiovascular and Metabolic Diseases, 430,000 deaths in China were attributed to coronary heart disease, stroke, and other cardiovascular diseases in 2017, which was 42% of all deaths. The burden of cardiovascular diseases was more than 85 million disability-adjusted life-years (DALYs), and cardiovascular and metabolic diseases are the main cause of death in China. Therefore, cardiovascular disease prevention, control, and treatment are very important.Recent research has established a strong link between lipid metabolism disorders, an illogical diet, inactivity, smoking, and excessive alcohol use and the sharp increase in cardiovascular metabolic diseases [3]. There is no disputing the fact that atherosclerosis is viewed as being primarily a lipid-driven disease due to the close connection between lipid accumulation and the onset of the disease. Low-density lipoprotein cholesterol (LDL-C) buildup has long been known to be the first step in the onset of atherosclerosis [2, 4, 5]. Numerous harmful stimuli (such as hyperlipidemia, smoking, hypertension, viruses, etc.) can harm endothelial cells, causing lipid to build up in the artery intima where it is then changed, aggregated, or oxidized [6]. LDL-C aggregates into complexes with sizes ranging from 100 nanometers to 1.0 millimeters, which immune cells in the basement space can process through cytotoxic or phagocytic action. Complexes with a size of 100 nm to 1 mm are created when LDL-C aggregates, and immune cells in the subordinated space can handle these complexes through cytologic or phagocytic action. When monocytes enter the subendothelial, they undergo macrophage differentiation and phagocytose modified LDL-C, in which extra cholesterol is esterified and stored in lipid droplets [7]. This causes macrophages to appear foamy. The recruitment of circulating immune cells and the release of cytokines and chemokines by foam cells result in the subsequent induction of inflammatory reactions [4]. The 2016 Chinese Guidelines for the Prevention and Control of Dyslipidemia in Adults recommend measuring triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and LDL-C as the basic screening indices of lipids. The clinical value of other lipid items such as ApoA1, apolipoprotein B, and lipoprotein a has also been reported. LDL-C has been considered the most important indicator in the early warning of ASCVD, drug counseling, and lipid-lowering monitoring work [6]. The clinical significance of additional lipid items such as ApoA1, apolipoprotein B, and lipoprotein a has also gained growing attention. High lipoprotein an is an independent genetic risk factor for ASCVD, and studies have shown that people with slightly to moderately high TG are at greater risk of developing ASCVD [8]. Almost half of the patients with ASCVD had LDL-C values below 100 mg/dL, suggesting that LDL-C only accurately reflects the risk of half of the ASCVD patients, according to international research that included 136905 individuals hospitalized for coronary artery disease. However, ASCVD events continue to happen regularly even in those with managed LDL-C. In the GLAGOV study, therapy with evolution plus statins lowered mean LDL-C to 0.95 mmol/l, but intravascular ultrasound analysis showed that approximately one-third of patients had no change in atherosclerotic volume or even development of atherosclerosis at 18 months. Despite attaining the levels of cholesterol management indicated by guidelines, many patients still have uncontrolled residual cardiovascular risk as a result of these data, which encourage aggressive LDL-C lowering to reduce cardiovascular events. In order to prevent and cure cardiovascular disease, it is now essential to look for better predictors of ASCVD risk as well as therapeutic approaches to manage risk variables.

3. Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy (NMRS) detection of lipid metabolism indexes: The revolutionary detection method known as NMRS, which has found extensive usage in the fields of chemical analysis, drug testing, life sciences, etc., is based on nuclear magnetic induction theory and quantum optics. [6] A non-destructive detection method that may be used to find specimens already in place is NMRS. Its ability to reflect the combined effects of various physiological conditions, phenotypes, and genomes, among other factors, on the human body makes NMR a unique platform for studying the relationship between the composition and fingerprinting of human body fluids and health and disease. The NMR fingerprint of a body fluid specimen is made up of more than a thousand different signals produced by thousands of metabolites. The method is particularly well suited for the detection and analysis of small molecules in complex solutions including plasma, serum, and urine [6]. It is quantitative, repeatable, non-selective, and non-destructive. The Bruker AVANCE IVDr system can target (exactly quantify) 114 hemolipoprotein subtractions and particles in human serum/plasma [9], and the technique has been approved by several labs across the world. For accurate and trustworthy findings, the procedure is highly standardized, automated, and has been verified in several labs across the world. The system has also developed a database of 850 reference compounds in biological fluids, which is suitable for biological fluid specimens as well as water-soluble specimens of tissues or cell extracts, and all steps can be traced back to meet the needs of our non-targeted analyses. This database has proven to be a reliable resource for the discovery of biomarkers linked to diseases [8].

4. Serum Lipid Profile

In order to comprehend the function and metabolic regulation of lipids, as well as to reveal lipid metabolic pathways, networks, and their relationship to physiological and pathological processes in the body, lipidosis is a novel discipline that provides a thorough analysis and identification of lipids and their metabolites [9]. The cornerstone of clinical assessment and management of atherosclerotic CVD risk is laboratory measurement of plasma lipids (mostly cholesterol and triglycerides) and lipoprotein lipids (primarily low density lipoprotein [LDL] and low density lipoprotein [HDL] cholesterol). HDL particles stop or reverse atherosclerosis by reversing cholesterol transport, in contrast to LDL and, to a lesser extent, very low-density lipoprotein [VLDL] particles. The proportion of "good" HLDL and "bad" LDL influences the overall risk of CVD. The ratio of "bad" LDL (and VLDL) to "good" HDL particles determines the overall risk of CVD [5]. NMR spectroscopy can now be used to directly rate lipoprotein particle counts.According to research by Monica M. Purmalek *et al*. [10], greater LDL particle counts are principally responsible for the positive correlation between dense calcified plaques and total and LDL particle counts as well as LDL cholesterol. Overall VLDL particle counts were favorably correlated with dense calcified plaques; this correlation was inversely correlated with VLDL particle size and driven by low VLDL particle counts. Additionally, LDL particle concentration demonstrated a larger connection with university diseases than did LDL cholesterol [11]. Small and dense LDL subtractions were found to be substantially linked with ASCVD in a study of lipoprotein subtractions, but they did not always fluctuate sympathetically with total LDL-C, which explains why clinically abnormal total LDL-C levels are only found in half of ASCVDs [10]. Because patients with similar cholesterol levels may differ in the number and size of lipoprotein particles transporting cholesterol and triglycerides, and as a result, may differ in CAD risk, conventional cholesterol levels may not accurately reflect the true atherogenesis of the plasma lipid profile [12]. Since LDL-P is a better predictor of cardiovascular events than is LDL, LDL-P is the actual amount of LDL particles (particle concentration). A potential target for screening, risk assessment, and treatment is LDL-C because it is a stronger predictor of the likelihood of having a cardiovascular event [13]. While the US guidelines for the prevention and management of atherosclerosis and hyperlipidemia point out that the different size distribution of LDL particles results in different atherosclerosis risk sizes, with the smaller the particles, the greater the risk with an approximation of a proportion of.05%, more recent studies have confirmed that LDL-P is directly involved in the pathological progression of atherosclerosis and is an independent risk factor for ASCVD [14]. As a result, investigating the serum lipid profile is useful for finding atherosclerosis biomarkers.

5. Detection of Lipoprotein Subclasses by Nuclear Magnetic Resonance Spectroscopy

On recently thawed frozen plasma samples, lipoprotein subclass profiles are assessed using proton nuclear magnetic resonance spectroscopy. Blood is often spun down to remove hemoglobin-containing red blood cells and leave the plasma before being tested as serum [9]. When necessary, salt must be added to the serum or plasma to maintain the osmotic pressure during any dilution in order to preserve structures like lipoproteins [9]. The NMR approach bases its measurement on the distinctive signals emitted by various lipoprotein subtypes. The total number of terminal methyl groups on the lipids in the particle is used to calculate each subclass signal [11]. Triglycerides and phospholipids each supply three methyl groups to the particle's core, whereas uncertified cholesterol and cholesteryl esters each contribute two methyl groups to the particle's surface shell. Regardless of variations in lipid composition, such as variations in the relative amounts of cholesteryl esters and triglycerides in the core of the particle, variations in the degree of unsaturation of the fatty acyl chains of the lipids, variations in phospholipid compositions, etc., the diameter of the particle determines the number of methyl groups present to approximate the approximation. Thus, the methyl NMR signal produced by each subclass acts as a direct indicator of the subclass's concentration [11]. The most remarkable and compelling feature of NMR is its capacity to obtain data from an incredibly broad range of samples without causing harm or destruction, whether those samples are found inside of organisms, like the human body, isolated organ preparations, intact tissue samples, like tumors, or in vitro, from homogeneous tissue extracts in solid, liquid, or gaseous form [9]. This adaptability can be shown in NMR spectroscopy, which uses both nuclear absorption and radiofrequency radiation emission [9].

The number and size of lipoprotein particles may be detected thoroughly and with high throughput using serum lipoprotein subtractions NMR spectroscopy [15]. Lipidosis is a thorough analysis and identification of lipids and their metabolites to comprehend the function of lipids and the pathways of metabolic regulation, as a way to reveal the lipid metabolic pathways, networks, and their relationship with the physiological and pathological processes of the body [9]. Lipidosis technology has greatly aided in the screening of the new generation of biomarkers for hyperlipidemia and cardiovascular diseases. Lipids are primarily moved about and dispersed in serum by LDL, HDL, and low density lipoprotein. As a result, it is helpful to examine the serum lipid profile while looking for atherosclerosis biomarkers. Conventional lipid markers like LDL and HDL can still be separated into different subtractions, as shown by lipoprotein electrophoresis [14]. Immunological techniques, gradient gel electrophoresis, gradient media centrifugation, nuclear magnetic resonance spectroscopy (NMRS), vertical gradient centrifugation, ultracentrifugation, and ion mobility shift (IMS) are some of the current methods for the detection of lipoprotein subtractions and particles. The only high-throughput, all-encompassing method for detecting lipoprotein subtractions and particles at the moment is NMRS. It is a subfield of spectroscopy in which, during the transition from a low-energy to a high-energy state, electromagnetic radiation is absorbed by molecules or nuclei with nuclear magnetism in a strong magnetic field. This resonance spectrum is digitally converted into a computerized mathematical model, which is then used to identify the quantity, kind, and relative position of the components of a mixture [16]. Since being standardized and employed for the detection of lipid subcomponent profiles in clinical practice, NMRS has been used for the first time to detect lipids in 1990 [17]. The application of NMRS for the detection of lipid subcomponent profiles is the foundation of NMRS theory. The idea is to use NMRS to count the protons in phospholipids, uncertified cholesterol, cholesteryl esters, and TG terminal methyl groups. The phospholipids and

uncertified cholesterol on the surface each have two terminal methyl groups, whereas the esterified cholesterol and TG have three terminal methyl groups each in the center of the lipid particles.

NMRS can quantitatively detect the average particle size of LDL, as well as the distribution of lipids in the subtypes of VLDP, MLDP, LDL, and HDL. It also determines the mass, number of particles, and particle size of lipoprotein subtractions through the detection of the number of protons of the four types of terminal methyl groups. The distribution of lipids was measured [18]. LDL-P is typically advised to be 1,200 nmol/L in patients with intermediate-risk of ASCVD and 1,000 nmol/L in patients with high-risk or very high-risk of ASCVD according to the ACCE Guidelines for the Prevention and Management of Dyslipidemia and Atherosclerosis in the United States of America [12]. The first lipid subtractions of the Chinese population were discovered by Lin Yahui [6] and colleagues, who also created reference intervals for lipid subtractions in healthy Chinese people. However, this method is solely applicable to scientific study and has not been applied in clinical practice.

By using nuclear magnetic resonance spectroscopy to remove serum lipoproteins, it is possible to access more potent pharmacogenetics biomarkers and create an early warning system for coronary artery stenosis [6]. Existing research have revealed that the number and particle size of lipoprotein particles are substantially linked with ASCVD, with smaller particle size and higher particle number having extreme anthropogenic impacts [3]. Different lipid subtractions are present in LDL-C with the same LDL-C content. Since there may be variations in the number and size of cholesterol-lipoprotein particles, patients with the same cholesterol values may have different risks of developing coronary artery disease (CAD). As previously mentioned, small and dense LDL subtractions are strongly associated with ASCVD, but they do not always vary sympathetically with total LDL-C. Since LDL-P is a stronger predictor of the risk of cardiovascular events than LDL-C, it may be a target for screening, risk assessment, and treatment [13]. LDL-P is the actual number of LDL particles (particle concentration). The American Guidelines for the Prevention and Management of Atherosclerosis and Dyslipidemia note that the different size distribution of LDL particles leads to a different magnitude of risk for atherosclerosis, with the smaller the particles, the greater the risk of a greater perceivable risk for atherosclerosis [14]. More recent studies have confirmed that LDL-P is directly involved in the pathological progression of atherosclerosis and is an independent risk factor for ASCVD.

Studies on the relationship between HDL2-C and HDL3-C and the prevalence of coronary heart disease have also revealed a negative correlation between large HDL-P and early-onset (45 years of age) coronary heart disease in the Chinese population. This is likely due to the smaller, denser HDL3-C levels that are the primary cause of the inverse association between HDL-C and sudden-onset coronary heart disease. Plasma sd-LDL is an independent risk factor for coronary heart disease and is linked to the onset of arterial coronary stenosis, which can predict the development of atherosclerosis and coronary heart disease. By using magnetic resonance spectroscopy to measure the subtractions of serum lipoproteins, one can investigate a more potent pharmacogenetics biomarker and develop an early detection system for coronary artery stenosis [19]. Large-scale investigations on humans have consistently linked overall HDL-C content to cardiovascular risk [20]. Smaller HDL subclasses tend to be more functional for numerous protective mechanisms, despite the fact that the link between HDL subclasses and clinical outcomes is ambiguous [14]. Greater antioxidant activity, greater anti-inflammatory activity, greater cholesterol efflux ability, and larger impacts on cytoarchitecture are all characteristics of smaller HDL subtypes [19].

Further research into more potent pharmacogenetics biomarkers is required. Lipid subgroups and particle levels are intimately associated to the association of cardiovascular events and the effectiveness of coronary heart disease treatment. The severity of coronary artery stenosis can be determined using the Gensini score, and the degree of stenosis can be determined using serum lipid profiling. By examining the relationship between lipid subgroups and particle levels and cardiovascular events and outcomes for high-risk ASCVD patients, new effective lipid-lowering monitoring indices can be discovered. By examining the relationship between lipid subgroups, lipid particle levels, and the severity of coronary artery stenosis, an early warning system for coronary artery stenosis can be established based on a coronary atherosclerosis cohort. In order to assess the auxiliary diagnostic value of the pertinent indexes for the degree of stenosis of coronary arteries, a coronary atherosclerosis cohort should be established to include patients with coronary atherosclerosis suggested by coronary angiography, and the correlation between the levels of lipid subtractions and lipid particles and the degree of stenosis detected by NMRS should be analyzed [17]. LDL-P may be a potential target for screening, risk assessment, and treatment because it has been further proven that it is a stronger predictor of the risk of cardiovascular events than LDL-C [14]. Meanwhile, Glyc A can be found simultaneously thanks to nuclear magnetic resonance spectroscopy. The numerous acute-phase inflammatory proteins haptoglobin, 1 -antitrypsin, 1 -anti chymotrypsin, and transferrin are among the abundant acute-phase inflammatory proteins that are reflected in the overall concentration and glycosylation status of GlycA [19], a novel systemic biomarker of inflammation. Glyc A offers the potential to detect inflammation and predict the performance of improved biomarkers for assessing inflammation and forecasting the course of cardiovascular disease. Due to its lower intra-individual variability and higher analytical precision when compared to other traditional markers of inflammation, GlycA can be employed as a trustworthy biomarker when assessed by nuclear magnetic resonance spectroscopy [21]. In future clinical applications, Glyc A is expected to work together with serum lipid subtractions to predict the probability of CVD development and guide medication.

6. Discussion

In order to accurately and quantitatively analyze a variety of LDL subtractions and particles in human serum and plasma, nuclear magnetic resonance spectroscopy (NMR), a new detection technology founded on the theories of quantum optics and nuclear magnetic induction, has been validated in numerous labs all over the world. The method also yields accurate and dependable detection results. The risk of atherosclerosis varies depending on the size and distribution of LDL particles; the smaller the particles and the higher the proportion of small particles, the greater the risk, according to the recommendations for the prevention and management

of atherosclerosis and hyperlipidemia. Therefore, the analysis of serum lipid profiles is advantageous for the detection of atherosclerosis biomarkers and aids in the development of a coronary artery stenosis early warning system. By mining additional biomarkers, we can also keep track of the meticulous association between lipid subgroups and particle levels with cardiovascular events and outcomes following treatment for coronary heart disease.

Few hospitals or healthcare facilities in Northwest China offer the ability to perform MRI spectroscopy, and routine subtractions and Glyc A measurements on patients are now prohibitively expensive. While the development of cardiovascular diseases has a strong correlation with the standard of living, lifestyle, emotions, and other external factors, it was discovered when reviewing the literature that the common sample collection was concentrated in a certain fixed hospital or a certain fixed group of medical checkups. As a result, it is important to take into account the differences in geographic areas and economic levels in the variables when collecting the samples and the data. By using such techniques, the monitoring and early warning role's credibility can be raised.

7. Summary

The investigation of serum lipoprotein subtractions by nuclear magnetic resonance spectroscopy is discussed in this research as a way to develop a pharmacogenetics biomarker pipeline and a coronary artery stenosis early warning system. We evaluated the value of the pertinent indices for the auxiliary diagnosis of the degree of coronary artery stenosis and may become a useful method for screening, risk assessment, and treatment by examining the correlation between lipid subtractions and lipid particle levels detected by NMRS and the degree of stenosis.

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The authors report there are no competing interests to declare.

Data availability statement

Not applicable

Authors' contributions

CW performed the literature search and selection, extracted and analyzed the data, and drafted the manuscript, YS, HZ, HF and YZ revised the manuscript. JD designed and supervised the study, analyzed the data, and revised the manuscript. All authors granted the final approval for submission.

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