Review Article

Nuclear Magnetic Resonance Spectroscopy as a Tool for the Characterization of Human Gallstones Diseases

N Dwivedi, Research Scholar;[1,2] M A Siddiqui, Research Scholar;[1,2] N Sinha, Professor.[1]

[1]Centre of Biomedical Research, SGPGIMS Campus, Lucknow, India

[2]Integral University, Lucknow, India

Mr. Navneet Dwivedi is currently working as DST INSPIRE Fellow and pursuing Ph.D. at Centre of Biomedical Research, Lucknow. He has done M.Sc. Physics (Gold-Medalist) from Integral University, Lucknow. His area of interest is the development and application of NMR spectroscopy in biological systems.

Corresponding author - Dr.Neeraj Sinha (neerajcbmr@gmail.com)

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Abstract

Gallstone disease is affecting a major population and emerging as a prevalent health problem all over the world. Gallbladder cancer (GBC) is often associated with gallstones. The mechanism involved in the formation of different types of gallstones is not completely understood. Several factors are reported to be linked with the variation of stones formation of different disease conditions such as chronic cholecystitis and gallbladder cancer. The pathogenesis of gallstone is quite complicated and still remains elusive. Nuclear magnetic resonance (NMR) is a tool that may be useful for the analysis of the composition of gallstones of different aetiology. In this review article, we illustrate the studies related to the application of NMR spectroscopy for unveiling the insight associated with gallbladder disease.

Keywords: Gallstones, Cholecystitis, GBC, NMR.

Introduction

Gallstone disease is a pervasive gastrointestinal disorder that affects millions of people throughout the world.[1] About 6% of the Indian population is affected by gallstone disease, which is about half of the gallstone disease prevalence in the world.[2] Cholecystectomy is the conventional treatment available for the people who suffered from gallstone disease. The gallbladder acts as a reservoir and concentrates the bile produced by the liver. During the digestive process, the gallbladder releases bile into the small intestine, which is required for breaking up the fats. Gallstones are formed by the agglomeration of the different constituents of the bile. The supersaturation of the cholesterol of the bile[3] and the defective bilirubin conjugation[4] leads to the formation of gallstones that are mainly composed of cholesterol and calcium bilirubinate.

Along with these two compounds, some minor compounds are also present in gallstones. The patients having a history of gallstone disease with GS size of more than 3.0cm are more susceptible to gallbladder cancer (GBC).[5] Although GBC is a rare disease, India contributes to nearly 10% of the global GBC burden.[6] The prevalence and the chemical composition of gallstones vary from the population of different geographical areas. It is also dependent on many factors like dietary habits, age, sex, etc.[7] The effect of dietary habits on gallstone composition could be associated with patients from different geographical regions. It is also reported that rates of formation of gallstones are two to three times higher among women than men. Increasing age for both sexes, obesity, family history, and genetics are some other risk factors linked with the occurrence of gallstones. The gallstone disease is also correlated with variations in environmental factors. This indicates the involvement of the multiple aetiological factors in the formation of gallstones and also the type of stones that formed.[8]

Based on their composition, gallstones can be divided into three types:cholesterol stones, pigment stones, and mixed stones.[9]

(a) Cholesterol stones

These types of stones are usually yellow, greenish, or cream-white. They are usually made of undissolved cholesterol. These stones contain more than 70% cholesterol.[10]

(b) Pigment stones

These are brown and black stones and usually small in size. They are composed primarily of bilirubin. These stones contain less than 25-30% of cholesterol.[10]

(c) Mixed Stones

These stones contain less than 30-70% of cholesterol.[10] The other constituents in the mixed stones are calcium bilrubinate and calcium carbonate.

Understanding gallstone composition provides insight into its formation mechanism and related diseases. This may provide clues to GS pathogenesis, so it is essential to determine the composition of GS. Nuclear Magnetic Resonance (NMR) is a sophisticated and advanced spectroscopic tool which is used to obtain detailed information like structure, chemical composition, the chemical environment of any system with minimum sample preparation and reproducibility. It has an applicability to explore biological samples in disease conditions. These characteristics make NMR spectroscopy a preferable method for clinical application. Figure 1 shows the structure of cholesterol and the ^{1}H NMR spectrum of gallstones dissolved in $CDCl_3$. The major resonances assigned in the NMR spectrum are associated with cholesterol.

In this review article, we describe the application and utilization of NMR spectroscopy in the gallbladder diseases by presenting the description of some significant studies available. A comprehensive literature search of articles was performed and the studies found relevant within the scope of this review are tabulated in Table 1 and elaborated below.

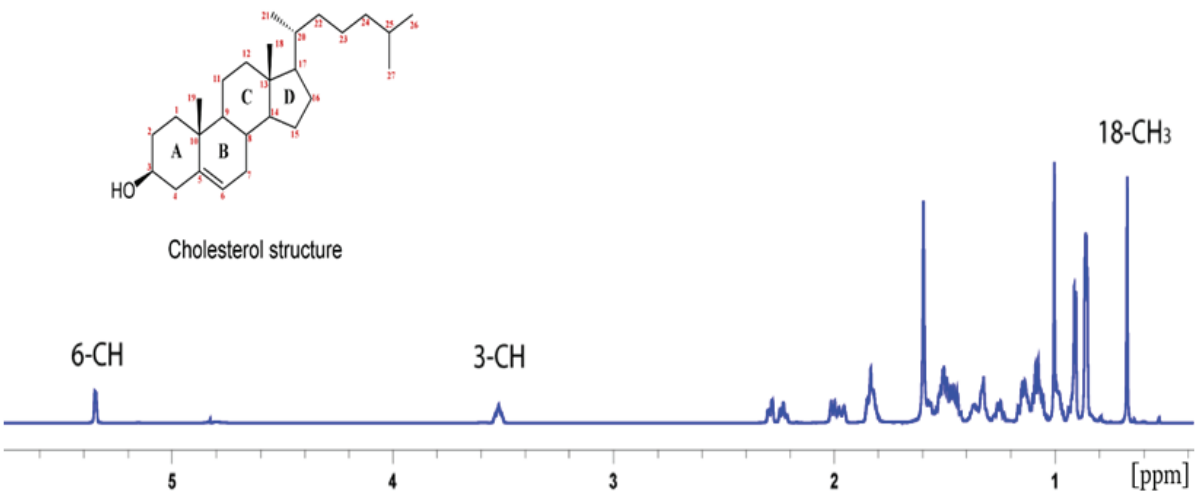

Figure 1: ^{1}H NMR spectrum of gallstones dissolved in $CDCl_3$. The assignments of various resonances are marked in the spectrum.

Authors	Gallstone Samples	Analytical technique	Outcome
Srivastava et al[11]	GBC n =11, CC n =23, XGC n =11	^{1}H NMR	Comparatively low level of cholesterol in GBC stones.
Jayalakshmi et al.[12]	GBC n =12, CC n =22, XGC n = 5	^{13}C NMR solid-state NMR	Main constituents:cholesterol. Exits in different polymorphs in CC.
Sharma et al.[13]	n = 134 North India (n = 67), UAE (n = 56), and South India (n =11)	^{13}C NMR solid-state NMR	North India: Cholesterol South India: Bilirubin UAE: Cholesterol & Bilirubin
Pichugina et al.[14]	n = 4	^{1}H NMR	Presence of cholesterol
Ramya et al.[15]	North India (n = 3), South India (n =2).	^{1}H and ^{13}C NMR	Cholesterol: significant constituent in North India

Table 1: Various NMR based studies on gallstones compositions.

Application of NMR spectroscopy in gallbladder disease

Srivastava et al. performed proton nuclear magnetic resonance spectroscopy to study the chemical composition of stones from cancerous and benign gallbladders. The study has analyzed 11 gallbladder cancer (GBC) stones, 23 chronic cholecystitis (CC) stone, and 11 xanthogranulomatous cholecystitis (XGC) stones. The comparison of the concentration of cholesterol, calcium, and magnesium in gallstones of GBC, CC, and XGC was made to figure out the differences among them. It was found that the quantity of cholesterol was significantly less in the gallstones of gallbladder cancer than in benign gallbladder diseases. The study has also revealed that both calcium and magnesium were significantly higher in cancerous gallstones than in benign gallstones.[11]

Jayalakshmi et al. had analyzed gallstones from cancer and benign gall bladder diseases using solid-state ^{13}C cross-polarized (CP) magic-angle spinning (MAS) nuclear magnetic resonance (NMR). In the study, 12 CC stone, 5 XGC, and 12 GBC stones collected from patients undergoing Cholecystectomy were subjected for the analysis. The significant finding of their work was that most of the gallstones have cholesterol as its main constituents with three different structural arrangements, such as anhydrous form, monohydrate crystalline with amorphous form, and monohydrate crystalline. They have also reported that chronic cholecystitis (CC) stones have mostly different polymorphs of cholesterol than stones associated with gall bladder cancer (GBC) patients.[12]

Sharma et al. employed ^{13}C NMR solid-state NMR in combination with FTIR spectroscopy to characterize human gallstones collected from three different geographical regions. They have incorporated a total of 134 gallstones from North India (n= 67), UAE (n=56), and South India(n=11) to analyze the prevalence of gallstone disease among the population of different geographical areas. The main constituent in gallstone from North India was found to be cholesterol, whereas bilirubin was present predominantly in gallstones from South India. The major constituents in the gallstones from UAE were both cholesterol and bilirubin.[13]

Pichugina et al. have carried out studies on the gallstones by X-ray diffraction, electron paramagnetic resonance, and nuclear magnetic resonance spectroscopy. The analysis of ^{1}H NMR spectra reveals the presence of cholesterol in the gallstones. Also, it provides an idea about the structures depicting a desmosterol transition, thus giving a clue for the mechanism for gallstone formation.[14]

Ramya et al. investigated chemical and structural analysis of gallstones from the northern and southern parts of India by utilizing different analytical methods. In this study, 20 gallstones from north India and 37 gallstones from south India were incorporated, and for the analysis two from south India and 3 from north India were adopted. They have recorded ^{1}H and ^{13}C NMR spectra and confirmed the presence of cholesterol as a principal constituent in the north Indian gallstones.[15]

Conclusion

To perform appropriate disease management and to control different complications related to acute gallbladder diseases that require immediate hospitalization and surgery, it is necessary to explore the illness in-depth and thus get the insight into the disease. In this direction, NMR spectroscopy is one of the valuable tools that have the strength to identify the significant compositional characteristics of different types of stones. It also provides information about the variation in the level constitutes of stones from cancerous and benign gallbladders and the correlation between the GS disease and GBC. NMR spectroscopy-based analysis of stones from different geographical areas was highlighted, and the prevalence of GS disease among the population also revealed. Thus it is emerging as a potential tool for furnishing a better understanding of the aetiopathogenesis of gallbladder disease. Furthermore, a comprehensive analysis of all the constituents present in the gallstone is also encouraged, which can be investigated by using several different analytical platforms like X-ray diffraction, Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscope (SEM) imaging.

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