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Proton nuclear magnetic resonance of Testicular cancer

Dina Ahmed Mohamed [1] , Jassim M. Najim [2] , Yousif Al Mashhadany [3]

[1,2] Physics Department, College of Science, University of Anbar, Ramadi, Iraq
[3] Electrical Engineering Department, College of Engineering, University of Anbar, Ramadi, Iraq

Email [1]: din19s2007@uoanbar.edu.iq

ABSTRACT

By utilizing a magnetic field and noticing the radio recurrence RF signal emitted by the specimen, are an equally efficient method is to change the frequency of the radio frequency radiation while keeping the outside field fixed in each state. The aim this research utilizing NMR to study the chemical and physical side for variation in the intensity , concentration of hydrogen atoms, chemical shift , Full width at Half Maximum (FWHM) of the H-NMR spectrum and to explain the number of peaks framed, by RF signal emitted by the specimen through the impact of the magnetic field, and afterward to make an compare between the ordinary state and the sick state (Testicular cancer) to arrive at the reason for this kind of illness and It can be applied to the remainder of different illnesses like leukemia and others, which we have arrived at a treatment or a more exact clarification of the sick condition. Through the outcomes got, there is a distinction between the sick condition and the normal condition in the cases above-mentioned.

Keywords: Proton nuclear magnetic resonance (H-NMR), Serum of Testicular cancer, lyophilization (Freeze drying), Serum powder.

Introduction

Proton nuclear magnetic resonance (proton NMR, hydrogen-1 NMR, or 1H NMR) is the utilization of nuclear magnetic resonance in NMR spectroscopy regarding hydrogen-1 cores inside the molecules of a substance, to decide the construction of its molecules[1],[2],[3]. Uncovering the structure and elements of biological macromolecules is fundamental for understanding their biological function. High-resolution Nuclear Magnetic Resonance (NMR) spectroscopy is a main strategy giving admittance to multidimensional protein structure and conformation[4],[5]. In specimens where natural hydrogen (H) is utilized, basically all the hydrogen comprises of the isotope 1H (hydrogen-1; for example having a proton for a nucleus)[6],[7],[8]. NMR spectra are recorded in solution, and solvent protons should not be permitted to meddle. Deuterated (deuterium = 2H, frequently represented as D) solvents particularly for use in NMR are liked, for example ,deuterated water, D_2O, deuterated acetone, $(CD_3)_2CO$, deuterated methanol, CD_3OD, deuterated dimethyl sulfoxide, $(CD_3)_2SO$, and deuterated chloroform, $CDCl_3$. In any case, a solvent without hydrogen, like carbon tetrachloride, CCl4 or carbon disulfide, CS2, may likewise be used [9],[10]. By and large, deuterated solvents were provided with a tiny quantity (ordinarily 0.1%) of tetramethylsilane (TMS) as an inside standard for calibrating the chemical shifts of each analyte proton [11],[12]. TMS is a tetrahedral molecule, with all protons being chemically equivalent, giving one single signal, used to characterize a chemical shift = 0 ppm. making sample recovery easy as well. Modern spectrometers are able to reference spectra based on the leftover proton in the solvent (for example the CHCl3, 0.01% in 99.99% CDCl3) [13],[11],[14]. Deuterated solvents are currently generally provided without TMS. 1H NMR spectroscopy, which is the most-utilized spectroscopic apparatus in organic chemistry [15],[16]. Chemical shift values, represented by δ, are not accurate, but typical - they are to be therefore regarded mainly as a reference. Deviations are in ±0.2 ppm range, sometimes more[17],[18],[19]. The accurate value of chemical shift depends on many factors like molecular structure, solvent, temperature, magnetic field in which the spectrum is being recorded and other adjacent functional groups[20],[21].Hydrogen nuclei are sensitive to the hybridization of the atom to which the hydrogen atom is attached and to electronic effects. Nuclei tend to be obscured by electron density pulling groups. Deshielded nuclei resonate at higher δ values, whereas shielded nuclei resonate at lower δ values[22],[23].

Testicular cancer

Testicular cancer occurs in the testicles (testes), which are situated inside the scrotum, the delicate skin sac under the penis. The testicles produce male sex hormones and sperm for reproduction. If compared with other kind of cancer, testicular cancer is uncommon [24],[25]. Yet, testicular cancer is the most well-known malignancy in males between the ages of 15 and 3. Testicular cancer is very treatable, even when the cancer has spread outside the testicle. Depending on the kind and stage of testicular cancer, you may receive one of several treatments, or a combination[26],[10]. Testicular cancer is often divided into two broad classification germ cell tumors and sarcomas. Germ cell tumors represent the majority of cases (90–95%) and have a good prediction with new therapies even at advanced stages [27],[28], [29],[30],[31],[32] . Although the World Health Organization (WHO) proposed huge changes to the classification system in 2016, germ cell tumors have historically been classified into two groups; seminoma and non-seminoma [33]. Stromal tumors (non-germ cell) are moderately uncommon, representing only 5–10%of all testicular cancer [34],[35]. The five-year endurance rates at beginning phases are still high,, but for the 10–20% of patients who develop malignant disease, survival is worse than their germ cell counterparts [36],[37],[38].

Material and Method

This research was carried out in the Quality Control Laboratories of the Ministry of Industry and Minerals - Industrial Research and Development Authority (Al-Razi Center) for Research and Production Medical Diagnostic Kits, and the accessible devices utilized in our measurements are: Electronic Analytical Scale, for the electric balance made by (KERN and Sohn), The sensitivity of this balance is doing (0.1 mg). This scale was utilized to measure the weight of normal and abnormal blood specimens. Christ Alph 1-2 LD freeze drying (freeze drying) involves removing water from products in a frozen state at extremely low pressures. The process is generally used to dry thermodegradable products that can be destroyed by heat drying. the samples were dried for three days and turned into a powder. As for the SIGMA 3-16L centrifuge, it is a device that uses centrifugal force to separate the different components of a liquid. This is achieved by circulating the liquid at a high speed within a container, thus separating liquids of different densities eg liquids from solids. It causes the denser particles to settle to the bottom of the tube, while the lower density ones rise to the top. the blood samples were separated to obtain blood serum and the samples were placed in a machine at 3000 r/m for 3 minutes. A water bath is laboratory equipment made from a container filled with heated water. It is used to incubate samples in water at a constant temperature over a long period of time. Blood samples were placed in the water bath to obtain a clot in order to ease the separation process. The samples were placed in the device for 5 minutes.

Sample preparation and conversion to powder

Blood samples were taken from patients with testicular cancer in the Anbar Specialized Center for Oncology. Other blood samples were taken from healthy people without testicular cancer. Samples are placed in a gel tube and then in a water bath for 5 minutes to obtain coagulated blood. Coagulated blood was removed and centrifuged at 3000 rpm for 3 min to separate the blood and obtain serum. The serum is placed in the white tube and kept at a temperature of -20°C for the purpose of storage. The serum was dried by Lyophilyzer Christ Alph 1-2 LD, which freezes liquid materials (converting them to powder by cooling and pressure) for 3 days. were separated and weighed with an electronic analytical balance as shown in the following table (1). In order to take the NMR spectra of a solid, it is usually necessary to dissolve it in a suitable solvent, such as deuterium oxide (D_2O). Since the deuterium isotope of hydrogen has a different magnetic moment and spin, it is invisible in a spectrometer tuned to protons.

Results and discussion

NMR spectroscopy uses the interaction between the nucleus which acts as a little magnet and an external magnetic field, making it appropriate for assessing chemical bonds and the nuclear environment and others [39].

By utilizing a magnetic field and noticing the radio recurrence RF (500MHz) signal produced by the specimen utilizing NMR to study the physical and chemical side for by in terms of difference, chemical shift, intensity, concentration of hydrogen atoms, the Full width at Half Maximum (FWHM) , integration ,multiplicity of the H-NMR spectrum between two cases (control, testicular) as shown fig.(1) and its results shown in the tables (1). By using Mestrenova NMR processing software, is spectral data

analyzing software, which can be run on Windows, when we take a larger section of the two cases, for pathological condition and the control condition as in the figure (2) , after compared according to the result, we show a highly significant valve in the intensity between a normal person with a testicular cancer patient, the valve increases for a testicular cancer patient than the normal person, the number of peaks for the normal person is about 36 peaks, while for testicular cancer patient 13 peaks this means the number of compounds appear less in the testicular cancer patient with a highly significant valve and a different in Full width at Half Maximum (FWHM) of the H-NMR spectrum as shown in table (1). The signal obtained from NMR spectroscopy gives information about the interactions among cores and electrons as well as interactions between cores, which can assist with deciding the construction of a chemical compound[40] [41]. The resulting NMR spectrum is a collection of one or more resonant peaks at a certain frequency.

1H-NMR spectroscopy is an analytical technique used to decide the structure of a compound depends on the kind of proton or hydrogen. The 1H-NMR spectrum gives information in regards to the number of proton types in a compound and the ecological properties of each sort of hydrogen proton. From the information of signal multiplicity and spin-spin splitting each signal in a proton NMR spectrum could conceivably be parted into at least one pinnacles. This is called signal multiplicity and gives rise to names such as singlet, doublet, triplet, quartet, pentet, and multiple. Every one of those protons is seen in two states which either support or go against the applied field, causing shielding and deshielding of the signal under observation. The multiplicity of the sign then, at that point, relies upon the number of such protons. The absorption peak's area of various NMR resonance signals is subject to both the outer magnetic field strength and the RF frequency. Since no two magnets will have the very same field, resonance frequencies will differ likewise and an alternative method for characterizing and indicating the area of NMR signals is required. Circulation of electrons about the actual protons produces a field-aligned in such a way that - at the proton - it go against the applied field the field felt by the proton is thus reduced, and the proton is said to be protected.[41],[42],[43],[44] according to this references there is some important information appearing in the ^{1}HNMR spectrum, such as

1. as shown in fig.1. For two cases the proton resonance is distributed along the frequency axis. Each proton is in a different chemical environment characterized by its chemical shift (δ).
2. Data is presented in table (1) different peaks in the spectrum can be seen appearing with different intensities which related to the number of protons giving rise to the signal as shown in fig. (1,2)
3. As we shown in fig.2.and data is presented in table (1) the difference in multiple proton resonances can interact with neighboring atoms. The degree of interaction or coupling is indicated by the coupling constant (J). The absorption peak that appears in the ^{1}H-NMR spectrum is represented by the difference in the resonance frequency of a nucleus against the standard in units of ppm or chemical shift (δ). The schematic of the 1H-NMR spectral peaks for various types of proton absorption is shown in Figure (2).
4. Figure 3 represents the relation between FWHM and chemical shift the Information from ^{1}H-nmr spectra from fig.3. and data is presented in table (1) the difference in Number of signals (How many different types of hydrogen's in the molecule) and Position of signals (chemical shift).
5. Figure 4 represents the relationship between intensity and chemical shift. Relative areas under signals (integration) how many hydrogens of each type. Splitting pattern (How many neighboring hydrogen's).

In both cases, they decrease by 10^{1} powers and this indicates a significant decrease in the hydrogen concentration in the testicular that led to a change in the composition. The relative intensities of the signals reflect the relative numbers of protons of different kinds present in the molecule in two cases. The term intensity, when used in reference to NMR signals, indicates the area under the peak. The areas under the peaks are given by an integrator when recording a spectrum. The relative intensities of the signals reflect the relative numbers of protons of different kinds present in the molecule. The following examples illustrate this point, as shown in fig. (1, 2) and data is presented in table (1).

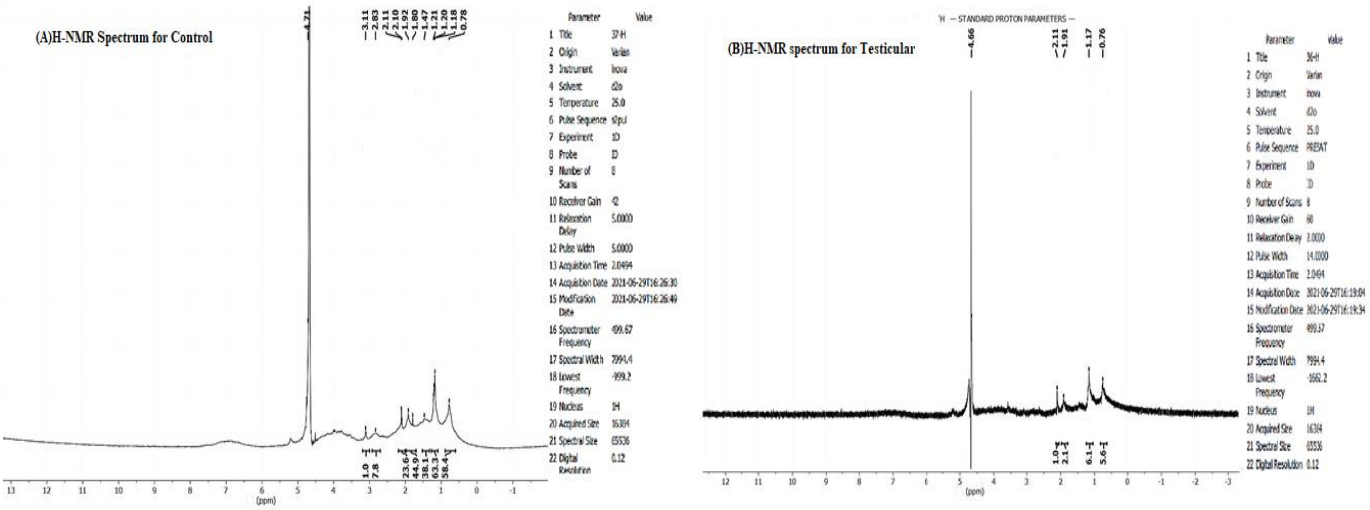

Figure 1. H-NMR Spectrum A: Control B: Testicular

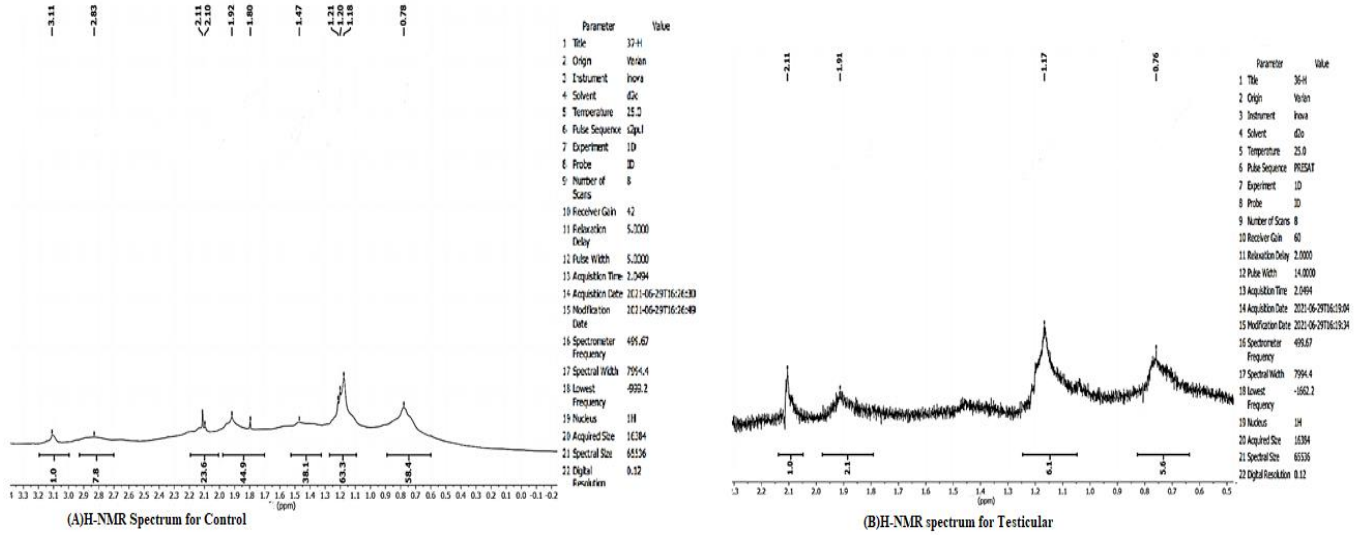

Figure.2. enlarged part from H-NMR Spectrum A: Control B: Testicular

Table (1) Control H-NMR spectrum for control and H-NMR Testicular

H-NMR (Control)								H-NMR (testicular)							
Number of peaks	**ppm**	**Hz**	**Intensity**	**Width**	**integration**	**Area**	**Kurtosis**	**Number of peaks**	**ppm**	**Hz**	**Intensity**	**Width**	**integration**	**Area**	**Kurtosis**
1	6.93	3462.2	0.6	382.27	1.00	2189.59	1.52	**1**	4.73	2364.3	10.4	39.92	1	5966.56	-0.84
2	5.2	2600.4	0.6	30.08	0.24	266.07	-0.86	**2**	4.68	2336.8	12.3	0.27	0.01	32.27	1.89
3	4.79	2392.7	1	63.24	2.16	909.14	-0.89	**3**	4.67	2335.9	31.5	0.27	0.03	79.2	2
4	4.76	2376.7	1	2.09	0.19	27.51	-0.34	**4**	4.67	2335	108.1	0.33	0.17	505.29	-0.65
5	4.75	2375.4	1.5	3.51	0.28	76.12	-1	**5**	4.67	2334.4	31.6	1.14	0.26	377.97	1.4
6	4.71	2355.3	4.6	5.06	1.32	287.46	0.25	**6**	4.67	2332.6	2.9	0.21	0.03	5.77	1.88
7	4.68	2336.7	855.3	1.96	44.13	20227.82	0.49	**7**	4.66	2328	30	10.72	0.92	3869.04	0.5
8	4.67	2335.4	1032.4	1.99	29.32	26166.14	0.07	**8**	4.63	2315.3	2.7	0.29	0.02	7.49	2
9	4.63	2314.5	0.7	1.68	0.04	14.95	-0.2	**9**	3.59	1793.8	2.7	2.91	0.11	116.48	-1
10	4.6	2297	0.5	1.2	0.01	6.69	1.11	**10**	2.1	1051.7	6.1	9.39	1.0	842.56	-1
11	4.59	2295	0.2	0.63	0.01	1.8	0.55	**11**	1.91	955.7	5	24.64	2.1	1594.93	0
12	4.59	2292.3	0.2	3.83	0.02	10.24	1.11	**12**	1.17	582.9	9.3	25.55	6.1	3475.1	-1
13	4.57	2282.5	0.4	2.99	0.01	13.95	0.74	**13**	0.76	377.6	5.1	43.33	5.6	3047.61	-0.5
14	4.52	2256.7	0.7	1.89	0.00	17.6	0.19								
15	4.51	2255	0.9	2.47	0.01	28.46	0								
16	4.47	2235.4	0.2	4.94	0.02	15.63	-0.4								
17	4.17	2081.1	0.3	52.5	0.30	202.5	-1								
18	3.99	1996.1	0.2	2.34	0.03	6.14	0.24								
19	3.98	1989.5	0.2	2.83	0.05	6.36	1.31								
20	3.89	1941.5	1	303.37	2.33	3643.02	0.56								
21	3.78	1890.2	0.2	16.08	0.13	38.5	0								
22	3.55	1771.8	0.3	26.49	0.06	113.44	-0.73								
23	3.24	1618.3	0.1	4.88	0.02	8	-0.12								
24	3.1	1551.4	0.9	21.77	1.0	235.81	0.4								
25	2.84	1416.8	0.6	107.63	7.8	795.38	0.69								
26	2.64	1320.7	0.2	21.65	0.07	47.09	0.2								
27	2.11	1055.5	1.5	2.55	23.6	37.71	1.88								
28	2.1	1047.8	0.5	10.37	23.6	72.87	-0.77								
29	1.92	959.2	1.4	38.66	44.9	790.55	-1								
30	1.8	897.8	0.8	3.01	44.9	24.25	1.73								
31	1.47	733.8	0.9	136.77	38.1	1655.28	-0.24								
32	1.22	607.2	0.9	3.47	0.30	31.37	1.39								
33	1.2	601.2	0.6	2.5	63.3	20.47	-0.52								
34	1.2	599.9	0.9	3.48	63.3	44.56	-0.75								
35	1.18	587.5	5.5	26.94	63.3	2152.68	-1								
36	0.78	388.4	3.2	69.02	58.4	3175.52	-1								

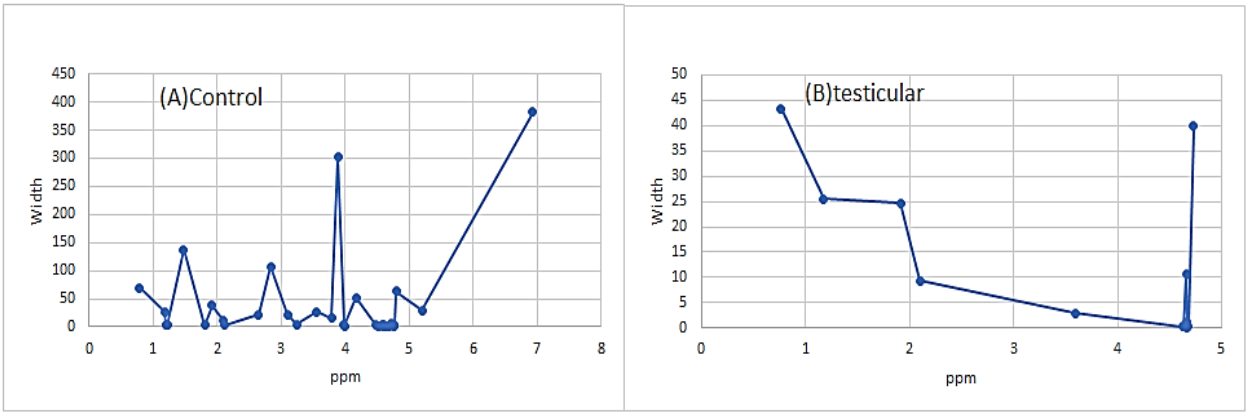

Figure.3. Relationship between FWHM and ppm (A): Control (B): Testicular

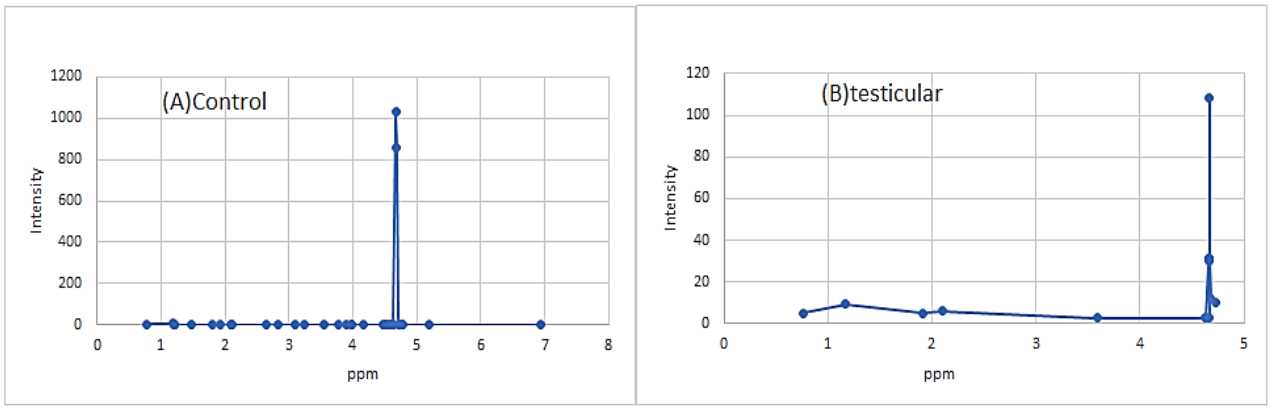

Figure.4. Relationship between Intensity and ppm (A): Control (B): Testicular

Conclusion

The study of the difference in spectral analysis such as H-NMR between two cases, the normal (control) state and the disease state is very important by studying the difference in the increase or decrease of some structure and compounds for the two cases, which can be generalized by studying other spectra such as C, F, Si, P and others in order to reach a diagnosis and that help us treat some diseases. With this paper, we believe that this paper can be used as a basis for understanding and interpreting H-NMR and others data.

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