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Detection of antinuclear antibodies in chronic *Hepatitis* (C) patients in Taif governorate

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Abstract
Objective: To investigate the prevalence and correlation of auto-antibodies in patient with chronic hepatitis C (CHC) and also to investigate the significance of auto-immune reaction in HCV infected patients.
Methods: Serum samples were collected from 300 subjects (representing the control and patient groups) visiting King Abdul-Aziz Specialist Hospital in Taif Governorate between June 2017 and December 2017 for detection of HCV by ELISA and RIBA. Serum Antinuclear antibodies (ANA) and Anti-smooth muscle antibodies (ASMA) were detected by IIFT.
Result: A total number of 127 (42.3%) of 300 subjects were positive for auto-antibodies. The prevalence rate of ANA was 58 (19.2%) and the prevalence rate of ASMA was 40 (13.3%). While the prevalence rate of ANA and ASMA together was 29 (9.66%).
Conclusion: This study denoted that ANA and ASMA are correlated with chronic hepatitis C patients and consider as a marker for autoimmune hepatitis (AIH). They are also more common in females than male patients. Moreover, it can be found in some normal persons.

Keywords: CHC, AIH, ANA, ASMA, IIFT

1. Introduction
Hepatitis C virus (HCV) infection is a severe life-threatening medical and public health problem worldwide, caused by hepatitis C virus. It is also known to be a primary cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma, and a primary signal for liver transplantation in the Western world [1].

The HCV is a small enveloped, single-stranded, positive-sense RNA virus belonging to the family *Flaviviridae* and genus *Hepacivirus* [2]. HCV is a roughly spherical [3]. There are two types of HCV infection, acute and chronic [4]. Asian and African countries have higher seroprevalence than the developed world including North America, Northern and Western Europe and Australia [5]. The predominant genotype in the Kingdom of Saudi Arabia (KSA) is genotype (4) [6].

HCV infection is affecting 170 million people all over the world [7]. Hepatitis C is usually turns into a chronic disease and leads to autoimmune disease [8] and the complications of chronic HCV cause deaths in about 500,000 individual every year [7]. The prevalence of CHC in the KSA has been falling gradually during the last years due to the implementation of blood donation screening programs [9].

Persistent chronic HCV infection is a major cause not only for liver diseases, but also for many extrahepatic autoimmune conditions [10]. Immuno-pathological disorders vary from production of autoantibodies such as non-organ specific autoantibodies (NOSA), Antinuclear antibodies (ANA) and Anti-smooth muscle antibodies (ASMA) and organ-specific autoantibodies such as thyroid autoantibodies [11].

Autoimmune hepatitis is characterized by generalized elevation of serum globulins, specially gamma globulin and immunoglobulin G (IgG) [12]. It is characterized by bad prognosis with end stage liver cirrhosis occurring in most of patients [8]. There are three types of AIH, type 1, 2 and 3. Type 1 is more common in adults and is characterized by the presence of ANA or ASMA in about 65% of patients [13]. Type 2 AIH is generally seen in children and is characterized by the presence of high anti-LKM (Liver kidney Microsome) antibodies. While autoimmune hepatitis Type 3 is characterized by autoantibodies against soluble liver antigens or to liver-pancreas antigen [14].

Serum ANAs have been reported to occur in 15% of the general healthy populations worldwide and particularly in older age individuals [15] and more common in females than in males with ratio of (3.5) to (1) [16]. Autoimmune hepatitis is generally responds to standard treatment and the disease can be controlled in most cases [17].

The limitation of HCV spread is depending mainly on prevention programs which are needed at the local, national, regional and global levels [18].

This study is aimed to considering the seriousness of hepatitis C virus infection, its negative effects on public health and its complications, so this study will explore the prevalence of (ANA) in chronic HCV patients in the general population of Taif Governorate using the available techniques to follow the development of AIH in HCV patients.

2. Materials and Methods
2.1 Subjects and Sample Collection
The study included a total of 500 subjects attending the Blood

Bank and the Internal Medicine and Gastroenterology Clinics at King Abdul-Aziz Specialty Hospital in Taif Governorate from June 2017 to December 2017.

All samples were extracted from these subjects after their consent and prepared as follow:

- 7 ml extracted from every subject was collected in Serum Separator Tubes (SST) or plain tube.
- In the laboratory, blood was centrifuged at 3000 rotation per minute (rpm) for 15 minutes and the serum was separated in dry clean tube and storage at 2-8°C for ≤ 7 days or storage at -20°C for > 7 days until used for analysis of the various parameters outlined below.

2.2 Detection of HCV antibodies by ELISA

Presence of Anti-HCV antibodies was assessed using by 3rd generation ELISA (Monolisa™ anti-HCV, Bio-Rad, France). The samples were considered positive for anti-HCV antibodies when the index values (S/CO) were ≥ 1, and negative when values were < 1.

2.3 Recombinant immunoblot assay (RIBA)

The results of the HCV antibodies for ELISA were confirmed by the recombinant immunoblot assay (RIBA), using INNO-LIA™HCV Score supplied by (Fujirebio Europe N.V., Belgium).

The INNO-LIA HCV Score is a 3rd generation line immunoassay which incorporates HCV antigens derived from the core region, the E2 hyper variable region (HVR), the NS3 helicase region, the NS4A, NS4B, and NS5A regions. Band reactivity is graded by visual calibration against IgG control bands present on each strip. The intensity of the colored bands is proportional to the amount of bound antibody and is graded as - (none), ± and 1+ to 3. Results interpretation.

1. A sample is negative for HCV antibodies
   - If all HCV antigen lines have a negative reactivity rating
   - If only one HCV antigen line has a reactivity of ±, except when the reactivity is observed for NS3.
2. A sample is POSITIVE for HCV antibodies:
   - If at least two HCV antigen lines have a reactivity of ± minimum or higher
3. A sample is considered indeterminate for HCV antibodies
   - If one HCV antigen line has a reactivity rating of 1+ or higher
   - If the NS3 line reacts with a reactivity of ± or higher and all other antigen lines are negative.

From the confirmed cases 200 (patients) positive samples (100 males + 100 females) and 100 (control) negative samples (50 males+50 females).Both groups were representing male and female equally and both were at the same age range (30 – 50 years).

2.4 Indirect Immunofluorescence Test (IIFT)

Presence of Autoantibodies was assessed using by Indirect Immunofluorescence using Immco Diagnostics Autoantibody test system. Reactions are observed under a fluorescent microscope equipped with appropriate filters. The presence of Anti-Nuclear Antibodies (ANA) and Anti-Smooth muscle Antibodies (ASMA) is demonstrated by an apple green fluorescence of specific histologic structures in the tissue**.**

2.5 Statistical analyses

Statistical analysis was done by computer using statistical package for social science (SPSS) version 22 for windows. The following tests were used: Descriptive statistics for all studied groups including frequency test, Mann-Whitney Test, Spearman's correlation.

3. Results

Table 1: Result of ELISA and RIBA tests

Test used	Total	Positive	Negative
ELISA	500	263	237
RIBA	500	256	244

* Table 1: the positive cases by using ELISA and RIBA (263 and 256) and negative are (237 and 244).

Table 2: ANA in Male and Female of control and patient groups by using IIFT

Group examined	Number	Positive Number	Percentage	Negative Number	Percentage
Male control group	50	2	4%	48	96%
Female control group	50	3	6%	47	94%
Male patient group	100	23	23%	77	77%
Female patient group	100	30	30%	70	70%

* Table 2: the number and percentage of positive ANA in male and female of control groups are 2(4%) and 3(6%) while the negative are 48(96%) and 47(94%) from the total male and female in control groups, and the number and percentage of positive ANA in male and female of patient groups are 23(23%) and 30(30%) while the negative are 77(77%) and 70(70%) from the total male and female in patient groups.

Table 3: ASMA in Male and Female of control and patient groups by using IIFT

Group examined	Number	Positive Number	Percentage	Negative Number	Percentage
Male control group	50	1	2%	49	98%
Female control group	50	2	4%	48	96%
Male patient group	100	12	12%	88	88%
Female patient group	100	25	25%	75	75%

* Table 3: the number and percentage of positive ASMA in male and female of control groups are 1(2%) and 2(4%) while the negative are 49(98%) and 48(96%) from the total male and female in control groups, and the number and percentage of positive ANA in male and female of patient groups are 12(12%) and 25(25%) while the negative are 88(88%) and 75(75%) from the total male and female in patient groups.

Table 4: ANA and ASMA together in Male and Female of control and patient groups by using IIFT

Group examined	Number	Positive Number	Percentage	Negative Number	Percentage
Male control group	50	0	0%	50	100%
Female control group	50	0	0%	50	100%
Male patient group	100	8	8%	92	92%
Female patient group	100	21	21%	79	79%
Male and female patient group	200	29	14.5%	171	85.5%

* Table 4: the number and percentage of positive ANA and ASMA together in male and female of control groups are 0(0%) and 0(0%) while the negative are 50(100%) and 50(100%) from the total male and female in control groups, and the number and percentage of positive ANA in male and female of patient groups are 21(21%) and 29(29%) while the negative are 88(88%) and 75(75%) from the total male and female in patient groups.

Mann-Whitney Test

Table 5: Mann –Whitney test for comparison between control groups (total male and female) and patient groups (total male and female) as regard ANA

Group	Number	Positive	Mean rank	2 tailed significance	Sig
control group (male and female) ANA	100	5	140.00	0.001	HS
patient group (male and female) ANA	200	53	171.50		

* Table 5: the comparison between control groups (total male and female) and patient groups(total male and female) as regard ANA revealed a high significant (HS) difference between them p= 0.001 (<0.05) with increase mean rank in patients than control.

Table 6: Mann –Whitney test for comparison between control groups (total male and female) and patient groups (total male and female) as regard ASMA

Group	Number	Positive	Mean rank	2 tailed significance	Sig
control group (male and female) ASMA	100	3	144.25	0.001	HS
patient group (male and female) ASMA	200	37	163		

* Table 6: the comparison between control groups (total male and female) and patient groups (total male and female) as regard ASMA revealed a high significant (HS) difference between them p= 0.001 (<0.05) with increase mean rank in patients than control.

Table 7: Mann –Whitney test for comparison between male and female patient groups as regard ANA

Group	Number	Positive	Mean rank	2 tailed significance	Sig
male patients ANA	100	23	94	0.042	S
female patients ANA	100	30	107		

* Table 7: the comparison between male and female patient group as regard ANA show significant (S) difference between them p= 0.042(<0.05) with increase mean rank in female patients than male patients.

Table 8: Mann –Whitney test for comparison between male and female patient groups as regard ASMA

Group	Number	Positive	Mean rank	2 tailed significance	Sig
male patients ASMA	100	12	93.5	0.030	S
female patients ASMA	100	25	107.5		

* Table 8: the comparison between male and female patient groups as regard ASMA show significant (S) difference between them p= 0.030 (<0.05) with increase mean rank in female patients than male patients.

Table 9: Mann –Whitney test for comparison between male and female patient groups as regard ANA and ASMA together

Group	Number	Positive	Mean rank	2 tailed significance	Sig
male patient (ANA and ASMA together)	100	8	46.56	0.000	HS
female patient (ANA and ASMA together)	100	21	70.42		

* Table 9: the comparison between male and female patient groups as regard ANA and ASMA together show high significant (HS) difference between them p= 0.000(<0.05) with increase mean rank in female than male.

Correlations

Table 10: Spearman's correlation between ANA and ASMA in studied patients

			M. patient. G. ANA	M. patient. G. ASMA	F.patient. GANA	F.patient. GASMA
Spearman's rho	M. patient.G. ANA	Correlation Coefficient	1.000	.326**	.738**	.455**
		Sig. (2-tailed)	0.0	.001	.042	.000
		N	100	100	100	100
	M. patient.G. ASMA	Correlation Coefficient	.326**	1.000	.281**	.508**
		Sig. (2-tailed)	.001	0.0	.005	.030
		N	100	100	100	100
	F. patient.G. ANA	Correlation Coefficient	.738**	.281**	1.000	.710**
		Sig. (2-tailed)	.042	.005	0.0	.000
		N	100	100	100	100
	F.patient.G. ASMA	Correlation Coefficient	.455**	.508**	.710**	1.000
		Sig. (2-tailed)	.000	.030	.000	0.0
		N	100	100	100	100

**. Correlation is significant at the 0.01 level (2-tailed).

* Table 10: the spearman's correlation between ANA and ASMA in studied patient groups revealed a strong positive correlation between male patients ANA and ASMA in male patient group (Rs=0.326,p = 0.001), (p <0.05). A strong positive correlation between male and female ANA, (Rs= 0.738, p = 0.042), (p <0.05). A strong positive correlation

between male patient ANA and female patient ASMA (Rs= 0.455, p = 0.000), (p <0.05). A strong positive correlation between male and female patient ASMA (Rs=.508, p = 0.030) (p <0.05), and male and female patient ANA (Rs= 0.281, p =0.005) (p <0.05).A strong positive correlation between female patients ANA and ASMA (Rs= 0.710, p =0.000), (p <0.05).

4. Discussion

Chronic hepatitis C usually develops long term complications such as cirrhosis, end-stage liver disease, and AIH [19].

Results of IIFT in this study for the control group revealed that ANA was detected in three (6%) female samples, while ASMA was detected in two (4%) female samples. ANA was detected two (4%) male samples, while ASMA was detected in one (2%) male sample, these results are agreed with Manns and Vogel **[15]** who mentioned that serum ANAs have been reported to occur in 15% of the general healthy populations worldwide and particularly in older age individuals.

For the patient group, ANA was detected in 30 (30%) female samples, while ASMA was detected in 25 (25%) female samples. ANA was detected 23 (23%) male samples, while ASMA was detected in 12 (12%) male samples. This are agreed with Czaja and Donaldson; Amin et al., **[16, 20]** who reported that AIH is more common in female than in males and also can occur in both children and adults as well. The use of IIFT in this study was very helpful in detection of ANA and ASMA in CHC patients and considers a good marker for AIH.

On the other hand, IIFT was also employed for the detection of both ANA and ASMA together and revealed that the positive number of ANA and ASMA together in female patient group is 21 (21%) from total female patient group, while the positive number of ANA and ASMA together in male patient group is 8 (8%) from the total male patient group and this are agreed with Heneghan et al., **[13]** who recorded the presence of ANA and ASMA in patient with CHC.

The positive number of ANA and ASMA together in control group (male and female) is 0 (0%), while the negative samples is 100 (100%).The positive number of ANA and ASMA together in patient group (male and female) is 29 (14.5%) from the total number of patient group. This is agreed with **[21]** who found that 13 patients (12.03%) of 108 (anti-HCV positive patients) were positive for at least one autoantibody, while there wasn't any autoantibody detected in the control group and their also confirmed that CHC is a lead cases of AIH.

Comparison of ANA between control group (Male and Female) and Patient group (male and female) revealed a high significant (HS) difference between them; p= 0.001 (<0.05). Also, comparison of ASMA between control group (male and female) and Patient group (male and female) revealed a high significant (HS) difference between them; p= 0.001 (<0.05), and it is agreed with **[22]** who reported that ANA alone were detected in 15% of Caucasians patients and ASMA alone was detected in 35% of them.

Comparison of ANA between male and female patient group showed significant (S) difference between them; p= 0.042(<0.05), which is confirmed by Guo et al., [23] who found that females had a higher prevalence of ANA than males (P < 0.01). Also comparison of ASMA between male and female patient group showed significant (S) difference between them; p= 0.030 (<0.05), which is not agreed with Teubner et al., [24] who didn't find any sex differences for ASMA.

Comparison of ANA and ASMA together between male and female Patient groups revealed a high significant (HS) difference between them; p= 0.000(<0.05), which is agreed with Amin et al., [20] who found that AIH was more common among females than in males (3:1) and at least one type of autoantibodies was found and the most prevalent one was ANA.

5. Conclusion and Recommendations

Since chronic liver diseases was responsible for large number of deaths all over the world during the last years because of cirrhosis and liver cancer, this study investigated the incidence of AIH in chronic hepatitis C patients in Taif Governorate through detection of autoantibodies (ANA and ASMA) in their blood.

Autoantibodies including ANA and ASMA could be detected separately or together in chronic HCV patients and in this case the patient is classified as Type 1 Autoimmune Hepatitis. Results of the study also approved that females are more likely suffering from AIH than males. ANA and ASMA are still normally present in some healthy persons as previously reported in many studies.

Early diagnosis are recommend for Hepatitis C to ensure successive treatment in order to decrease the incidence of Chronic Hepatitis and subsequently decrease the incidence of Auto Immune Hepatitis by screening of Chronic Hepatitis C patients for ANA and ASMA in their serum.

6. Abbreviations

HCV: Hepatitis C virus; CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; NOSA: non-organ specific autoantibodies; ANA: Antinuclear antibodies; ASMA: Anti-smooth muscle antibodies; RIBA: Recombinant immunoblot assay; IIFT: Indirect immune-fluorescent technique; S/C: Sample\cut off; HVR: hyper variable region; SPSS :statistical package for social science; NS: nonstructural region.

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9. Competing interests

The authors declare that they have no competing interests.

10. Authorship and Contributorship

All authors play a main role in study design, in the collection, analysis and interpretation of data.

All authors in read and approved the final manuscript.

11. Patients consent

Oral approval was taken from participants in the study. As no personal data was used for the participants.

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