ORIGINAL ARTICLE

Anti-rods and rings autoantibodies can occur in the hepatitis c-naïve population

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Key words

Autoimmunity • Autoantibodies • Hepatitis C

Summary

Introduction. *The anti-Rods and Rings autoantibody recently described in clinical populations is thought to occur in the setting of hepatitis C treatment, specifically in the context of cytidine triphosphate (CTP) and guanosine triphosphate (GTP) synthetic pathway inhibitors, and is important in its potential impact on response to therapy. This study asks the question: what is the epidemiology of anti-RR autoantibody in the general, non-clinical population?*
Materials and methods. *This is a cross-sectional study using the National Health and Nutrition Examination Survey (NHANES). Immunofluorescence assay for anti-Rods and Rings autoantibody were performed by NHANES labs and the results made publically available. Sample weights were used to calculate the prevalence and distribution of the autoantibody across demographics. A medication profile of the autoantibody positive population was also constructed.*
Results. *The study sample consisted of 4738 persons over the age of 12 years. Anti-Rods and Rings autoantibodies were found in 39 persons representing 1.3 million persons in the United States population. 38 of 39 persons with anti-Rods and Rings autoantibody had no prior history of hepatitis C virus infection. A majority of these persons were found to have poly-pharmacy.*
Discussion. *This is the first study to show that anti-RR can occur in the general population without evidence of hepatits C virus infection, and that the majority of persons with anti-RR in the population have no evidence of prior hepatitis C infection. This indicates that there may be another undetermined etiology for anti-rods and rings autoantibodies besides the currently accepted exposure etiology of hepatitis C virus infection and treatment found in clinical studies.*

Introduction

Studies have recently described a new cytoplasmic organellar complex that is morphologically toroidal, also occurring as rods and rings [1, 2]. These structures have been shown to be evolutionarily conserved and have been observed in a number of organisms including Drosophila [3],rats [1] and yeast [4] in addition to their detection in humans [2, 5-7]. Clinical studies have found that patients positive for anti-rods and rings autoantibodies (anti-RR) tend to be HCV positive [2], have been treated by combination interferon (IFN) and/or ribavarin therapy [2, 8, 9], and are poorer responders to therapy [9]. It is thought that occurrence of anti-RR in persons with HCV may be secondary to its treatment using IMDPH2 inhibitors such as ribavarin leading to intracellular aggregation of IMPDH2 thereby altering its immunogenicity [8]. Past clinical studies have shown that anti-RR can be found in up to 35% of HCV infected persons [8], most often in those being treated with IFN/ribavarin combination therapy and not typically in uninfected persons or prior to antiviral therapy [9]. Studies thus far have been conducted primarily on clinical populations. We set out to answer the question: what is the epidemiology of anti-RR in the general population? We intend to investigate the prevalence of anti-RR in the US non-clinical population, investigate the distribution of this autoantibody by population demographics and examine what proportion has evidence of prior HCV infection. This investigation is relevant to medical practice because amongst the HCV infected persons where it has been studied anti-RR status is thought to impact response to therapy [9].

Materials and methods

Study design

This study is a cross-sectional analysis of data from multiple years (1999-2004) of the National Health and Nutrition Examination Survey (NHANES) administration.

Survey design and data collection

The NHANES has a non-random, complex, multi-stage, probability sampling design [15]. The sample is representative of the non-institutionalized US population with over-sampling of persons 60 and older, African Americans, and Hispanics [14, 15]. Interview and examination data is collected based on Institutional Review Board approval from the National Center for Health Statistics Research Ethics Review Board.
Given that anti-RR antibodies are rare phenomenon, 3 cycles of the continuous NHANES were combined to

produce estimates with greater statistical reliability. In total, 31,126 persons participated in the NHANES 1999-2004 [15]. Of these, 29402 persons (94.5%) completed both the interview and the examination portions, from which 7106 persons (23% of total sample) were randomly selected to be included in the subsample whose sera was tested for autoantibodies. From this subsample 4738 persons (15% of total sample) had complete data for anti-RR and HCV antibody, constituting the study sample [15].

Laboratory testing

Data on anti-RR autoantibodies were obtained from the 1999-2004 NHANES files. The details of the procedures involved in testing of IgG autoantibodies to human cellular antigens can be found in the respective documentation on the NHANES website [10] as well as in the study on US prevalence of Antinuclear Antibodies in the US by Satoh et al. [13]. Immunofluoresecence assays were used by NHANES associated labs to detect autoantibodies using anti-human IgG [13], with staining intensities for anti-RR of 1 or more defined as positive and codified in a separate variable [15].

HCV antibody testing was conducted on all examinees 6 years and older. Antibodies directed against hepatitis C virus (anti-HCV) were measured from serum or plasma using direct solid -phase enzyme immunoassay with the anti-HCV screening ELISA. Positive specimens were reanalyzed with the same procedure, with those that were repeatedly positive tested using the Chiron RIBA Processor System (Chiron Corporation, Inc.), an in vitro qualitative enzyme immunoassay for the detection of anti-HCV [14]. Samples where the RIBA result was positive were reported as confirmed positive for antibody to HCV, while those with a negative RIBA result were reported as negative, and indeterminate results are reported as indeterminate [14].

C-Reactive Protein (CRP) in serum was quantified using latex-enhanced nephelometry, in which a dilute solution of test sample is mixed with latex particles coated with mouse monoclonal anti-CRP antibodies, allowing CRP present in the test sample to form an antigen-antibody complex with the latex particles [12].

Interviews for demographic and health information were conducted by the NHANES in respondents' homes using the computer-assisted personal interviewing (CAPI) method. Health examinations were performed in mobile examination centers. All lab procedures were performed by NHANES associated labs with the resulting variables available in datasets categorized by iteration.

Statistical analysis

Statistical analysis was conducted taking into account the non-random, multi-stage probability sampling methodology of the NHANES dataset. Sampling errors were estimated using the Taylor series (linearization) method with survey design variables for strata and primary sampling units included in statistical survey procedures using the respective variables provided in the data files. Appropriate subsample weights associated with the antinuclear antibodies (ANA) data were used to generate national weighted estimates and to account for the complex survey design (including oversampling), survey non-response, post-stratification, stage of selection and additional non-response for the subsample. The prevalence of anti-RR in the US population was calculated stratified by demographic characteristics. (Tab. I) Demographic characteristics of subjects with and without Anti-RR antibodies were analyzed and Chi-Square tests were performed for comparison with a $p < 0.05$ desig-

Tab. I. Demographic characteristics by anti-Rods and Rings autoantibody (anti-RR) status.

DEMOGRAPHIC CHARACTERISTICS		(-) Anti-RR†		(+) Anti-RR†		
		%	SE*	%	SE*	p-value
TOTAL		**4699**		**39**		
Age,y (mean)		42.9	0.4	48.1	2.6	0.05
Gender						**0.5**
	Male	48.2	0.9	54.6	10.1	
	Female	51.8%	0.9	45.4%	10.1	
Ethnicity						**0.2**
	Non-Hispanic White	70.5	1.8	52.6	11.5	
	Hispanic	13.2	1.5	20.1	8.1	
	Non-Hispanic Black	10.7	1.1	20.7	6.0	
	Other	5.6	0.6	6.6	5.2	
Education						**0.2**
	Less than highschool	26.3	0.9	35.9	9.2	
	High school diploma	23.4	1.2	10.9	5.4	
	More than high school	50.2	1.2	53.2	9.7	
Family Income-to-Poverty Ratio						**0.9**
	At or Above Poverty Level	85.6	1.0	86.8	6.8	
	Below Poverty	14.4	1.0	13.2	6.8	

† anti-Rods and Rings autoantibodies; * weighted Standard Error.

nating statistical significance. (Tab. II) The distribution of anti-RR over the known etiologies of HCV infection and treatment were calculated using survey questionnaire, medication use data and serum antibodies for HCV status. (Tab. III) Additionally, the medication usage profile of the anti-RR positive, HCV naïve population was investigated. (Tab. IV) Appropriate sample weights, stratification, and clustering design variables were incorporated into all SAS survey procedures to ensure correct estimation of sampling error. Special Dioxin subsample weights are required to analyze the NHANES ANA data as it was generated using a randomly selected 1/3 subsample of each 2-year NHANES cycle (the Dioxin subsample). The 6-year subsample, interview and examination weights were calculated for the combined 1999–2004 data by following the NHANES

Tab. II. Estimated US prevalence of anti-rods and rings autoantibodies (anti-RR*) by demographic characteristics.

DEMOGRAPHIC CHARACTERISTICS		n	Population positive for Anti-RR* (n)	% Population positive for Anti-RR* (95%CI†)
TOTAL		**4738**	**39**	**0.7 (0.4,1.1)**
Age, years				
	12-19	1188	7	0.6 (0, 1.2)
	20-29	684	4	0.5 (0,1.1)
	30-39	639	5	0.3 (0,0.6)
	40-49	578	2	0.5 (0,1.3)
	50-59	474	11	1.8 (0.5,3.0)
	60-69	523	3	0.7 (0,1.4)
	> 70	652	7	1.1 (0.2,1.9)
Gender				
	Male	2276	22	0.8 (0.4,1.3)
	Females	2462	17	0.7 (0.2,1.1)
Ethnicity				
	Non-Hispanic White	2111	13	0.6 (0.2,1.0)
	Hispanic	1446	13	1.1 (0.3,2.0)
	Non-Hispanic Black	992	11	1.4 (0.6,2.3)
	Other	189	2	0.9 (0,2.1)
Education				
	More than high school	1658	17	0.8 (0.3,1.3)
	High school diploma (GED)	896	5	0.3 (0,0.7)
	Less than highschool	2178	17	1.0 (0.4,1.6)
Family Income-to-Poverty Ratio				
	At or Above Poverty Level	3357	30	0.7 (0.4,1.0)
	Below Poverty	980	4	0.6 (0,1.4)

*anti-Rods and Rings autoantibodies; †Weighted Confidence Interval.

Tab. III. Estimated US prevalence of anti-Rods and Ring autoantibodies by selected anti-HCV* antibodies status, CRP† levels and anti-viral therapies.

		n	(+) Anti-RR‡ (n)	% (+) Anti-RR‡ (95%CI§)
anti-HCV*				
	Absent	4658	38	0.8 (0.4,1.1)
	Present	80	1	0.1 (0,0.3)
CRP† Categories				
	< 1	4286	34	0.8 (0.4,1.1)
	1 to 3	387	4	0.4 (0,1.0)
	> 3	65	1	0.8 (0,2.2)
Interferon + Ribavirin combination use				
	Absent	4752	39	0.7 (0.4,1.1)
	Present	2	0	-
Interferon use only				
	Absent	4749	38	0.7 (0.4,1.1)
	Present	5	1	-
Ribavirin only				
	Absent	4753	39	0.7 (0.4,1.1)
	Present	1	0	-

*Hepatitis C Virus; †C-Reactive Protein; ‡anti-Rods and Rings autoantibodies; §Weighted Confidence Interval.

Tab. IV. Medication Profile of Hepatitis C virus-naïve, anti-Rods and Rings autoantibody (+) Population.

	n	%
MEDICATION CLASSES		
Antihypertensives	8	38.1
Anti-GERD*	5	23.8
Albuterol	3	14.3
Synthetic hormones	3	14.3
Anti-clotting medications	2	9.5
Other	11	52.4
POLY-PHARMACY		
4+	4	19.0
3	4	19.0
2	4	19.0
1	9	42.9

* GERD: Gastroesophageal Reflux Disease.

analytic and reporting guidelines. The Dioxin weights (WTSPO4YR for 1999-2002 and WTSC2YR for 2003-2004) were used for the subsample. One-third of the respective 2-year weights for 2003–2004 were used and merged with two-thirds of the 4-year 1999–2002 respective weights [18]. All analysis was performed using SAS 9.2 (SAS Institute, Cary, NC).

Results

In this sample of 4738 persons over the age of 12 years, 39 persons were found to be positive for anti-RR (Tab. I), the outcome of interest, representing a prevalence of 1.3 million (95% CI: 0.7-1.9 million) persons in the US population. The mean age of the population was comparable across anti-RR status (42 years old *vs.* 48 years old for negative and positive respectively). The two groups had approximately equal proportions of males and females, were more likely to be non-Hispanic White, with more than high school level of education, and above the poverty level (Tab. II).

Of all person who were anti-RR positive (n = 39), only one was found to have had prior HCV infection. Conversely, anti-HCV was found in 80 persons who were almost all anti-RR negative (79 negative *vs.* 1 positive). The use of HCV therapy was examined to determine the influence of CTP and GTP synthesis inhibitors. However, of participants currently using Ribavarin and/or IFN, only one also tested positive for anti-RR. Inflammation, marked by CRP, was not associated with anti-RR status.

Because prior studies have shown an association with medication usage (for HCV therapy) and anti-RR status, medication use amongst HCV-naïve but anti-RR positive persons was calculated. Medication use was reported by 21 of the 39 persons positive for anti-RR with one person reporting IFN usage (Tab. IV). Over half of these (11/21) had polypharmacy and over a third (8) were antihypertensives. Usage of medications with CTP or GTP synthetic pathway inhibition activity was not prevalent in this population.

Discussion

In this analysis of the US non-institutionalized population, the overall prevalence of anti-RR was found to be 0.74%, signifying 1.3 million persons. Persons with and without anti-RR were found to be similar across demographic characteristics.

This study found that the majority of anti-RR prevalence was found to occur without prior exposure to HCV and its therapy. In contrast, prior studies of clinical populations have suggested that a majority of anti-RR occurs in the context of HCV infection and treatment [1, 2, 8, 9]. The fact that more persons with a past history of HCV were not found to have anti-RR may be partly explained by the transience of some forms of anti-RR as reported in prior literature [17]. The fact that more persons with anti-RR and HCV infection were not found in the general population would not be surprising if anti-RR typically cleared in the post-therapy period. From this context, it is likely that the ever-positive status of anti-RR could significantly be higher in the population of persons having been infected by HCV and having gone through its treatment. This association would subsequently be hard to detect in the general, non-clinical population as the baseline levels of the autoantibody would exist with no trace of induced antibodies in the ever-positive population. Although this hypothesis was tested by examining the population of persons currently using IFN, or ribavirin or a combination of both, the analysis was limited by the small proportion of persons reporting usage of these medications.

In regards to the existence of anti-RR in persons without evidence of prior HCV infection, this finding is corroborated by implicit findings of an earlier study that showed that anti-RR can in fact also occur in persons independent of an antecedent HCV infection. In a 2010 study by Carcamo et al it was demonstrated that 15/23 persons with anti-RR had prior infection with HCV, indicating that 8/23 persons had anti-RR without prior HCV infection [17].

The question that arises from our findings is whether there is a difference between the type of anti-RR found in persons infected with HCV with subsequent treatment versus HCV-naïve persons. A difference in the etiology or physiology is suggested by the high prevalence of anti-RR without evidence of prior HCV infection. If HCV infection played a more substantial role then its history would be more prominent amongst anti-RR positive persons, even if the antibodies are transient. However if the transience of the antibodies is impacted by their cause (HCV treatment versus other than HCV treatment) then it better explains the findings of a large number of people having those antibodies but no history of HCV. The implications for the finding of anti-RR in HCV-naïve persons is that either these antibodies are elicited through the same pathways associated with HCV treatment or by another pathway that has not yet been characterized. If the former is considered, then anti-RR may be induced in HCV-naïve persons by diseases, medications or environmental factors that inhibit the CTP and GTP synthesis pathways.

This study has several limitations including the small population prevalence of anti-RR, which prevented a more thorough statistical analysis for associations. Furthermore, the primary risk factor for anti-RR has been found to be ribavarin and IFN combination therapy, however the prevalence of this was also very low. Additionally, given the cross-sectional study design it was not possible to determine the transiency or permanence of anti-RR, which can impact prevalence and associations.

While the limitations and/or differing mechanisms prevent verification of findings derived from prior studies of clinical populations, the strength of this study is that its large nationally representative sample indicates that where findings are positive these are worthy of further investigation. One such finding of importance is that anti-RR positivity can in fact occur in the general population outside of the context of hepatitis C infection and its treatment. This indicates that in addition to the identified pathways in HCV positive persons, there may possibly be other factors or pathways in HCV-naïve persons, possibly involving polypharmacy or chronic conditions, which may be associated with cytoplasmic rods and rings induction and/or anti-RR formation. Furthermore, this study presents a baseline population estimate of anti-RR prevalence so that future studies can monitor trends. Since anti-RR and their target cytoplasmic rods and rings structures have only recently been identified, the cause of these newly discovered autoantibodies in the general population, their role in health and disease, the clinical consequences of their presence, and their natural history are all questions that remain and require further investigation.

Conclusions

In conclusion, this study found that there are approximately 1.3 million persons in the US who are positive for anti-RR, the vast majority of whom have no circulating antibodies to the hepatitis C virus and therefore no evidence of a prior immune response to its infection and no active treatment individually or in combination with ribavarin and IFN. This study is significant in its finding that the anti-RR autoantibody normally associated with HCV infection and treatment can also be found in the general HCV-naïve population. This study has implications on the possible temporal dependency of anti-RR and additional etiologies for its onset, thereby providing directions for future investigation: long-term follow-up studies can help establish the relationship of time with detectability of anti-RR; further investigation of non-HCV related anti-RR may help identify relationships with clinical presentations, particularly with chronic disease given the extent of prescription usage found in this study; and comparisons of non-HCV and HCV related anti-RR can help determine if there is a difference in anti-RR transience based on etiology and, if so, factors promoting clearance versus persistence may reveal insights into autoimmunity and therapy.

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