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Hypothesis

Further evidence for association of hepatitis C infection with parenteral schistosomiasis treatment in Egypt

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Abstract

Background: Hepatitis C virus (HCV) infection and schistosomiasis are major public health problems in the Nile Delta of Egypt. To control schistosomiasis, mass treatment campaigns using tartar emetic injections were conducted in the 1960s through 1980s. Evidence suggests that inadequately sterilized needles used in these campaigns contributed to the transmission of HCV in the region. To corroborate this evidence, this study evaluates whether HCV infections clustered within houses in which household members had received parenteral treatment for schistosomiasis.

Methods: A serosurvey was conducted in a village in the Nile Delta and residents were questioned about prior treatment for schistosomiasis. Sera were evaluated for the presence of antibodies to HCV. The GEE2 approach was used to test for clustering of HCV infections, where correlation of HCV infections within household members who had been treated for schistosomiasis was the parameter of interest.

Results: A history of parenteral treatment for schistosomiasis was observed to cluster within households, OR for clustering: 2.44 (95% CI: 1.47–4.06). Overall, HCV seropositivity was 40% (321/796) and was observed to cluster within households that had members who had received parenteral treatment for schistosomiasis, OR for clustering: 1.76 (95% CI: 1.05–2.95). No such evidence for clustering was found in the remaining households.

Conclusion: Clustering of HCV infections and receipt of parenteral treatment for schistosomiasis within the same households provides further evidence of an association between the schistosomiasis treatment campaigns and the high HCV seroprevalence rates currently observed in the Nile delta of Egypt.

Background

Infection with the hepatitis C virus (HCV) occurs primarily through percutaneous exposure to contaminated blood or blood products. Unlike most other viral hepatitis infections that tend to be acute, hepatitis C infections are often chronic and persist for decades [1–3]. The long-term sequelae of chronic HCV infections include increased risks of liver cirrhosis and hepatocellular carcinoma [4,5].

HCV infection is a major public health problem in Egypt [6–8]. Blood bank and community-based surveys conducted in Egypt have reported sero-prevalence rates of HCV to be as high as 40% in some parts of the country [9–12]. These rates are substantially higher in the Nile Delta region compared with the rest of the country [13]. Schistosomiasis is a parasitic infection transmitted to humans from snails that harbor the parasite. Most rural and peri-urban areas located in the Nile Delta are in close proximity to the distributaries of the Nile River or irrigation canals drawn from the Nile. These slow flowing waters are infested with snails that serve as the vector for the schistosomal parasite. Schistosomiasis infections, in addition to HCV are hyperendemic in the Nile Delta region [14].

In the 1960s, 1970s and early 1980s, mass campaigns were conducted to treat schistosomiasis infections in these areas, during which individuals older than 5 years of age were treated with tartar emetic injections [15]. Sero-surveys conducted in the 1990's in Egypt have reported positive associations between HCV infections and a history of schistosomiasis or a history of having received injections for the treatment of schistosomiasis [9,12,13,16]. Based on this evidence, the studies suggest that inadequately sterilized needles and syringes used during the campaign were probable causes for transmission of HCV in the region.

Since all the studies were conducted as cross-sectional surveys, it is not possible to know whether the HCV infections were pre-existing at the time of the treatment campaigns or whether they were incident infections as a consequence of the campaigns. The studies have also reported low prevalence rates in the younger age groups and high prevalence rates for the older ages suggesting that individuals infected between the 1960's to the early 80's are now older and more likely to be sero-positive compared to those born after the campaigns. A limitation of most of the published studies is the implicit assumption in their analyses that all the individuals in the survey are independent of each other and that infection rates are uniformly distributed both within and across all households in the community. If the suggested nosocomial mechanism of transmission were true, the assumption that infection rates are uniformly distributed would not be valid, because HCV infections will tend to cluster within households that participated in the campaign compared to households that did not. The uniform distribution assumption is further complicated by the existence of subgroups of high risk and low risk individuals within each house based on their date of birth. If a household participated in the campaign and has an infected member, there is a greater likelihood that another member who resided in the same house at the time of the campaign will be infected, but a low likelihood that an individual born within the same house after the campaign period will be infected.

We postulate that an entire household was more likely to have participated in the campaign if at least one member of the household had a history of schistosomiasis and received treatment by injections, compared to houses in which no member reported a past history and parenteral treatment for schistosomiasis. Based on this premise, we would expect clustering of HCV infection in households in which one or more members reported having received parenteral treatment for schistosomiasis, if the underlying reason for the spread of HCV infection was in fact attributable to the treatment campaign.

Using the HCV infection status of individuals participating in a community based sero-survey conducted in the Nile delta of rural Egypt, we tested the following hypotheses to assess if the schistosomiasis treatment campaigns were associated with transmission of HCV:

i) Participation by village residents in the schistosomiasis treatment campaigns were likely to cluster within houses, with members of a house more likely to participate in the campaign if at least one member participated.

ii) Clustering of HCV infections within houses is more likely among houses that participated in the treatment campaigns compared to those that did not.

Methods

Site

The study was conducted in Kalama, a semi-urban area in Qalyub District of the Qualyabia Governorate. The village is located on the south-east edge of the Nile River delta, approximately 19 km north of Heliopolis, Cairo, Egypt. Irrigated farmlands and canals surround the village, a feature typical of the Nile delta. The village has a population of approximately 17 000 residents living in about 2400 houses. Agriculture is the primary occupation. Residents live in small single story homes either as single nuclear families or extended families with multiple related nuclear families residing within the same house. The median household size was 7 persons at the time of the study.

Population and sero-survey

Details of the study have been described elsewhere [16]. Briefly, after conducting a census in December 1994, the village was divided into 12 geographic sectors. Every 15th house was then systematically sampled for participation in a sero-survey. Of a total of 172 sampled houses, 168 (98%) household heads agreed to participate. Between January and June 1995, after obtaining written informed consent, we conducted a sero-survey and administered a questionnaire to the study participants, soliciting medical

and behavioral risk factors for liver disease. History of schistosomiasis was obtained by interview in which participants were asked whether they had ever received a diagnosis of schistosomiasis. Regardless of their histories, all participants were further queried about past treatment for schistosomiasis and whether the treatment was with oral drugs, injections or both. Approximately 7 ml of blood was then obtained via a venipuncture from each participant. Persons under 10 years of age were excluded from the sero-survey because earlier work in Kalama demonstrated HCV infection to be rare in this age group [12]. Baseline socio-demographic information was collected at the time of the census. Household socio-economic status was classified as high, medium or low according to a locally developed scale. Details of the laboratory methods used to detect anti-HCV antibodies have been described elsewhere [16]. Briefly, serum from the blood samples was screened for anti-HCV antibodies by EIA (Abbot Laboratories). All EIA-reactive sera were evaluated with either the second or third generation recombinant immunoblot assays (Chiron Corporation) as confirmatory tests. Positive results by either of the immunoblot assays were considered to be HCV positive (positive for anti-HCV antibodies).

Statistical methods

For baseline comparisons of the HCV positive and negative individuals, we used the chi-square test (or Fisher's Exact test when there was sparse data) for categorical variables. For dimensional variables, the t-test was used to compare the two groups when the data was normally distributed, and the Mann-Whitney-U test, when assumptions of normality were not met.

To test for intra- and inter-class clustering of HCV infections, we used a modified formulation of the Generalized Estimating Equations (GEE). The original formulation of GEE also referred to as GEE1 [17] is used in the analysis of correlated data in which clustering is treated as a nuisance factor that needs to be taken into account at the analysis stage. This approach uses a working correlation matrix to model the correlation between the repeated or related observations for an individual or a cluster while simultaneously adjusting for covariates in a generalized linear model. With the new formulation, also referred to as GEE2 or Alternating Logistic Regression (ALR), clustering can be treated as a parameter of interest on which we can perform hypothesis testing [18–20]. Instead of specifying a correlation matrix, an OR is computed for each cluster in this approach to detect clustering among related observations. Since the outcome of our study is binary (HCV positive or negative), we describe the approach for binary outcomes.

For a family of size n, if Yj is the HCV status (0 = negative, 1 = positive) of the j^{th} individual, we define

$$\log [Pr(Yj = 1)/Pr(Yj = 0)] = \beta 0 + \beta 1x1j + \beta 2x2j + ... + + \beta mxmj \quad (1)$$

where the x's are m covariates for the j^{th} individual. For most analyses, the x's are variables that describe the baseline characteristics or are confounding variables. These variables could be specific to the j^{th} individual (such as age and gender) or could be common for the entire family (e.g. race or socio-economic status of the family). The β's are the regression parameters to be estimated. We next define the odds ratio (OR) between the j^{th} and k^{th} members of the family as

$$OR_{jk} = \frac{\Pr\left(Y_j = 1, Y_k = 1\right)\Pr\left(Y_j = 0, Y_k = 0\right)}{\Pr\left(Y_j = 1, Y_k = 0\right)\Pr\left(Y_j = 0, Y_k = 1\right)} \qquad (2)$$

The odds ratio is a commonly used measure of association for categorical variables and assumes the values between zero and infinity. An odds ratio equal to one suggests the lack of an association between individuals. In equation (2), an OR = 1 for each of the NC2 (number of ways in which we can select 2 out of N) pairs of members within a house indicates the absence of clustering of disease within the family. On the other hand, an odds ratio greater than one will indicate clustering of disease within the families. It should be noted that the OR defined in (2) is not the same OR that one conventionally uses to measure the association between exposure and disease in case-control or observational studies. The OR in (2) describes the odds of disease or no disease for another member in the same cluster, given that a member is affected or unaffected. We henceforth refer to this OR as 'OR for clustering'. Such ORs are commonly used to describe familial aggregation of disease in genetic studies [21]. The OR can be modeled for subclasses or sub-clusters within the main cluster as

$$\log ORjk = \gamma z \quad (3)$$

Where z indicates the class membership of the (j, k) pair within the house. For example, if we consider adults within a house to be members of class 'a' and children to be members of class 'c', then log(ORjk) = γaa, γcc, or γac depending on whether the (j, k) pair are adults, children, or an adult-child pair. From equation (3) we thus obtain two intra-class ORs namely $e^{\gamma aa}$ and $e^{\gamma cc}$ for adults and children respectively and one inter-class OR namely $e^{\gamma ac}$ between adults and children. A significant γaa or γcc is suggestive of clustering within adults or within children, whereas a significant γac is indicative of the existence of a

correlation between adults and children. When z = 1, there is a single class and the equation models a constant log odds across all the clusters. Estimates for β's and γ's are obtained by simultaneous estimation of the parameters from equations (1) and (3), thereby adjusting the ORs for covariates introduced in (1). Further details on the formulation, estimation methods, features of the model, and properties of the estimators have been published elsewhere [18–20].

To model sub-groups of high and low risk groups within houses, we chose an age cutoff of 15 years based on an examination of the age-specific prevalence within our study and based on reports stating that parenteral treatment of schistosomiasis was stopped between 1982 and 1986, with the introduction of praziquintal, an oral drug used to treat the disease [22]. Further, children under 5 years of age were not eligible for receiving injections during the campaigns. Since children born in 1981 would have been 14 years of age at the time of our 1995 HCV study, it can be safely assumed that they were not present at the time of the schistosomiasis campaigns. This age cutoff has also been used in other studies [13].

We conducted our analysis using the ALR implementation of GEE in SAS V8.02 (SAS Institute Inc., Cary, NC). Since we assume that the parenteral treatment for schistosomiasis in the campaigns for the entire house occurred around the same time, to model the correlation between the members of the house, ordering of individuals within the house (cluster) is not relevant. Hence, to model the OR for clustering of schistosmiasis treatment in the house, an exchangeable log odds ratio was assumed. With an exchangeable log odds ratio, a constant OR for clustering is assumed for all houses. To estimate the degree of clustering of HCV infections, a history of parenteral treatment of schistosomiasis in the house was considered to be a blocking factor. Separate log odds ratios were estimated for each block with the assumption that the log odds ratios are constant within each block.

Results

The study sample contained 910 age eligible individuals residing in 168 houses. Of these, 810 (88%) agreed to participate in the study and provided blood samples. The number of participants within these houses ranged from 1 to 20 members with a median (inter-quartile range) of 4 (2 – 6) members. Anti-HCV antibodies were evaluated for 796 (98%) of the blood samples collected. A total of 321 (40%) subjects were sero-reactive to HCV. Individuals positive for HCV were significantly older than those who were negative ($p < .001$). Other variables measured at baseline were comparable for the two groups. Although not statistically significant, HCV seropositive individuals were more frequently males and of low socio-economic status (table 1). Age specific HCV positive prevalence rates increase around 15 years of age suggesting the possibility of a cohort effect (table 2). The sero-prevalence of anti-HCV antibodies was 11% (95%CI: 6% – 16%) for children under 15 years of age compared to 47% (95%CI: 43% – 51%) for those 15 years or older ($p < .0001$).

Among the 746 individuals who reported no history of schistosomiasis or no parenteral treatment for diagnosed schistosomiasis, the prevalence of HCV was 38% compared to 68% for the 50 individuals who reported receiving the treatment ($p < .0001$). Examination of the degree of clustering of HCV infections among houses with members that received parenteral treatment indicated that there was a significant degree of clustering of HCV infections in such houses. After adjusting for individual level effects of age and gender, and household characteristics such as socio-economic status and risk group based on eligibility for participation in the schistosomiasis treatment campaigns, all of which were significant confounding variables, the OR for clustering of HCV infections was 1.76 (95% CI: 1.05 – 2.95; $p < .05$) (table 3). In contrast, the OR for clustering of HCV infections among houses in which no member reported a history of parenteral treatment for a past schistosomiasis infection was 1.37 (95% CI: 0.92 – 2.05; $p = 0.12$). If we inappropriately used logistic regression and treated all participants to be independent of each other, the odds of HCV infection among members reporting previous parenteral schistosomiasis treatment compared to those who did not, after adjusting for the same confounding variables, was 1.55 (95% CI: 0.81 – 2.98; $p = 0.19$).

Fifty adults reported receiving injections for the treatment of schistosomiasis. Houses in which an adult member reported receiving parenteral treatment were more likely to have other adult members with histories of parenteral treatment for schistosomiasis. The OR for clustering of individuals receiving parenteral treatment was 2.44 (95% CI: 1.47 – 4.06; $p < 0.001$), suggesting that past participation in the treatment campaigns tended to cluster within houses.

Discussion

Our analysis reveals that there is significant amount of clustering of HCV infections within houses of the study village and that the infections are not distributed uniformly across the village. We also demonstrate that some households were more likely to have participated in the schistosomiasis treatment campaigns compared to others and that the clustering of HCV infections were more likely among the households that participated in the treatment campaigns. In the general population, based on the time period during which the treatment campaigns were conducted and the ensuing age distribution of the HCV infec-

Table 1: Sociodemographic features of the study population by HCV serostatus, Kalama, Egypt 1995.

Sociodemographic Feature	HCV Positive (N = 321)	HCV Negative (N = 475)
Median Age (yrs)	35[a] (25–47) [b]	20 (14–30)
Female	46%	51%
Occupation of Household Head[c]:		
Farmer	24%	17%
Manual Laborer	18%	13%
Civil Servant	19%	26%
Pensioner	8%	8%
Other	31%	36%
Type of Household Dwelling:		
Village House	44%	45%
Flat	41%	37%
Other	15%	18%
Household Ownership of Dwelling[d]:		
Owned Exclusively	86%	83%
Owned Partially	11%	11%
Rented	3%	6%
Household Luxury Items[e]:		
Electric Oven	64%	65%
Color Television	27%	33%
Washing Machine	61%	63%
Refrigerator	40%	44%
Truck	1%	2%
Car	3%	4%
Primary Source of Drinking Water in Household[e]:		
Municipal	68%	66%
Water Truck	32%	34%
Primary Defecation Site in Household[e]:		
Western Toilet	3%	3%
Asian Toilet	97%	97%
Household SES[f]:		
Low	42%	36%
Medium	56%	60%
High	2%	4%

[a] $P < 0.001$ (2-tailed) [b] Interquartile range. [c] Information available for 318 HCV positive and 469 negative individuals. [d] Information available for 310 HCV positive and 459 negative individuals. [e] Information available for 318 HCV positive and 464 negative individuals. [f] Socioeconomic scale (SES); complete information available for 310 HCV positive and 459 HCV negative individuals

Table 2: Age-specific HCV seroprevalence rates, Kalama, Egypt 1995

Age in years	Total Tested	HCV Positive	% Positive (95% CI)[a]
10–14	153	17	11% (6% – 16%)
15–24	222	63	28% (22% – 34%)
25–34	132	58	44% (36% – 52%)
35–44	116	70	60% (51% – 69%)
45–54	86	63	73% (64% – 83%)
55 +	87	50	57% (47% – 68%)
Total	796	321	40% (37% – 44%)

[a] % positive for HCV (95% Confidence Interval)

Table 3: Evaluation of clustering of HCV seropositivity within houses that participated in the schistosomiasis treatment campaigns, Kalama, Egypt 1995.

	Houses with treatment[a]	Houses with no treatment[b]
Number of Houses	36	132
HCV infections	94	227
Odds Ratio for Clustering	1.76	1.37
95% Confidence Interval	1.05 – 2.95[c]	0.92 – 2.05[d]

[a]Houses that had one or more members reporting a diagnosis of schistosomiasis and having received treatment with injections [b] Houses with no member being treated with injections for schistosomiasis [c] $p = 0.03$ [d] $p = 0.12$

tions obtained from multiple cross-sectional surveys, a temporal and possible causal relationship between these two events has been suggested [13]. Our study adds to this finding by studying these two events within the same population, thus providing further evidence that the spread of HCV in the Nile Delta region was associated with the schistosomiasis treatment campaign.

The observation that households were more likely to have participated in the treatment campaign if any member within the house participated in the campaign is corroborated by the manner in which the treatment campaigns were conducted. Unlike mass campaigns conducted for vaccinations, where house-to-house visits are made and residents are immunized, the treatment campaigns were conducted at centralized health care facilities such as hospitals and clinics. In some instances, these clinics were rolling clinics established temporarily at the villages. This situation makes it likely that entire households rather than random individuals would have visited the clinics based on factors such as proximity, risk of schistosomiasis infection to the household, and socio-economic status. Indeed, our analysis finds that socio-economic status of a house was strongly predictive of whether an individual tests positive for HCV, after adjusting for the effects of clustering.

Potential limitations of this study need to be mentioned. Since reports of receiving injections for treatment of schistosomiasis were by recall, it is possible that inaccuracies in the recall might have biased the results of the study. Additionally, it is possible that a report by one member of the family potentially influences the response of another family member during the interview process. Such related responses will artificially tend to show clustering of participation in the treatment campaigns within the houses. However, since testing of sera for HCV antibodies for the members of the family was done without knowledge of which sera belonged to which family, and since clustering of HCV infections was observed within families independent of the participation in the treatment campaigns, it is unlikely that the clustering of the individuals' participation in the treatment campaigns were solely due to biased reporting since there is an *a priori* reason to believe that such clustering is possible on the basis of the manner in which the campaigns were conducted. It could be stated that familial clustering of HCV infections may be due to other factors or behaviors that are related to an increased risk of schistosomiasis as well as an increased risk of HCV infections. Alternatively, certain household behaviors such as health care practices or tendency to visit providers who use unsafe treatment methods, could also result in clustering of HCV infections within the families. If this were true and was the sole explanation for the clustering of HCV infections within the household, we would expect to see HCV infections in the younger children too who were not present in the households at the time of the treatment campaigns within such families. However, our previous work in this village found HCV infections among children less than 10 years of age to be extremely rare, thus making it less likely that unmeasured factors might be influencing this association.

Despite the use of oral drugs for the treatment of schistosomiasis during the past two decades, HCV antibodies were detected in some children below 15 years of age. Vertical transmission of HCV from infected mother to infant occurs in approximately 5% of HCV infected mothers and is reported to increase with increasing viral loads [23]. Prospective studies could help determine the relative contribution of vertical and horizontal transmission of HCV infections observed in these children.

Most previous studies that have that have found associations between histories of schistosomiasis and HCV have either been designed as surveys or have used ecologic associations. While ecologic associations are very useful for generating hypotheses, one should be cautious in trying to make individual level inferences from population based observations because of the multiple sources of biases associated with such study designs [24]. After using the appropriate analytic methods, our results provide further evidence for an association between the parenteral

schistosomiasis treatment campaigns and the transmission of HCV infections in Egypt.

Competing interests

None declared.

Authors' contributions

MR conceptualized the problem, wrote the manuscript and conducted the analysis. MD and ND collected the data and performed the laboratory analysis. AN, ES, JC and RE participated in the design, conduct, and monitoring of the study assisted in the preparation of the manuscript. All authors have read and approved the final manuscript.

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