AIM OF THE WORK

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The aim of this work was to assess different risk factors for HCV infection, possibility of HCV intrafamilial transmission and risk behaviors associated with increasing intrafamilial co-infection among children attending the outpatient clinic of Alexandria University Children's Hospital.

SUBJECTS

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The study was conducted on family members of 75 children with chronic HCV infection “persistently positive HCV PCR for at least 6 months" aged 2 to 18 years old, 46 were males and 29 were females (cases). The chronic HCV cases including in the study were recruited from hepatology outpatient clinic of Alexandria University Children's Hospital. The study was also including family members of 106 randomly selected children with negative anti-HCV antibody (controls) from those attending the outpatient clinics of Alexandria University Children's Hospital.

Exclusion criteria:

* Concomitant HBV infection.

* Age of the index or controls more than 18 years or less than 2 years.

METHODS

METHODOLOGY

Sample size:

A sample of 500 persons was needed to identify the prevalence of HCV sero-positive cases = 14.8 % [126] with a precision of 3%, alpha error = 0.05

The sample units were randomly selected using a suitable random selection method. However our study was including 850 persons.

The study was conducted on family members of 75 children with chronic HCV infection “persistently positive HCV PCR for at least 6 months" aged 2 to 18 years old, 46 were males and 29 were females (cases). The chronic HCV cases including in the study were recruited from hepatic outpatient clinic of Alexandria University Children's Hospital. The study was also including family members of 106 randomly selected children with negative anti-HCV antibody (controls) from those attending the outpatient clinics of Alexandria University Children's Hospital. All children and their families were subjected to the following:

1. Thorough history taking through a special questionnaire. The questionnaire emphasized on the following:

- Demographic data including: age, sex, the residence of the patient whether urban or rural, the socioeconomic class and occupation that was calculated according to the modified social score for family social leveling (modified after Fahmy and El-Sherbini 1983). Appendix C

- The risk factors for HCV infection including;

* Intravenous access (history of blood or blood product transfusion including albumin, IV catheters and previous intravenous injections) and its site.

* Previous hospital admission and its site.

* Surgical procedures (circumcision, sutures, abscess drainage, surgical biopsy, dental maneuvers, urinary catheter and endoscopy) and its site.

* Folk medicine practice, tattooing, shaving at barber, pedicure, manicure, needle pricks including ear piercing.

* Household practice (living with a household with HCV infection).

- Whether family members (sleeping together, eating together) or no.

- Domestic high risk behaviors as: sharing of nail trimmers or other grooming items such as razors or toothbrushes, sharing personal tools as combs or soap, shared

cottons, sharing food utensils as spoons or glasses, accidental exposure to blood or any of body fluid of infected persons and being bitten by HCV infected patients.

- Family history of chronic liver disease, repeated blood transfusions for any of family members, hemodialysis patients or drug addicts within the family.

- If there is more than one member in the family positive for HCV PCR how long they have been staying together in the same house.

2. Full clinical examination stressing on the condition of liver and spleen and whether there is jaundice or ascites.

3. All studied children and their family members were screened for anti-HCV antibodies using ELISA test and all positive cases were confirmed by using HCV PCR.

4. If more than one family member were positive by HCV PCR, they were subjected to phylogenetic analyses (study of gene sequences of isolated viruses from different hosts).

5. An oral consent was obtained from all studied persons.

6. All positive cases were followed in the hepatology outpatient clinic for further assessment.

HCV antibody test

All family members of 75 positive cases and all family members of 106 negative cases underwent testing of HCV antibody using EIAgen HCV Ab (v.4) Kit manufactured by Adaltis Italia S.p.A.

Phylogenetic analysis

Eleven samples (3 index cases and their mothers, 1 index case and his father and 2 siblings and their mother) were studied for the HCV subtype by HCV isolation, RNA reverse transcription, PCR amplification and sequencing.

RNA was extracted from 250 μL serum using RNeasy Mini kit (Qiagen cat#) following the manufacturer instructions. Samples stored at -80 °C. Extracted RNA (5 µL) was reverse transcribed for 45 min at 42 °C. Samples were heated for 5 min at 95

°C. PCR was performed in 25 µL aliquots containing 5 µL of cDNA, 20 pmol of sense and antisense primers, (Applied Biosystems), and 12.5 µL buffer (Promega), in a GeneAmp PCR system 2400 (Perkin Elmer). The following cycling parameters were used for *NS5B* amplification. Denaturation at 94 °C (30 s), annealing at 54±64 °C (45 s) and elongation at 72 °C (1 min) was done. During the first five cycles, the annealing temperature was 64 °C; this was then reduced by 3 °C per cycle for 30 cycles (touch-down PCR). The last five cycles were performed at 54 °C. After 40 cycles, a final extension cycle was carried out at 72 °C (4 min).

Purified DNA *NS5B* fragments were directly sequenced on the positive strand using an ABI Prism 377 automated sequencer (Perkin Elmer) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer) [(205)]. Table 1

Table 1: Sequence of primers used for PCR amplification of the NS5B of HCV

Name	Gene	Sequence (5`→3`)*
Position.		
Sn755	*NS5B*	TATGAYACCCGCTGYTTTGACTC
7915±7937		
Asn1121	*NS5B*	GCNGARTAYCTVGTCATAGCCTC
8281±8303		

* Y¯C or T; R¯A or G; S¯G or C; B¯C, G or T; V¯A, C or G; N¯A, T, G or C.

Sequence analysis

Sequence analysis was performed at Clinilab Research Laboratories in Cairo. The following protocol was applied:

The DNA amplicons were purified using QIAquick PCR Purification Kit (Qiagen) following the manufacturer instructions.Table 2-3

Table 2: 2 Cycle Sequencing Reaction Using Bigdye Terminator V 3.1 Cycle Sequencing Kit:

Component	Volume	**Or** Volume
BigDye Terminator	8 ul	4 ul
5X Sequencing Buffer	--	4 ul
Primer (3.2 pmol)	1 ul	1 ul
Template	20 ng	20 ng
Nuclease free water	Up to 20 ul	Up to 20 ul
Total	20 ul	20 ul

Table 3: Thermal Profile of the Cycle Sequencing

Stage	Description	Temperature	Time
1	Denaturation	96	1 min
2	Amplification Annealing	96	10 sec
			5 sec
		60	4 min
3	Hold	4	Pause

Sequence alignment and phylogenetic analyses.

Viral sequences were aligned using BLASTx to create aligned protein sequences that were then analyzed on CLUSTAL W2 software, version 1.8 to create the phylogenetic tree [(205)].

Statistical analysis

After data were collected it was revised, coded and fed to statistical software SPSS version 20. The given graphs were constructed using Microsoft excel software.

All statistical analysis was done using two tailed tests and alpha error of 0.05.

The following statistical tests were used:

A. Descriptive statistics: Frequency and percent were used to describe the categorical data.

B. Analysis of categorical data

a. Pearson's chi square test: it is a non parametric statistic that is used to test for the association (or relationship) between the categories of two independent samples (row and column variables) to reflect a real association between these 2 variables in the population.
b. **Mont Carlo exact test and Fishers exact test:** they are alternatives for the Pearson's chi square test if there were many small expected values.
c. **Odds Ratio (OR): it** is a measure of association between the different studied risk factors and the outcome (HCV infection). The OR represents the odds that HCV infection will occur given a particular exposure, compared to the odds of the HCV infection occurring in the absence of that exposure. It is an effect size to quantify the relation between exposure and outcome. 95% confidence interval is the interval at which the true degree of association lies with a certain confidence.
d. **Multiple stepwise logistic regression: :** logistic regression can be used to predict a dependent variable on the basis of continuous and/or categorical independents and to determine the effect size of the independent variables on the dependent; to rank the relative importance of independents and to understand the impact of covariate control variables. The impact of predictor variables is usually explained in terms of odds ratio (OR) which mean the amount of increased risk at the exposed groups relative to the reference one. The stepwise regression methods were used to determine automatically which variables to add or drop from the model and identifying the most significant predictors.

C. Correlation analysis: correlation is used to test the nature and strength of relation between two quantitative / ordinal variables. The spearman correlation co efficient (rho) is expressed as the Pearson co efficient. The sign of the co efficient indicates the nature of relation (positive / negative) while the value indicates the strength of relation as follow: Weak correlation for rho less than 0.25, intermediate correlation for rho of value between 0.25-0.74 and strong correlation for values between 0.75-0.99.

RESULTS

RESULTS

Serology and questionnaire data were available from family members of 75 children with chronic HCV infection “persistently positive HCV PCR for at least 6 months" (cases). The study was also including family members of 106 randomly selected children with negative anti-HCV antibody (controls) from those attending the outpatient clinic of Alexandria University Children's Hospital.

All participants answered orally for administered standardized questionnaires covering socio-demographic characteristics, present and past health conditions, and exposure to established or potential risk factors for viral hepatitis. Risk factors were categorized as high risk and low risk parenteral exposures based on the magnitude of the associations with HCV transmission documented in previous studies. High risk parenteral exposures include surgery, blood transfusion or any of its derivative, albumin transfusion, biopsy, endoscopy, urinary catheter, history of previous hospital admission including neonatal intensive care unit, history of parenteral injection, catheter, dental drug and abscess drainage. Low risk parenteral exposures include ear piercing, folk medicine, tattooing, manicure, pedicure, shaving at barber, living with HCV infected patient and family history of chronic liver disease, hemodialysis or a patient on repeated blood transfusion. Also history of high risk behaviors of intrafamilial transmission include contact time with HCV infected patients, sleeping together, eating together, sharing spoons, drinking together, sharing towels, sharing personal tools, sharing sharp instruments including razors, scissors or pedicure tools, sharing shaving instruments, accidental exposure to blood of infected patients and being bitten by infected patients.

Prevalence of HCV infection in our study .

Seroprevalence of HCV infection among families of index cases was 15.9% (39 persons out of 245) and 8.6% by using PCR (21 persons out of 245). Seroprevalence was distributed as that 11% among parents and 4.9% among siblings and prevalence by using PCR was distributed as that 6.9% among parents and 1.7% among siblings.

Seroprevalence of HCV infection among families of controls was 3.7% (16 persons out of433) and 2.8% by using PCR (12 persons out of433). Seroprevalence was distributed as that 3.2% among parents and 0.5% among siblings and prevalence by using PCR was distributed as that 2.8% among parents and 0% among siblings.

Personal and sociodemographic risk factors:

1- Age.

Prevalence increased by age, in our study positive cases aged 1-5 years were 9.7% while negative cases were 90.3%, from those who aged 6-10 years positive cases were 41.1% while negative cases were 58.9% and from those who aged 11-18 years positive cases were 52.1% while negative cases were 47.9%. Those who aged 6-10 years were 6 times risk to acquire infection than aged 1-5 years (OR=6.5, 95% CI=1.8 to 23.9) and those who aged 11-18 years were 10 times risk to acquire infection than who aged 1-5 years (OR=10.2, 95% CI=2.9 to 35.7).Table 4

So increasing age is a probable risk factors for acquiring HCV infection (p=0.001).

2- Sex.

In our participants, male positive cases were 45.1% in comparison to negative males who were 54.9%. Females positive patients were 36.7% in comparison to negative females who were 63.3%. Table 4

No statistically significance difference in the risk of acquisition of HCV was found between males and females (p=0.256).

3- Residence.

From rural areas 38% were positive patients while 62% were negative. Meanwhile those from urban areas 46.6% were positive while 53.4% were negative. Table 4

However no statistically significance difference in the risk of acquisition of HCV was found between urban and rural areas in our study (p=0.256).

4- Education.

From those who were illiterate 27.5% were positive while 72.5 were negative. And from those who were educated 45.4% were positive while 54.6% were negative. Educated child was 2 times risk to acquire HCV infection than illiterate one (OR=2.2, 95% CI=1.1 to 4.7). Table 4

Thus education is considered a probable risk factor for acquisition of HCV infection a (p=0.0 34).

5- Social standard.

Of those with low socioeconomic standard 44.9% were positive to HCV infection while 55.1% were negative, those with middle social standard 35.5% were positive while 64.5% were negative and those with high social standard none was positive and all cases were negative. Table 4

However no statistically significance difference in the risk of acquisition of HCV was found between different social standards in our study (p=0.333).

Table 4: Personal and sociodemographic risk factors .

Demographic data	Group				X^2 (P)	*OR (95% CI)*
	Negative		Positive			
	No	%	No	%		
Age (years)						
▪ 1-5	28	90.3	3	9.7	17.3 (0.001)*	1
▪ 6-10	33	58.9	23	41.1		6.5 (1.8-23.9)*
▪ 11-18	45	47.9	49	52.1		10.2 (2.9-35.7)*
Sex						
▪ Male	56	54.9	46	45.1	1.3 (0.256)	
▪ Female	50	63.3	29	36.7		
Residence						
▪ Rural	67	62.0	41	38.0	1.3 (0.249)	
▪ Urban	39	53.4	34	46.6		
Education						
▪ Illiterate	29	72.5	11	27.5	4.1 (0.034)*	2.2 (1.1-4.7)*
▪ Educated	77	54.6	64	45.4		
Social standard						
▪ Low	65	55.1	53	44.9	0.333^	
▪ Middle	40	64.5	22	35.5		
▪ High	1	100.0	0	0.0		

^: P value based on Mont Carlo exact probability * P < 0.05 (significant)

OR: odds ratio C.I: confidence interval

Major risk factors for HCV acquisition:

1- **History of previous hospital admission, including NICU.**

In our study positive cases with previous history of hospital admission were 54.1% while negative cases with previous history of hospital admission were 45.9%. Those positive cases without previous history of hospital admission were only 15.3% while negative cases without previous history of hospital admission were 84.7%. Child with previous history of hospital admission was 6 times risk for acquisition of HCV than those with negative history of hospital admission (OR=6.5, 95% CI=2.9 to 14.4).Table 5

So history of previous hospital admission including NICU was a probable risk factor for HCV acquisition (p=0.001).

2- History of intravenous access.

Positive cases with no history of intravenous access were only 10.2% while negative cases no history of intravenous access were 89.8%. Positive cases with history of intravenous access were 53% while negative cases with history of intravenous access were 47%. Those with history of intravenous access were 10 times more risk for HCV acquisition than those with negative history (OR=9.9, 95% CI=3.7 to 26.6). Table 5

Thus history of intravenous access was a probable risk factor for HCV acquisition (p=0.001).

3- History of parenteral injection.

Positive cases with no history of parenteral injection were only 10.2% while negative cases with no history of parenteral injection were 89.8%. Although positive cases with history of parenteral injection were 53% while negative cases with history of parenteral injection were 47%. Child with history of parenteral injection was 10 times more risk for HCV acquisition than that with negative history (OR=9.9, 95% CI=3.7 to 26.6). Table 5

Thus history of parenteral injection was a probable risk factor for HCV acquisition (p=0.001).

4- History of blood transfusion or any of its derivatives.

Positive cases with no history of blood transfusion were only 29% while negative cases with no history of blood transfusion were 71%. Although positive cases with history of blood transfusion were 68.4% while negative cases with history of blood transfusion were 31.6%. Those with history of blood transfusion were 5 times more risk for HCV acquisition than that with negative history (OR=5.3, 95% CI=2.7 to 10.5). Table 5

Thus history of blood transfusion or any of its derivatives was a probable risk factor for HCV acquisition (p=0.001).

5- History of albumin transfusion.

Positive cases with no history of albumin transfusion were 38% while negative cases with no history of albumin transfusion were 62%. Although positive cases with history of albumin transfusion were 100% while no body with history of albumin transfusion was negative to HCV infection. Those with history of albumin transfusion were 16 times more risk for HCV acquisition than that with negative history (OR=16.3, 95% CI=2.4 to 47.8). Table 5

Thus history of albumin transfusion was a probable risk factor for HCV acquisition (p=0.001).

6- History of circumcision.

Positive cases with no history of circumcision were 34.2% while negative cases with no history of circumcision were 65.8%. Positive cases with history of circumcision were 46.7%% while negative cases with history of circumcision were 53.3%. Those with history of circumcision were 2 times more risk for HCV acquisition than that with negative history (OR=1.7, 95% CI=0.91 to 3.1). Table 5

However, no statistically significance difference in the risk of acquisition of HCV was found between circumcised and uncircumcised child (p=0.093).

7- History of sutures.

Positive cases with no history of sutures were 38.6% while negative cases with no history of sutures were 61.4%. Positive cases with history of sutures were 73.3%% while negative cases with history of sutures were 26.7%. Those with history of sutures were 4 times more risk for HCV acquisition than that with negative history (OR=4.4, 95% CI=1.3 to 14.4). Table 5

Thus history of sutures was a probable risk factor for HCV acquisition (p=0.009).

8- History of surgical procedures and diagnostic biopsy.

Positive cases with no history of surgical procedures or diagnostic biopsy were 36.1% while negative cases with no history of surgical procedures or diagnostic biopsy were 63.9%. Positive cases with history of surgical procedures or diagnostic biopsy were 52.5% while negative cases with history

of surgical procedures or diagnostic biopsy were 47.5%. Those with history of surgical procedures or diagnostic biopsy were 2 times more risk for HCV acquisition than that with negative history (OR=1.9, 95% CI=1.1 to 3.6). Table 5

Thus history of surgical procedures or diagnostic biopsy was a probable risk factor for HCV acquisition (p=0.035).

9- History of endoscopy or urinary catheter.

Positive cases with no history of endoscopy or urinary catheter were 38.7% while negative cases with no history of endoscopy or urinary catheter were 61.3%. Positive cases with history of endoscopy or urinary catheter were 76.9% while negative cases with history of endoscopy or urinary catheter were 23.1%. Those with history of endoscopy or urinary catheter were 5 times more risk for HCV acquisition than those with negative history (OR=5.3, 95% CI=1.4 to 19.9). Table 5

Thus history of endoscopy or urinary catheter was a probable risk factor for HCV acquisition (p=0.007).

10- History of dental extraction.

Positive cases with no history of dental extraction were 40% while negative cases with no history of dental extraction were 60%. Positive cases with history of dental extraction were 45.1%% while negative cases with history of dental extraction were 54.9%. Table 5

However, no statistically significance difference in the risk of acquisition of HCV was found between child who exposed to dental extraction and those who do not.

11- History of abscess drainage.

Positive cases with no history of abscess drainage were 41.6% while negative cases with no history of abscess drainage were 58.4%. Positive cases with history of abscess drainage were 33.3%% while negative cases with history of abscess drainage were 66.7%. Table 5

However, no statistically significance difference in the risk of acquisition of HCV was found between child who exposed to abscess drainage and those who do not.

Minor risk factors for HCV acquisition:

1- History of ear piercing.

Positive cases with no history of were ear piercing 45.1% while negative cases with no history of ear piercing were 54.9%. Positive cases with history of ear piercing were 36.7%% while negative cases with history of ear piercing were 63.3%. Table 5

However, no statistically significance difference in the risk of acquisition of HCV was found between child who exposed to ear piercing and those who do not.

2- History of folk medicine.

None of the participants were exposed to folk medicine. Table 5

3- History of sharing instruments (scissors, eating intruments, hair instruments).

Positive cases with no history of sharing instruments were 33.3% while negative cases with no history of sharing instruments were 66.7%. Positive cases with history of sharing instruments were 41.7%% while negative cases with history of sharing instruments were 48.3%. Table 6

However, no statistically significance difference in the risk of acquisition of HCV was found between child with history of sharing instruments and those who do not.

4- Manicure and pedicure.

None of the participant children were exposed to Manicure and pedicure. Table 6

5- History of shaving at a barber.

Positive cases with no history of shaving at a barber were 34% while negative cases with no history of shaving at a barber were 66%. Positive cases with history of shaving at a barber were 49.4% while negative cases with history of shaving at a barber were 50.6%. Those with history of shaving at a barber were 2 times more risk for HCV acquisition than those with negative history (OR=1.9, 95% CI=1.1 to3.4). Table 6

Thus history of shaving at a barber was a probable risk factor for HCV acquisition (p=0.036).

6- History of living with HCV infected persons.

Positive cases with no history of living with HCV infected persons were 40.1% while negative cases with no history of living with HCV infected persons were 59.6%. All children who lived with HCV infected persons were HCV positive. Those with history of living with HCV infected persons were 5 times more risk for HCV acquisition than those with negative history (OR=4.8, 95% CI=1.6 to22.8). Table 6

Thus history of living with HCV infected persons was a probable risk factor for HCV acquisition (p=0.016).

7- Family history of risky patient (chronic liver disease patient, hemodialysis patient or patient on repeated blood transfusion).

Positive cases with no family history of risky patient were 40.1% while negative cases with no family history of risky patient were 59.9%. Positive cases with family history of risky patient were 46.4%% while negative cases with family history of risky patient were 53.6%. Table 6

However, no statistically significance difference in the risk of acquisition of HCV was found between child with family history of risky patient and those who do not.

Table 5: Major risk factors for HCV acquisition

Risk factors	Group				X^2 (P)	*OR (95% CI)*
	Negative		Positive			
	No	%	No	%		
History of previous hospital admission(including NICU),site						
▪ No	50	84.7	9	15.3	24.7 (0.001)*	6.5 (2.9-14.4)*
▪ Yes	56	45.9	66	54.1		
History of I.V access						
▪ No	44	89.8	5	10.2	27.1 (0.001)*	9.9 (3.7-26.6)*
▪ Yes	62	47.0	70	53.0		
History of Parenteral injection(site, route)						
▪ No	44	89.8	5	10.2	27.1 (0.001)*	9.9 (3.7-26.6)*
▪ Yes	62	47.0	70	53.0		
History of blood transfusion, its derivatives						
▪ No	88	71.0	36	29.0	24.9 (0.001)*	5.3 (2.7-10.5)*
▪ Yes	18	31.6	39	68.4		
History of albumin transfusion						
▪ No	106	62.0	65	38.0	14.9 (0.001)*	16.3 (2.4-47.8)*
▪ Yes	0	0.0	10	100.0		
Circumcision						
▪ No	50	65.8	26	34.2	2.8 (0.093)	1.7 (0.91-3.1)
▪ Yes	56	53.3	49	46.7		
History of any sutures(site, place)						
▪ No	102	61.4	64	38.6	6.8 (0.009)*	4.4 (1.3-14.4)*
▪ Yes	4	26.7	11	73.3		
History of surgical procedures(place),diagnostic biopsy						
▪ No	78	63.9	44	36.1	4.4 (0.035)*	1.9 (1.1-3.6)*
▪ Yes	28	47.5	31	52.5		
Endoscopy, urinary catheter						
▪ No	103	61.3	65	38.7	7.3 (0.007)*	5.3 (1.4-19.9)*
▪ Yes	3	23.1	10	76.9		

* P < 0.05 (significant) OR: odds ratio C.I: confidence interval

Table 5: Major risk factors for HCV acquisition (continued)

Risk factors, continued	Group				X^2 (P)
	Negative		Positive		
	No	%	No	%	
Dental extraction					
▪ No	78	60.0	52	40.0	0.39 (0.531)
▪ Yes	28	54.9	23	45.1	
Abscess drainage					
▪ No	104	58.4	74	41.6	0.774!
▪ Yes	2	66.7	1	33.3	
Ear piercing					
▪ No	56	54.9	46	45.1	1.3 (0.256)
▪ Yes	50	63.3	29	36.7	
Folk medicine					
▪ No	106	58.6	75	41.4	-
▪ Yes	0	0.0	0	0.0	

!: p value based on Fisher exact probability OR: odds ratio

C.I: confidence interval * P < 0.05 (significant)

Table 6: Minor risk factors for HCV acquisition (continued).

Risk factors, continued	Group				X^2 (P)	*OR (95% CI)*
	Negative		Positive			
	No	%	No	%		
Shared instruments(scissor, eating instruments, hair instruments)						
▪ No	4	66.7	2	33.3	0.16 (0.682)	
▪ Yes	102	58.3	73	41.7		
Manicure pedicure						
▪ No	106	58.6	75	41.4	-	
▪ Yes	0	0.0	0	0.0		
Shaving at a barber						
▪ No	62	66.0	32	34.0	4.4 (0.036)*	1.9 (1.1-3.4)*
▪ Yes	44	50.6	43	49.4		
Living with HCV infected patient, for how long						
▪ No	106	59.9	71	40.1	0.016*!	4.8 (1.6-22.8)*
▪ Yes	0	0.0	4	100.0		
Family history of risky patients						
▪ No	91	59.9	61	40.1	1.8 (0.405)	
▪ Yes	15	53.6	14	46.4		

!: p value based on Fisher exact probability

* P < 0.05 (significant)

OR: odds ratio

C.I: confidence interval

Logistic regression results for risk factors of HCV infection among children.

Logistic regression technique was adopted to identify independent predictors of HCV acquisition. Among the previous studied risk factors we found that 5 risk factors are independent predictors for HCV infection acquisition by using logistic regression technique or the multi-variant models in which we study the effect of each risk factor separately.

1- Age.

By using logistic regression technique or the multi-variant models each year increasing in the age of the child leads to increase risk of HCV infection acquisition by 1.3 times (OR=1.28, 95% CI=1.15 to 1.43, p=0.000). Table 7

2- Previous history of hospital admission.

By using logistic regression technique or the multi-variant models those with previous history of hospital admission were 4 times more risk for HCV infection acquisition than those with no history (OR=4.25, 95% CI=1.4 to 12.8, p=0.011). Table 7

3- History of blood transfusion.

By using logistic regression technique or the multi-variant models those with previous history of blood transfusion were 5 times more risk for HCV infection acquisition than those with no history (OR=4.67, 95% CI=1.97 to 11.04, p=0.000). Table 7

4- History of sutures.

By using logistic regression technique or the multi-variant models those with previous history of sutures were 4 times more risk for HCV infection acquisition than those with no history (OR=3.64, 95% CI=1.01 to 16.43, p=0.050). Table 7

5- History of invasive procedures.

By using logistic regression technique or the multi-variant models those with previous history of invasive procedures were 9 times more risk for HCV infection acquisition than those with no history (OR=8.64, 95% CI=1.92 to 38.81, p=0.005). Table 7

Table 7: Results of multiple stepwise logistic regression analysis for risk factors of HCV infection among children

Risk factor	B	S.E.	Sig.	OR	95.0% C.I for OR	
					Lower	Upper
Age	0.25	0.06	0.000*	1.28	1.15	1.43
Previous hospitalization	1.45	0.57	0.011*	4.25	1.40	12.87
Blood transfusion	1.54	0.44	0.000*	4.67	1.97	11.04
Sutures	1.29	0.77	0.050*	3.64	1.01	16.43
Invasive procedure	2.16	0.77	0.005*	8.64	1.92	38.81
Constant	-4.88	0.80	0.000	0.01		
Level significance	P<0.001*					
Classification accuracy (Model validation)	82.1%					

* P < 0.05 (significant)

SE: standard error

OR: odds ratio

C.I: confidence interval

Prevalence of HCV intra-familial co-infection .

Seroprevalence of HCV intrafamilial trasmission in our study was 46.7% (35 positive families for intrafamilial co-infection for HCV infection out of total 75 positive index families) but number of cases of intrafamilial co-infection were 41 cases, owing to occurrences of more than one case in 6 families(3 families had both mother-child co-infection and child-child co-infection, 2 families had 2 cases of child-child co-infection and 1 family had both father-child co-infection and mother-child co-infection). Thus, results yielded 17 cases (42%) positive for mother-child co-infection, 14 cases (34%) positive for child-child co-infection and 10 cases (24%) positive father-child co-infection out of 75 total index positive families. Figure 1

By using PCR prevalence of HCV intrafamilial trasmission in our study was 31% (21 positive families for intrafamilial co-infection for HCV infection out of 75 total index positive families), but number of cases of intrafamilial coinfection by PCR were 23 cases, owing to occurrence of more than one case in 2 families (1 family had both mother-child co-infection and child-child co-infecion and the other family had 2 cases of child-child co-infection). So, results were as such 12 cases (52%) for mother-child co-infection, 6 cases (26%) for child-child co-infection and 5 cases (22%) for father-child co-infection out of 75 total index positive families.

Mother was more risky for intrafamilial co-infection to HCV infection than father or sibling whether by ELISA(42%) or by PCR(52%).

By using logistic regression models mother-child co-infection had 65 times to occur in comparison to negative family for co-infection (OR=65.3, 95% CI=9.8 to 437.1), father-child co-infection had 23 times to occur in comparison to negative family for co-infection (OR=23.3, 95% CI=4 to 135) and child-child co-infection had 42 times to occur in comparison to negative family for co-infection (OR=42, 95% CI=6 to 292.4). Table 8

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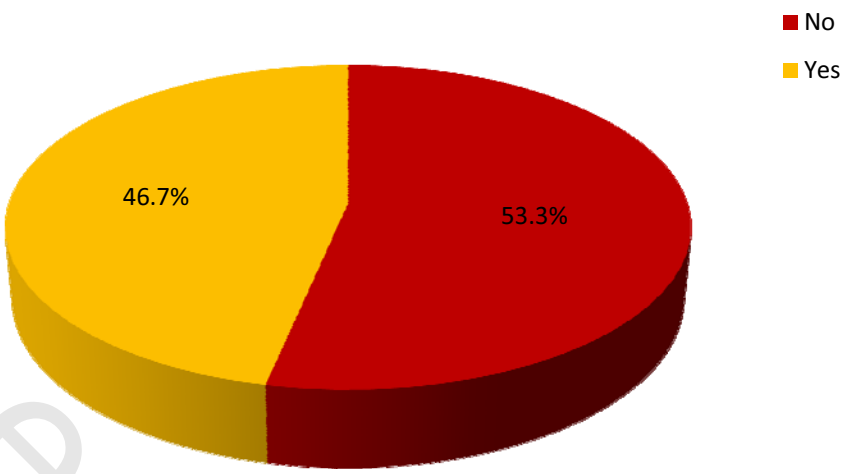


Figure 1: Family co-infection seroprevalence in families of index cases.

Table 8: Logistic Regression of distribution of intra-familial transmission in families of index cases .

Variables in the Equation				
		OR	**95.0% C.I.for EXP(B)**	
			Lower	**Upper**
Step 1	**Father--child**	23.3	4.0	135.0
	Mother--child	65.3	9.8	437.1
	Sib--sib	42.0	6.0	292.4

Relation between co-infection rate and index gender .

From families of positive intrafamilial co-infection, 45.7% were male and 54.3% were females, while from families of negative intrafamilial co-infection 75% were males and 25% were females. Thus female index case was more risky for intrafamilial co-infection than male (p= 0.002).Table 9, Figure 2

Table 9: Relation between co-infection rate and index gender .

Gender	Positive co-infection		Negative co-infection		*MCP*
	No	%	No	No	
Male	16	45.7	30	75.0	
Female	19	54.3	10	25.0	0.002*
Total	35	100	40	100	

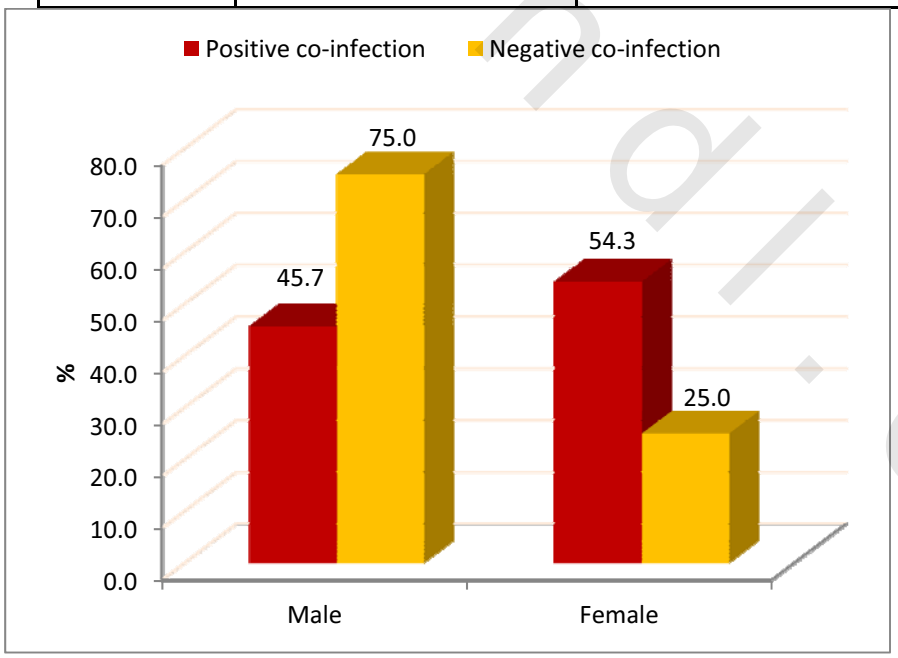
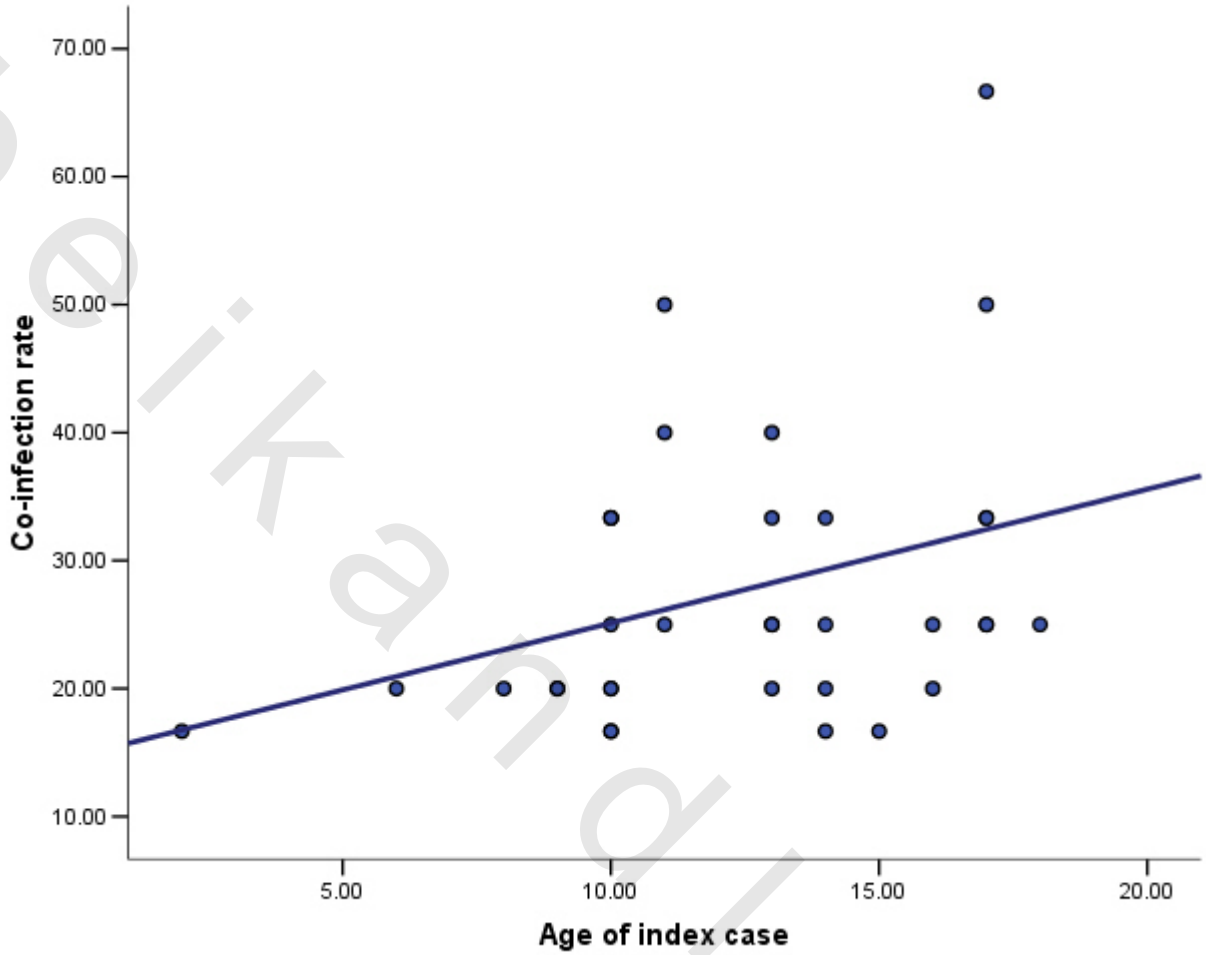


Figure 2: Relation between co-infection rate and index gender .

Relation between co-infection rate and index age .

With increasing of the age of index case, the co-infection rate was increasing. The correlation co-efficient was positive (r=0.37; P<0.05), which indicates that it is a reciprocal relation. Figure 3

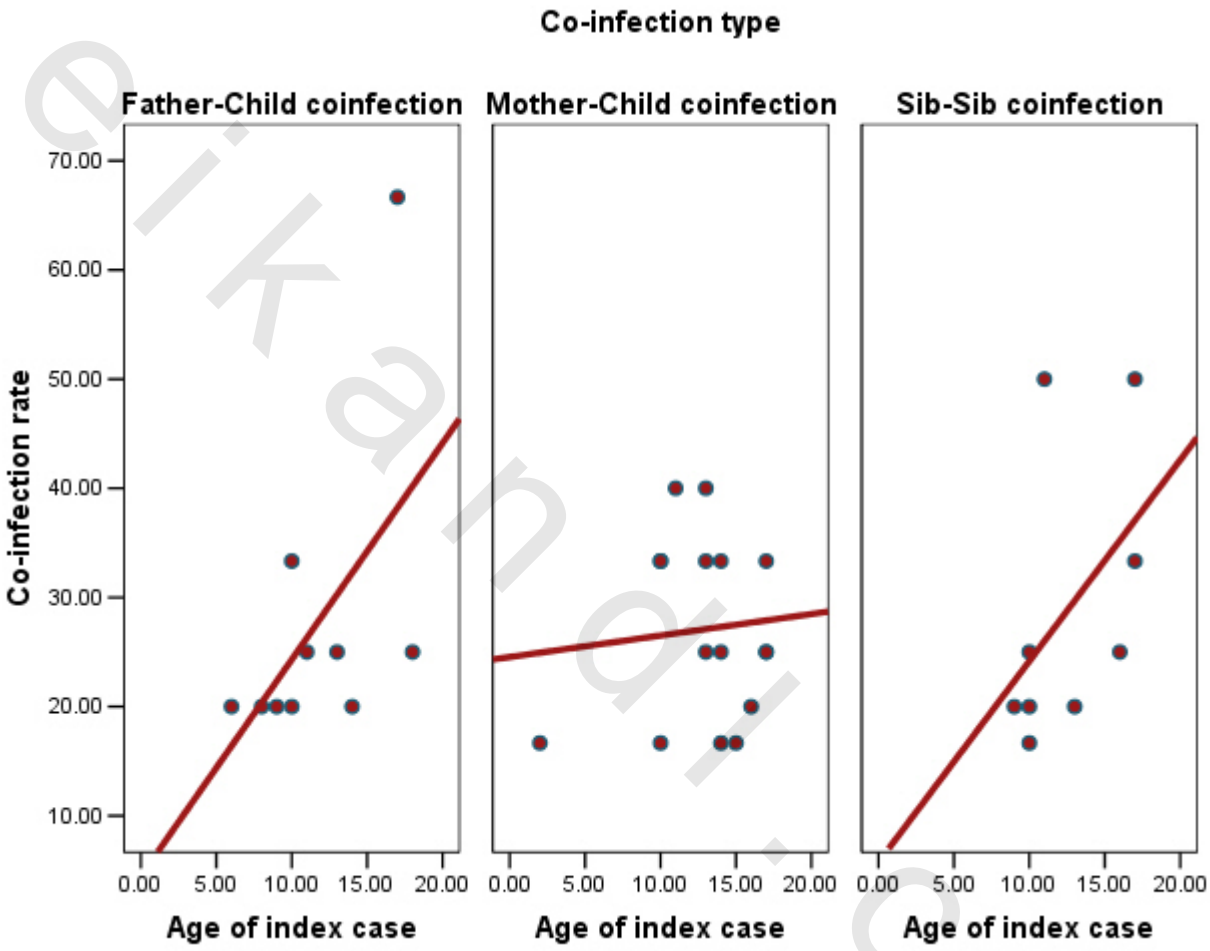


(r=0.37; P<0.05*)

Figure 3: Relation between age of index case and co-infection rate

Relation between co-infection rate and index age as regard different types of intra-familial transmission .

We studied co-infection rate in relation to index age in each type of intrafamilial transmission, where we found that in all relations there was an increase in infection rate with increasing index age (r positive in all relations), but the relation was much more evident in father-child co-infection (r=0.58; P=0.075) and sib-sib co-infection (r=47; P=202) than for mother-child co-infection (r=0.09; P=0.740).
Figure 4

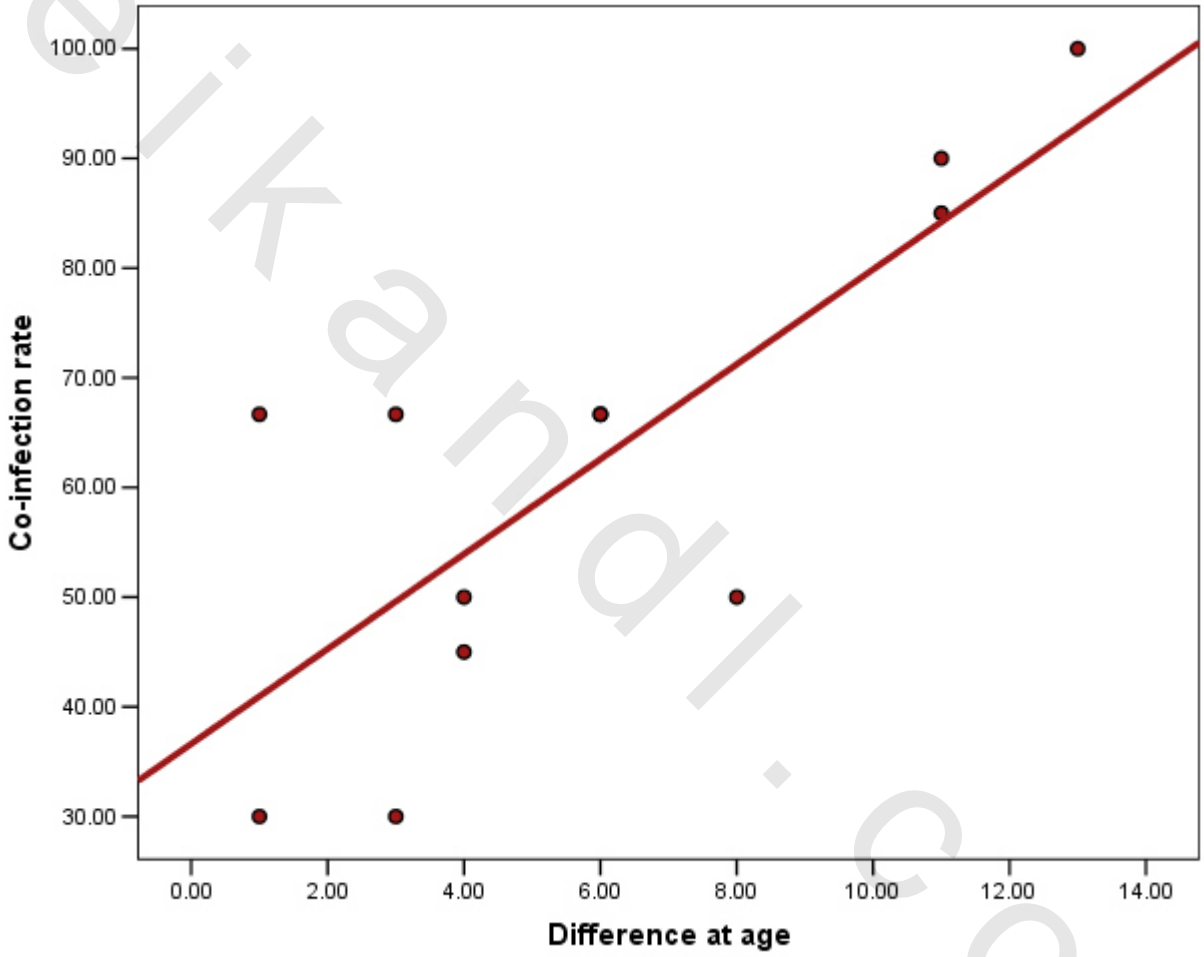


r=0.58; P=0.075 r=0.09; P=0.740 r=47;P=202

Figure 4: Relation between age of index case and co-infection type

Relation between sib-sib co-infection rate and difference in age between co-infected siblings .

With increasing difference in age between co-infected sibling, sib-sib co-infection rate was increasing. The correlation co-efficient was positive (r=0.58;P<0.05) which indicates a reciprocal relation. Figure 5 That was very evident with difference in age more than 5 years than those with difference in age less than 5 years. Those with difference in age more than 5 years had sib-sib co-infection rate 76 times (co-infection rate mean =76.39) in comparison to those with difference in age less than 5 years who had 48 times co-infection rate (co-infection rate mean =48.06).Table 10



r=0.58; P<0.05*

Figure 5: Relation between sib-sib co-infection and difference in age of the infected siblings

Table 10: Relation between sib-sib co-infection rate and difference in age of infected siblings

		Co-infection rate	
		Mean	**Standard Deviation**
Difference at age	**< 5 years**	48.06	16.48
	> 5 years	76.39	18.45

Relation between sib-sib co-infection rate and difference in age of infected siblings and numbers of siblings co-infected in the family.

The infection rate was highest with increasing age difference between infected siblings and with presence of 4 co-infected siblings within the family(r=0.74; P=0.055). Also it is high with presence of 2 co-infected siblings in the family(r=0.51; P=0.059) but not to the previous extent. Also the correlation coefficient is still positive for families with 3 co-infected siblings (r=0.37; P=0.354) but with lowest co-infection rate. Figure 6

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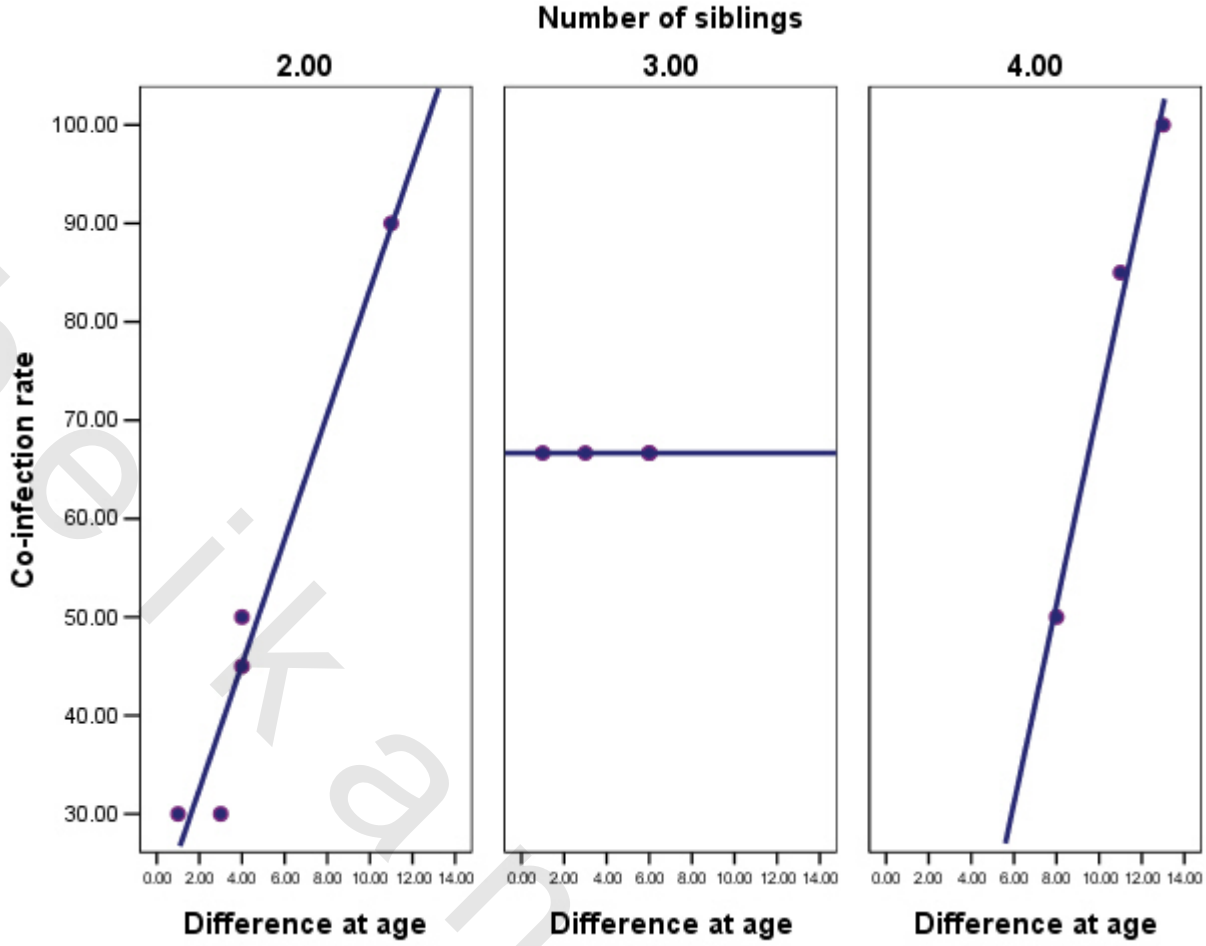


r=0.51; P=0.059 r=0.37; P=0.354 r=0.74;P=0.055

Figure 6: Relation between sib-sib co-infection rate and difference in age of the infected siblings and number of siblings co-infected in the family

Risk factors for intra-familial co-infection .

I- Contact risk factors.

1 -Contact times by hours per day.

Those with contact time <5 hours per day were 56% positive for intrafamilial co-infection while 44 % were negative for intrafamilial co-infection, those with contact time 5-6 hours per day were 35.7% positive for intrafamilial co-infection while 64.3% were negative for intrafamilial co-infection and those with contact time >7 hours per day were 50% positive for intrafamilial co-infection while 50% were negative for intrafamilial co-infection. Table 11

With no statistical significance difference was observed between the 3 groups.

2- Sleeping.

From those who sleep alone 58.3% were positive for intrafamilial co-infection while 41.7% were negative for intrafamilial co-infection, those who sleep with their siblings 42.5% were positive for intrafamilial co-infection while 57.5% were negative for intrafamilial co-infection and those who sleep with their parents 36.4% were positive for intrafamilial co-infection while 63.6% were negative for intrafamilial co-infection. Table 11

With no statistical significance difference was observed between the 3 groups.

II- Eating habits risk factors.

1- Eating together.

From those who were eating together 50% were positive for intrafamilial co-infection while 50% were negative for intrafamilial co-infection. Those who were not eating together 46.3% were positive for intrafamilial co-infection while 53.7% were negative for intrafamilial co-infection. Table 12

With no statistical significance difference was observed between who were eating together and who were not.

2- Sharing spoons.

From those who were sharing spoons 42.9%were positive for intrafamilial co-infection while 57.1% were negative for intrafamilial

co-infection. Those who were not sharing spoons 51.5% were positive for intrafamilial co-infection while 48.5% were negative for intrafamilial co-infection. Table 12

With no statistical significance difference was observed between who were sharing spoons and who were not.

3- Drinking together.

From those who were drinking together 64.3%were positive for intrafamilial co-infection while 35.7% were negative for intrafamilial co-infection. Those who were not drinking together 42.6% were positive for intrafamilial co-infection while 57.4% were negative for intrafamilial co-infection. Table 12

With no statistical significance difference was observed between who were drinking together and who were not.

III- Risky behaviors for HCV co-infection.

1- Sharing towels.

From those who were sharing towels 40.9%were positive for intrafamilial co-infection while 59.1% were negative for intrafamilial co-infection. Those who were not sharing towels 49.1% were positive for intrafamilial co-infection while 50.9% were negative for intrafamilial co-infection. Table 13

With no statistical significance difference was observed between who were sharing towels and who were not.

2- Sharing personal tools (combs, hairbrushes, toothbrushes, soap, shared cottons).

From those who were sharing personal tools 56.5%were positive for intrafamilial co-infection while 43.5% were negative for intrafamilial co-infection. Those who were not sharing personal tools 42.3% were positive for intrafamilial co-infection while 57.7% were negative for intrafamilial co-infection. Table 13

With no statistical significance difference was observed between who were sharing personal tools and who were not.

3- Sharing sharp instruments (scissors, pedicure tools)

From those who were sharing sharp instruments 44% were positive for intrafamilial co-infection while 56% were negative for intrafamilial co-infection. Those who were not sharing sharp instruments 48% were positive for intrafamilial co-infection while 52% were negative for intrafamilial co-infection. Table 13

With no statistical significance difference was observed between who were sharing sharp instruments and who were not.

4- Sharing shaving instruments.

From those who were sharing shaving instruments 70% were positive for intrafamilial co-infection while 30% were negative for intrafamilial co-infection. Those who were not sharing shaving instruments 43.1% were positive for intrafamilial co-infection while 56.9% were negative for intrafamilial co-infection. Table 13

With no statistical significance difference was observed between who were sharing shaving instruments and who were not.

IV- Direct exposure.

1- Accidental exposure to the blood or any secretions to the skin or the eye.

From those who were accidentally exposed to the blood of the infected index case 48.9%were positive for intrafamilial co-infection while 51.1% were negative for intrafamilial co-infection. Those who were not accidentally exposed to the blood of the infected index case 42.9% were positive for intrafamilial co-infection while 57.1% were negative for intrafamilial co-infection. Table 14

With no statistical significance difference was observed between who were accidentally exposed to the blood of the infected index case and who were not.

2- History of bite.

From those who were exposed to bite by the infected index case 47.4% were positive for intrafamilial co-infection while 52.6% were negative for intrafamilial co-infection. Those who were not exposed to bite by the infected index case 44.4% were positive for intrafamilial co-infection while 55.6% were negative for intrafamilial co-infection. Table 14

With no statistical significance difference was observed between who were exposed to bite by the infected index case and who were not.

Table 11: Risk factors for intrafamilial co-infection (contact risk factors)

Contact	Total	Family co-infection				X^2 (P)
		No		Yes		
		No	%	No	%	
Contact time by hours						
▪ <5	25	11	44.0%	14	56.0%	2.3 (0.313)
▪ 5-6	28	18	64.3%	10	35.7%	
▪ 7+	22	11	50.0%	11	50.0%	
Sleeping						
▪ Alone	24	10	41.7%	14	58.3%	2.1 (0.357)
▪ With sibling	40	23	57.5%	17	42.5%	
▪ With mother & father	11	7	63.6%	4	36.4%	

Table12: Risk factors for intrafamilial co-infection (eating habits risk factors).

Eating habits	Total	Family co-infection				X^2 (P)
		No		Yes		
		No	%	No	%	
Eating together						
▪ Yes	8	4	50.0%	4	50.0%	0.842!
▪ No	67	36	53.7%	31	46.3%	
Sharing spoons						
▪ No	33	16	48.5%	17	51.5%	0.56 (0.456)
▪ Yes	42	24	57.1%	18	42.9%	
Drinking together						
▪ No	61	35	57.4%	26	42.6%	2.1 (0.143)
▪ Yes	14	5	35.7%	9	64.3%	

!: p value based on Fisher exact probability

Table13: Risk factors for intrafamilial co-infection (risky behaviors for HCV co-infection).

Behaviour	Total	Family co-infection				X^2 (P)
		No		Yes		
		No	%	No	%	
Sharing towels						
▪ No	52	27	50.9%	26	49.1%	0.42 (0.520)
▪ Yes	23	13	59.1%	9	40.9%	
Sharing personal tools						
▪ No	52	30	57.7%	22	42.3%	1.3 (0.255)
▪ Yes	23	10	43.5%	13	56.5%	
Sharing sharp instruments						
▪ No	50	26	52.0%	24	48.0%	0.11 (0.743)
▪ Yes	25	14	56.0%	11	44.0%	
Sharing shaving instruments						
▪ No	65	37	56.9%	28	43.1%	0.112!
▪ Yes	10	3	30.0%	7	70.0%	

!: p value based on Fisher exact probability

Table 14: Risk factors for intrafamilial co-infection (direct exposure).

Direct exposure	Total	Family co-infection				X^2 (P)
		No		Yes		
		No	%	No	%	
Accidental exposure to others blood						
▪ No	28	16	57.1%	12	42.9%	0.26 (0.610)
▪ Yes	47	24	51.1%	23	48.9%	
Bite						
▪ No	18	10	55.6%	8	44.4%	0.05 (0.828)
▪ Yes	57	30	52.6%	27	47.4%	

!: p value based on Fisher exact probability

Logistic regression results for risk factors of HCV intrafamilial co-infection.

Logistic regression technique was adopted to identify independent predictors of HCV intrafamilial co-infection risk factors. Among the previous studied risk factors we found that, 3 risk factors were independent predictor risk factors for HCV intrafamilial co- infection by using logistic regression technique or the multi-variant models in which we study the effect of each risk factor separately.

1- Sharing drink.

By using logistic regression technique or the multi-variant models those with history of sharing drink were 4 times more risk for HCV intrafamilial co- infection than those with no history (OR=4.4, 95% CI=1.28 to 19.1, p=0.042). Table 15

Thus sharing drink was independent predictor for HCV intra-familial co-infection.

2- Sharing towels.

By using logistic regression technique or the multi-variant models those with history of Sharing personal tools were 14 times more risk for HCV intra-familial co- infection than those with no history (OR=14.2, 95% CI=1.08 to 69.7, p=0.047). Table 15

Thus sharing towels was independent predictor for HCV intra-familial co-infection.

3- Sharing shaving instruments.

By using logistic regression technique or the multi-variant models those with history of Sharing shaving instruments were 4 times more risk for HCV intra-familial co- infection than those with no history (OR=4.4, 95% CI=1.1 to 21.5, p=0.049). Table 15

Thus sharing shaving instruments was independent predictor for HCV intra-familial co-infection.

Table 15: Results of multiple stepwise logistic regressions for risk factors of interfamilial infection

Risk factor	B	SE	P	OR	95.0% C.I for OR	
					Lower	Upper
Sharing drink	1.5	0.76	0.042*	4.4	1.28	19.1
Sharing towels	1.3	0.69	0.047*	14.2	1.08	69.7
Sharing Shaving items	1.5	0.82	0.049*	4.4	1.1	21.5
Constant	-2.04	1.15	0.077	0.130		
Model significance	0.044*					
Model classification accuracy	74.6%					

* P < 0.05 (significant)

SE: standard error

OR: odds ratio

C.I: confidence interval

Results of phylogenetic analyses:

Eleven serum samples were studied. Six (6) samples only yielded an amplicon that was visualized by electrophoresis on 2.5% agarose. The Purified DNA NS5B fragments were directly sequenced on the positive strand using an ABI Prism 377 automated sequencer (Perkin Elmer) with the ABIPrism BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer).Table 16, Figure 7-12

Table 16: Results of sequencing of NS5B in six positive cases

Case 2
GGGGGAGGCA TCACCGACAG TAGGCGAGGT CTATCAGTGT TGTGACCTGG AGCCCGACGC TCGCAAGGTT
ATTGCCGCCC TCACAAACAG ACTCTATGTG GGTGGCCCCA TGCACAACAG CAAGGGAGAC CTTTGTGGGT
ATCGGAGGTG CCGCGCAAGC GGCGTCTTTA CCACCAGCTT CGGGAACACA CTGACGTGCT ATCTTAAAGC
CACGGCCGCC ACTAGGGCGG CGGGGCTGAA AGACTGCACT ATGCTGGTTT GCGGCGACGA CTTAGTCGTT
ATCGCTGAGA GCGATGGCGT GGAGGAGGAT AACCGAGCCC TCCGAGCCTT CACGGAGGCT ATGACCAGGT
ACTCCGCA
Case9
GCTCGGGGGG TTTTGACTTC AGTAGTGATG GGGTCTTCGT GTTGTGATCT GGAGCCCGAG CCCGCAGGTT ATTACCGCCC
TCACAGAAAG ACTCTACGTG GGCGGTCCCA TGCATAACAG
CAAGGGGGAC CTTTGTGGGT ATCGGAGATG CCGCGCAAGC
GGCGTCTATA CAACCAGCTT CGGAAACACA CTGACGTGCT
ATCTCAAAGC CACAGCCGCC ATCAGAGTGG CGGGGCTGAG
AGACTGCACT ATGCTGGTTT GCGGTGACGA CTTAGTCGTT
ATCGCTGAAA GCGACGGCGT GGAGGAGGAT AACCGAGCCC TCCGAGCCTT CACGGAGGCT ATGACCAGGT
ACTCCGCA
Case4
GATATCACCA GTTAAATCAA AACTGGTCTA TCAGTGTTGT GACCTGGAGC CCGACGCCCG CAAGGTTATT ACCGCCCTCA
CAAACAGACT CTATGTGGGT GGTCCCATGC ACAACAGCAA
GGGAGACCTT TGTGGGTATC GGAGATGCCG CGCAAGCGGC
GTCTTTACCA CTAGCTTCGG GAACACACTG ACGTGCTATC
TTAAAGCCAC GGCCGCTACT AGAGCGGCGG GGCTGAAAGA
CTGCACTATG CTGGTTTGCG GCGACGACTT AGTCGTTATC
GCTGAGAGCG ATGGCGTGGA GGAGGATAAC CGAGCCCTCC GAGCCTTCAC GGAGGCTATG ACCAGGTACT
CGGCA

Case 5

GAACCCTAAA AAGCGAATTT TATGCTGGTC TATCAGTGCT
GTGACCTGGA GCCCGAGGCC CGCAAGGCTA TCACCGCCCT
CACAGAGAGA CTCTACGTGG GCGGCCCCAT GCACAACAGC
AAGGGAGACC TTTGTGGGTA TCGGAGATGT CGCGCGAGCG
GCGTCTACAC CACCAGCTTC GGAAACACAC TGACGTGCTA
TCTCAAAGCT ACGGCCGCCA TTAGAGCGGC GGGGCTGAAA
GACTGCACTA TGCTGGTTTG CGGTGACGAC CTAGTCGTTA
TTGCTGAGAG CGACGGCGTG GAGGAGGACA GCCGAGCCCT
CCGAGCCTTC ACGGAGGCTA TGACCAGGTA
CTCCGCAA

Case 6

GGGTGTTTTG CTCTTCAGAT GGTGTATGCT GGTCTATCAG
TGTTGTGACC TGGAGCCCGA AGCCCGCAAG GTTATTACTG
CCCTCACGGA GAGACTCTAT GTGGGCGGCC CCATGTATAA
CAGCAAGGGA GAACTTTGTG GGTATCGGAG ATGCCGCGCA
AGTGGCGTTT ACACGACCAG CTTCGGAAAC ACGCTGACAT
GCTATCTCAA AGCCACAGCC GCTATTAGAG CGGCGGGCCT
GAGAGACTGC AGCATGCTGG TTTGCGGTGA TGACTTAGTC
GTTATCGCTG AGAGCGATGG CGTGGAGGAG GATAACCGAG
CCCTCCGAGC CTTCACGGAG GCTATGACCA
GGTACTCCGC A

Case 8

GACCTTTACA GTCTAACTCT ATGCTTGGTC TACCAGATGT
TGTGACCTGG AACCTGAAGC ACGCAAGGCC ATATCCGCCC
TCACGGAGAG ACTCTATGTG GGTGGCCCCA TGTATAACAG
CAAGGGAGAC CTATGTGGCC AGCGGAGATG CCGTGCAAGC
GGCGTCTTCA CCACCAGTTT CGGGAACACA CTGACGTGCT
ATCTTAAGGC CACGGCTGCC ACCAGGGCGG CCGGCCTAAA
AGACTGCACC ATGCTGGTCT GCGGCGACGA TTTGGTCGTC
ATCGCCGAAA GCGCTGGCAC CCAAGAGGAT GCCCAAGCCC
TCCGAGCCTT CACGGAGGCT ATGACCAGGT
ACTCCGCA

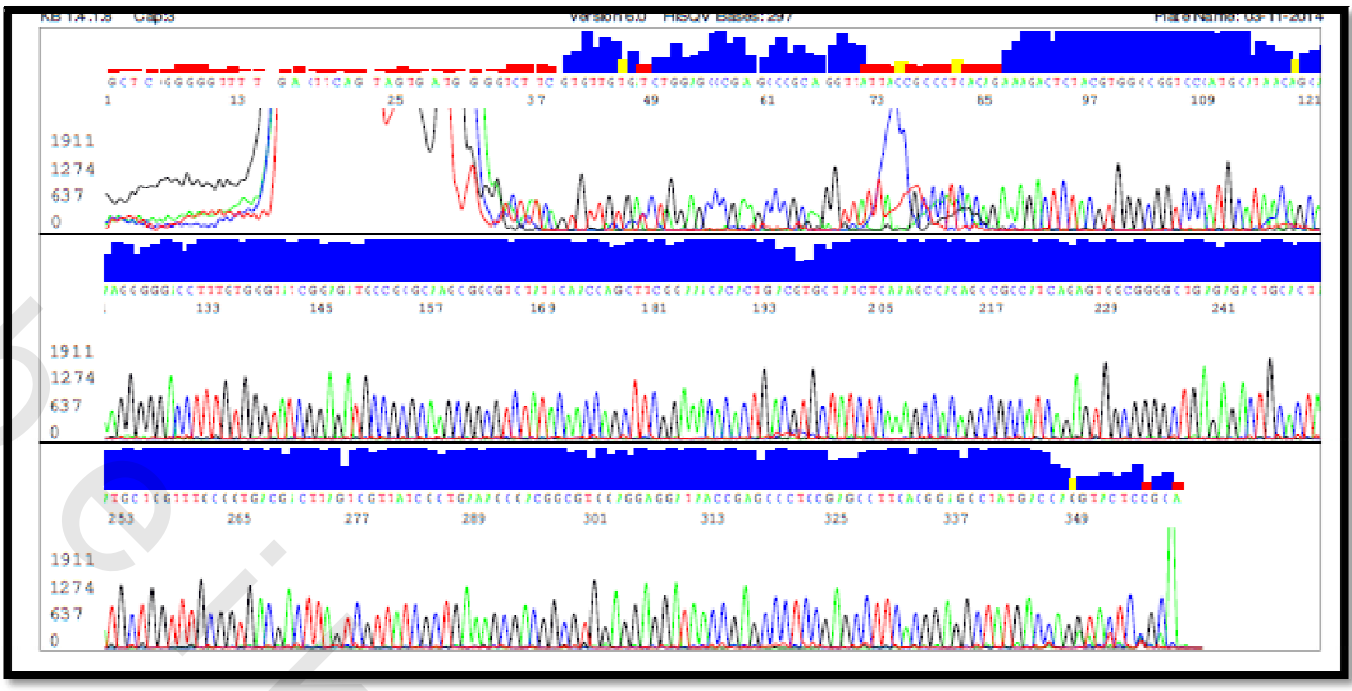


Figure 7: Results of sequencing of NS5B (Case 9)

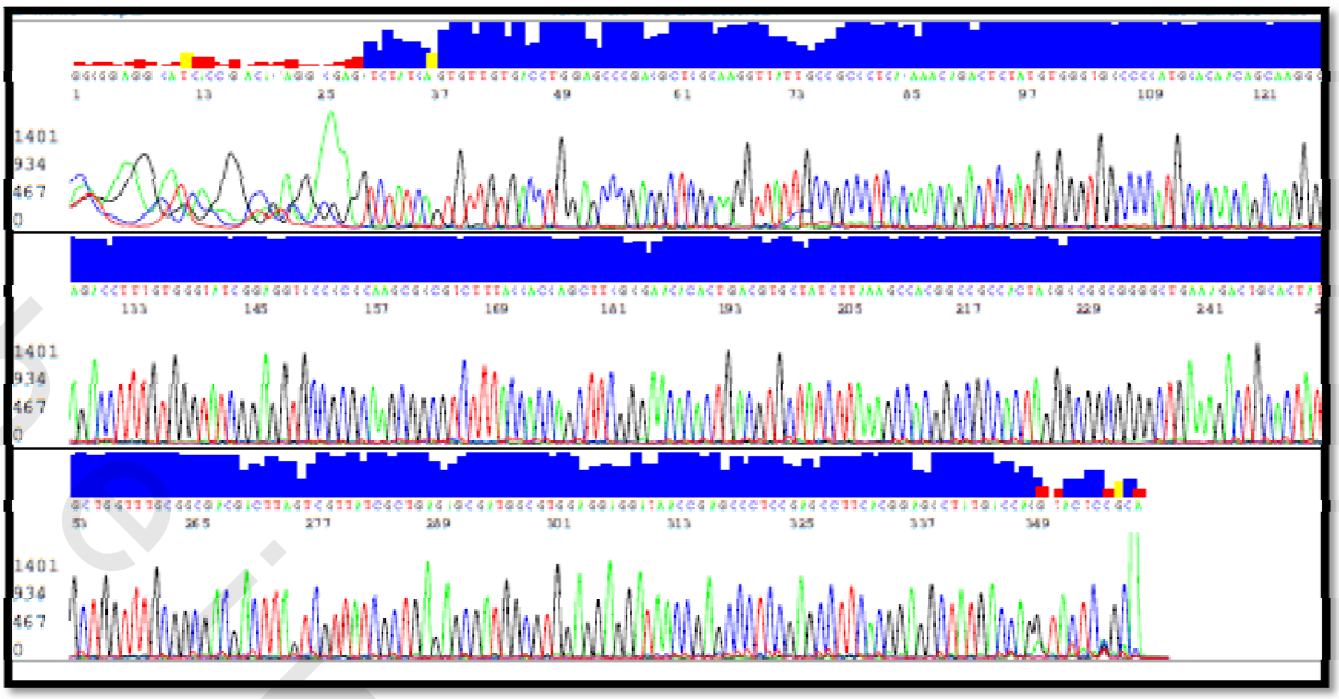


Figure 8: Results of sequencing of NS5B (Case 2)

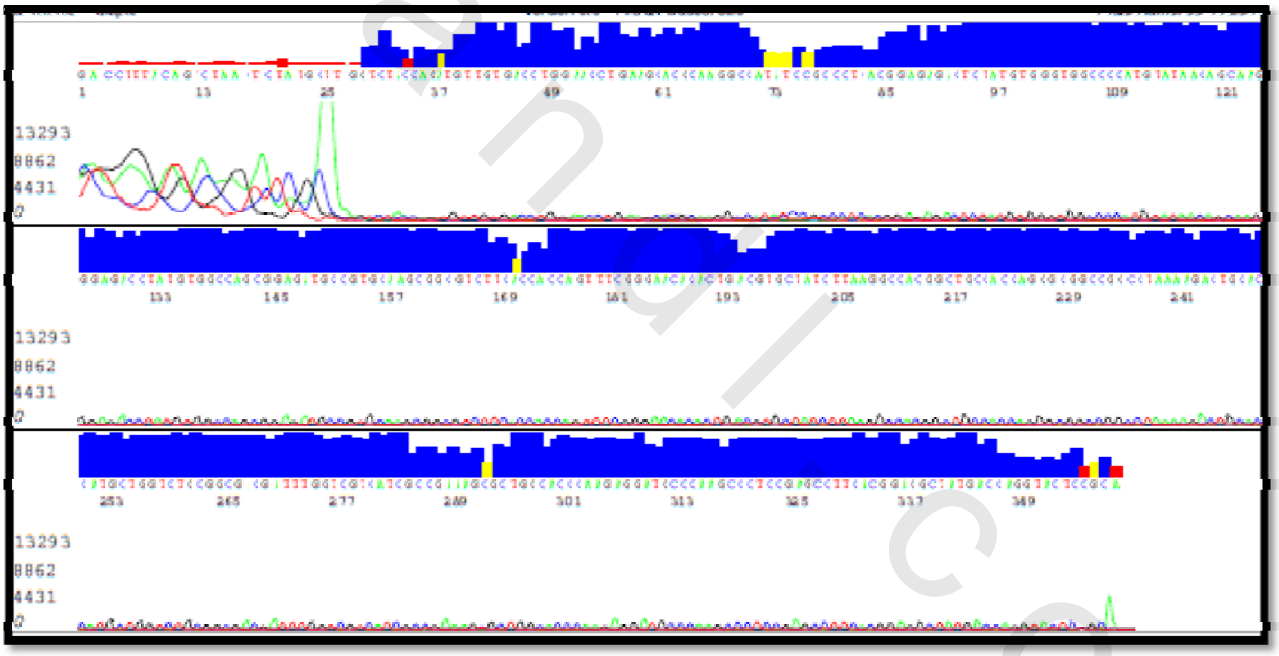


Figure 9: Results of sequencing of NS5B (Case 8)

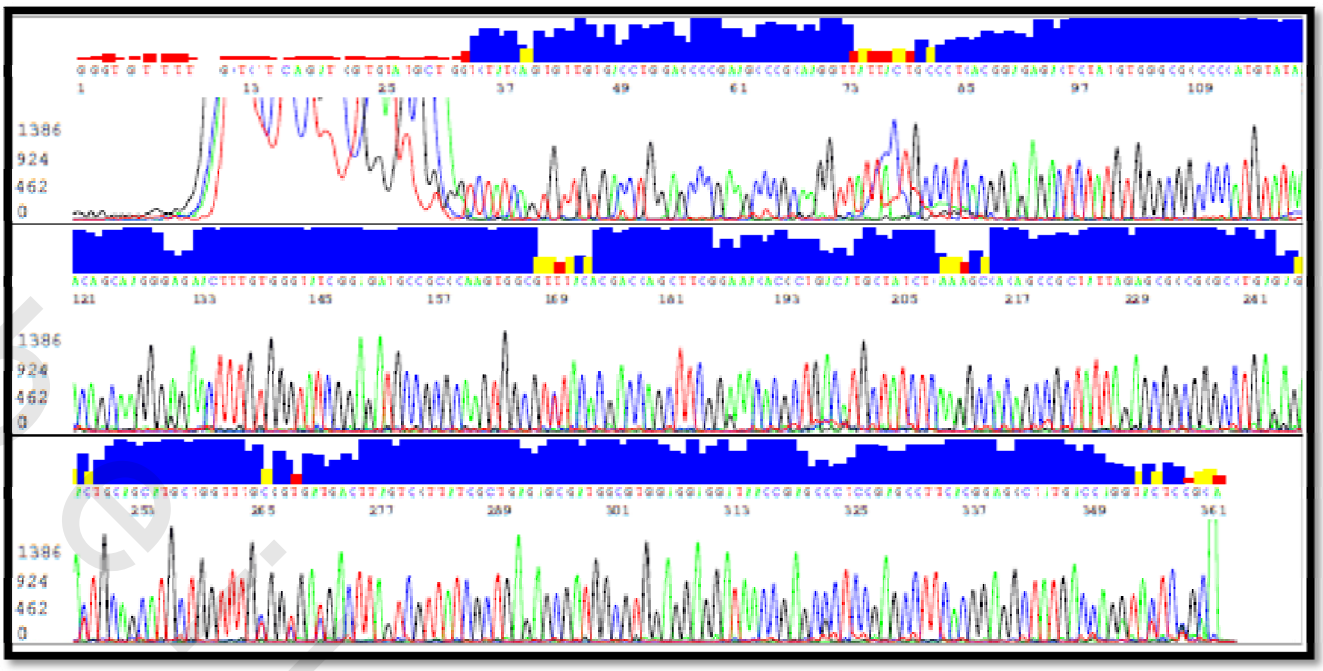


Figure 10: Results of sequencing of NS5B (Case 6)

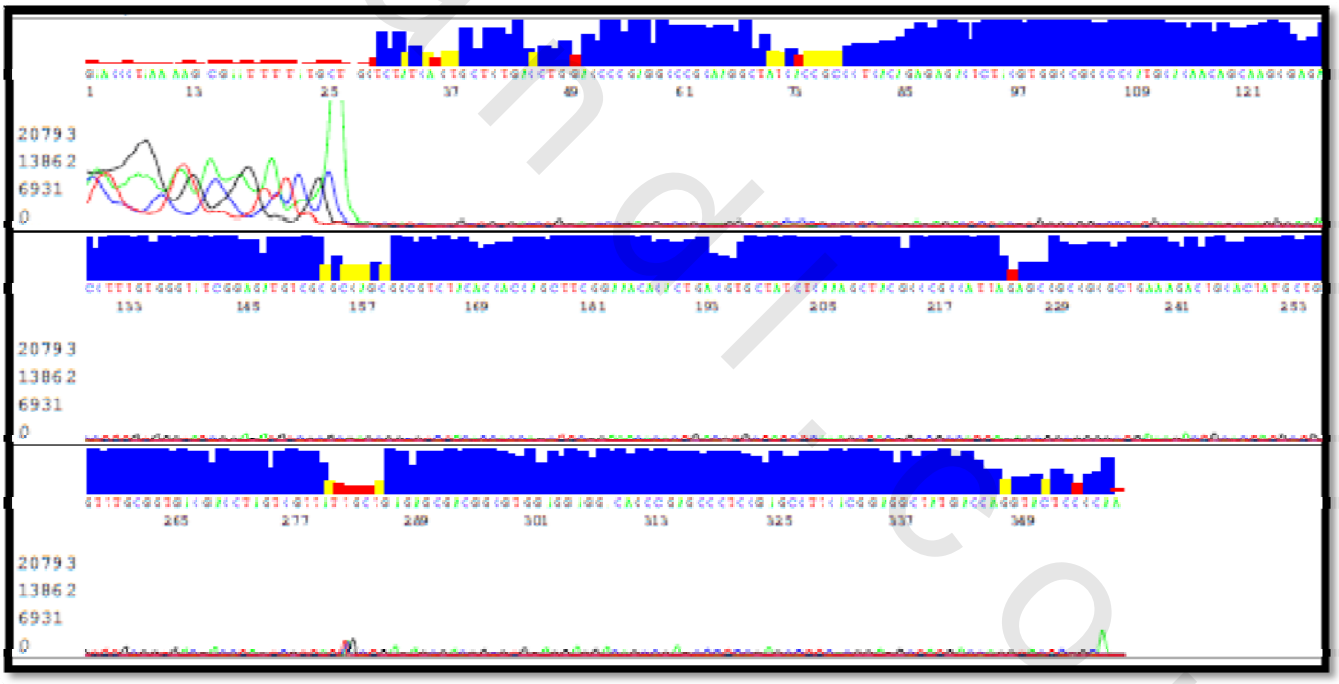


Figure 11: Results of sequencing of NS5B (Case 5)

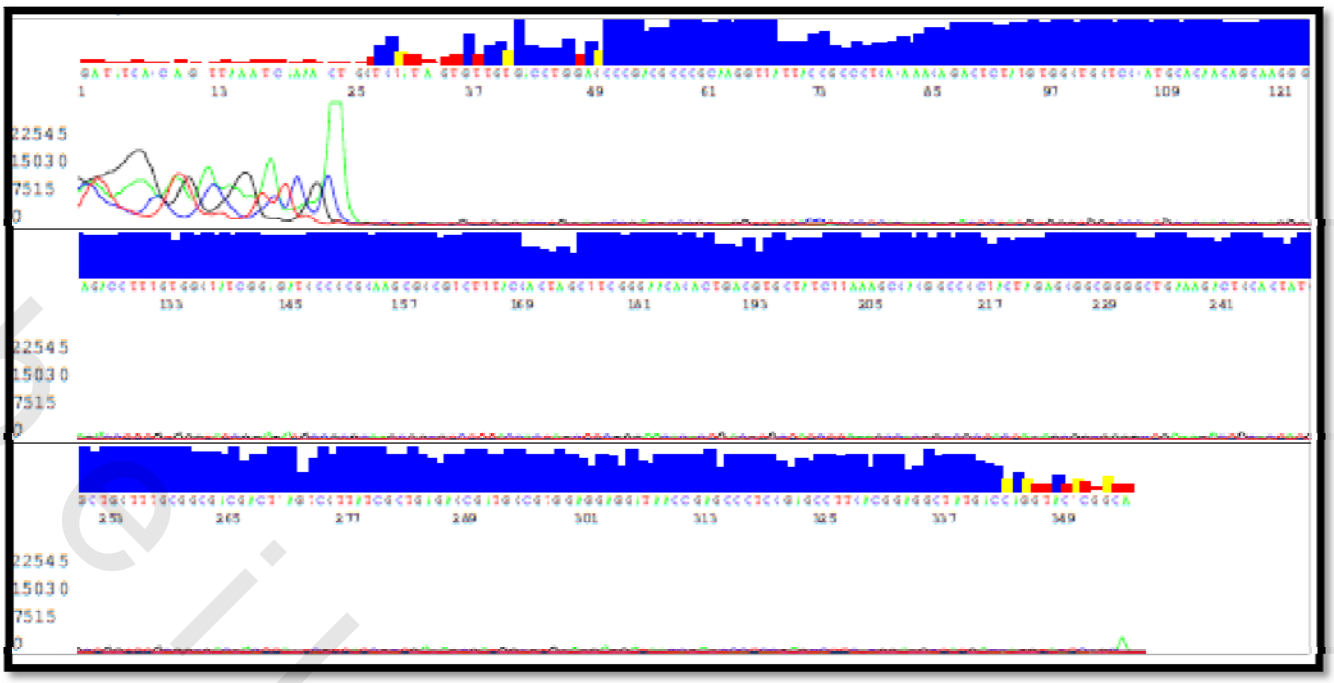


Figure 12: Results of sequencing of NS5B (Case 4)

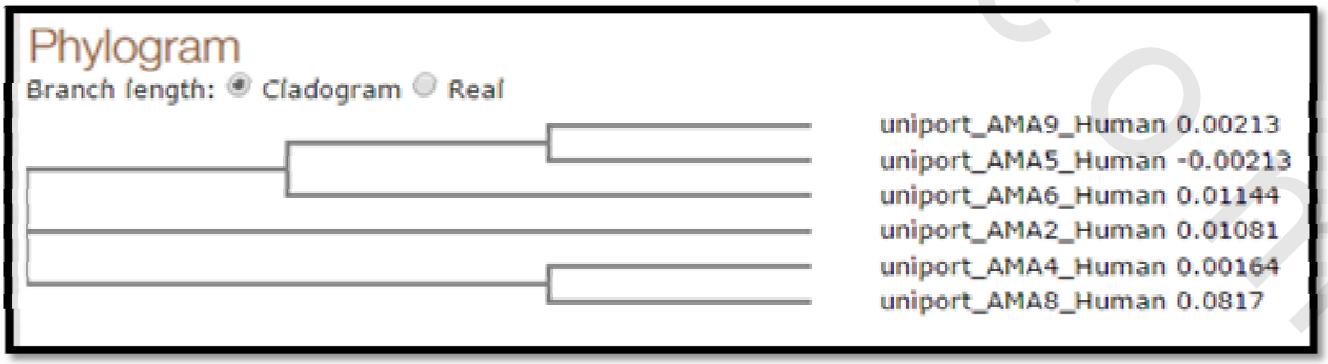
Results of phylogenetic analyses .

We studied 75 cases of chronic HCV infection with their household members. 35(38%) index with chronic HCV had at least one household member with anti-HCV antibodies and 6 (8%) had two household members with anti-HCV antibodies. 21 (28%) had at least one HCV viraemic household member and 2 (2%) had two viraemic household members. We collected samples within 1 and half year where more than half of index cases became negative for HCV PCR whether spontaneously or with treatment. 11 cases underwent PCR amplification (5 index cases and co-infected family members (2 families with mother-child, 2 families with father-child, 1 family had both sib-sib and mother-child co-infection). 5 out of 11 samples could not be sequenced which may be due to low virus titre or mismatch of the primers. BLASTx program was used first where sequence of the nucleotide was read as protein.Table 17 Viral sequences were aligned using CLUSTAL W2 software, version 1.8, the number of sequences analyzed was limited to all HCV-4 sequences that were available in the NS5B region. All trees were visualized with Tree View in which it provides a lot of information about the evolutionary relationship between a set of virus protein. Results yielded no genotype-concordant between index case and household co-infected members (the 2 sister groups with sequence homology were not belonged to the same families and the branches of co-infected family members were distant from each other).Figure 13

Table 17: Results of protein sequences of the 6 positive cases using BLASTx program

Case 2 EVYQCCDLEPEARKVIAALTERLYVGGPMHNSKGDLCGYRRCRASGVYTTS FGNTLTCYLKATAATRAAGLRDCTMLVCGDDLVVIAESDGVEEDNRALRAF TEAMTRYSA
Case 9 KVITALTERLYVGGPMHNSKGDLCGYRRCRASGVYTTSFGNTLTCYLKATA AIRAAGLRDCTMLVCGDDLVVIAESDGVEEDNRALRAFTEAMTRYSA
Case 4 VYQCCDLEPEARKVITALTERLYVGGPMHNSKGDLCGYRRCRASGVYTTSF GNTLTCYLKATAATRAAGLKDCTMLVCGDDLVVIAESDGVEEDNRALRAFT EAMTRYSA
Case 5 VYQCCDLEPEARKVITALTERLYVGGPMHNSKGDLCGYRRCRASGVYTTSF GNTLTCYLKATAAIRAAGLRDCTMLVCGDDLVVIAESDGVEEDNRALRAFTE AMTRYSA
Case 6 VYQCCDLEPEARKVITALTERLYVGGPMHNSKGELCGYRRCRASGVYTTSFG NTLTCYLKATAAIRAAGLKDCTMLVCGDDLVVIAESDGVEEDNRALRAFTE AMTRYSA
Case 8 QCCDLEPEARKVISALTERLYVGGPMYNSKGDLCRPRRCRASGVYTTSFGNT LTCYLKATAATRAAGLKDCTMLVCGDDLVVIAESAGTQEDCQALRAFTEAM TRYSA

Figure 13: Tree view of the six positive cases using CLUSTAL W2 software,



version 1.8

Results of the clinical examination and laboratory tests:

Out of the 75 index cases 64(85%) had hepatomegaly and 48(64%) had splenomegaly. ALT was increasing in 54(72%) cases out of 75 index positive cases.