DISCUSSION

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Hepatitis C virus (HCV) affects over 200 million people all over the world; about 3% of the world's population [122]. It has been estimated that HCV accounts for 27% of cirrhosis and 25% of hepatocellular carcinoma cases worldwide [3].Global and region-specific estimates of HCV prevalence vary greatly. The HCV-estimated prevalence in economically developed countries is relatively low with 1%-2% of the adult population whereas 5%-10% in less developed countries [123-125]. The countries with higher reported prevalence were located in Africa, Eastern Mediterranean, South- East Asia and the West Pacific [124,125], areas with lower prevalence included North America, Northern and Western Europe and Australia. But the highest prevalence (15– 20%) has been reported from Egypt [126]. In a previous Egyptian study accomplished among pediatric population, HCV seroprevalence was 5.8% [128].

The study was conducted on family members of 75 children with chronic HCV infection 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 also included 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.

In our study seroprevalence of HCV infection among families of index cases was 4 times in comparison to seroprevalence among families of controls (15.9% versus 3.7%). Seroprevalence among parents was as that 11% among families of index cases versus 3.2% among families of controls .While seroprevalence among children was as that 4.9% among families of index cases and 0.5% among families of controls.

The results obtained by using PCR were almost the same where prevalence among families of index cases was 3 times in comparison to prevalence among families of controls (8.6% versus 2.8%). The prevalence by using PCR was distributed as that 6.9% among parents of index cases while it was 2.8% among parents of controls and 1.7% among children of families of index cases and none of children of families of controls was positive by PCR.

The present work studied the different risk factors affecting HCV acquisition, the factors favoring HCV intrafamilial transmission, its prevalence and its corporation to the overall HCV risk of transmission.

Our study revealed that HCV prevalence increases with age. The risk of HCV acquisition increases by 1.3 times with each year increasing in the age of the patient.

The increase in HCV acquisition risk by age has been reported in several studies from Egypt [128], a study done in Egypt in 2013 revealed that prevalenc rises steeply with age, anti-HCV antibodies was detected in 2 to 7% in children under 10 years, about 10% in those 10–20 years of age, and in more than half the individuals between the age of 40 and 50 years in rural areas in the Nile Delta region[13,19,129,130]. That was consistent with many studies from other developing countries as Taiwan [131]. The rise in anti-HCV positivity with age could be explained by increasing exposure to community and health care related risk factors as age increases or it may suggest reduction in transmission in recent years [20].

Among the studied risk factors in our work neither the gender nor the residence nor the social standard affects HCV acquisition risk. That goes hand in hand with results of a previous Egyptian study among children which reported that prevalence did not differ by sex [20].However results from Egypt among adult populations highlight the effect of gender on HCV prevalence with higher prevalence observed in males and rural dwellers compared to females and individuals living in urban areas[126,132-134].

Our results as regard association of HCV infection with residence was inconsistent with results of most Egyptian studies where it yielded an association between residence in rural areas and increasing HCV prevalence among those below 19 years old (9% and 3% in the Nile Delta and in Upper Egypt, respectively for those under 19 years) [13,19]. A previous Egyptian study among pediatric population revealed a significant association between residence in rural areas and HCV seropositivity where the risk of HCV acquisition increases by 2.5 times in residence in rural areas [128].

Also a more recent Egyptian study among children stated a significant association between residence in rural areas and HCV seropositivity[20]. That was consistent with studies among adults which confirmed the overall prevalence in rural areas averaged about 20% [156], which is higher than the national average. A previous study conducted in Kalama, a village in the Nile Delta, reported HCV prevalence of 40% among village residents [135].

As regard the socioeconomic state many studies searched the relation between it and the risk of HCV acquisition. In a recent Brazilian study among adult population, the socioeconomic marker of extreme poverty (no sewage disposal) remained associated with anti-HCV prevalence after adjusting for confounding factors [136]. In a study accomplished in Egypt among adults, nearly one fifth (21.6%) of low socio-economic level were positive to HCV antibody [137]. Many previous studies from the United States indicated that lower education and poverty [138,139] are risk factors for positive anti-HCV. The same results were obtained from different countries of the

world where residing in highly deprived area [140] was a strong risk for HCV acquisition.

Surprisingly, education in our study was associated with increasing risk for HCV acquisition (p=0.0 34). That may be explained by increasing awareness of the importance of education nowadays as comparing to the previous century. Also it may be due to the age group of the index cases in our study (≤18 y) and most children of that age group attend schools.

As HCV is mainly transmitted by parenteral route, being blood transfusion and intravenous drug use are the most frequent risk factors [141]. Our study confirmed the significant association between HCV acquisition and hospital admission, history of intravenous access and parenteral injections.

We found that studied children with previous history of hospital admission were 4 times more risk for HCV infection acquisition than those with no history. That was consistent with the results of the previous studies where a substantial HCV prevalence was observed among Egyptian patients attending hospitals [156], ranging between 0% [142] and 72.8%[143].

As regard parenteral injection as an important risk factor for HCV infection in Egypt, our work confirmed its association with HCV seropositivity, where those with history of parenteral injection were 10 times more risk for HCV acquisition than those with negative history. A previous Egyptian study among children found that a history of receiving injections from informal healthcare providers was a significant risk factor for HCV infection [128].

In developing countries, HCV transmission is mainly by unsafe therapeutic injections [144]. Unsafe injections, defined as reuse of syringes or needles from patient to patient without sterilization, resulted in 2.3-4.7 million HCV infections every year approximately [145]. Transmission of HCV through contaminated injection equipment has been recognized in most developing countries [146,147]. A recent study from Germany stated that the risk of HCV transmission estimated per exposure to a contaminated syringe is 5-fold to 20-fold higher than that of HIV [148]. Recently, Paintsil et al found that, HCV survival in the syringe depends on, syringe type, time, and temperature. Infectivity could be detected for up to 63 days in high void volume tuberculin syringes [149]. The results indicated that the injection equipment were contaminated or non-disposable, which resulted in the spread of HCV. People with increased frequencies of injections for therapeutic purposes had elevated cumulative risks of HCV infection. The evidence suggested that it is important to reduce injection reuse and overuse in the prevention aspect of HCV control, especially in areas with limited disposable injection equipment and health professionals [131].

In developed countries post transfusion hepatitis C has become relatively rare. Incidence of transfusion associated hepatitis, traced from 1970 to 1998, demonstrated a decrease from 33% to nearly eliminated HCV transmission caused by the effectiveness of a series of donor screening intervention [150]. In developing countries where HCV testing in blood donation has not been feasible, receiving blood products remains a dominant source of HCV infection. Most of these countries are located in Africa and Asia, where blood safety is threatened by poverty, insufficient instruments and laboratory reagents, limited supply of trained professionals, traditional cultural barriers, and difficulties in mobilizing volunteer donors [151,152].

In a recent Egyptian study among children, they found that blood transfusion was the exposure with the highest odds ratio among the studied children [128]. Also another Egyptian study among pediatrics reported that, using multivariate logistic regression analysis, in which acquiring HCV infection is the dependent variable; blood transfusions was almost twice as common to have occurred among anti-HCV positive children than normal children[157]. This is also true in other developing countries like Pakistan where blood transfusion is one of the main risk factors for HCV acquisition [153,154]. In a previous study done among adult Brazilian population [155], they found that 28.9% of infected persons had a history of transfusion of blood and/or derivatives, with 70.9% (39/55) occurring before 1993, no discrimination was observed regarding the number of transfusion episodes, and blood transfusion were 3.9 times more frequent among the infected subjects than others.

In our work we confirmed the results of the previous studies where blood transfusion or any of its derivatives was a strong risk factor for HCV acquisition. Our study revealed that those with history of blood transfusion were 5 times more risk for HCV acquisition than that with negative history and those with history of albumin transfusion were 16 times more risk for HCV acquisition than that with negative history .

The majority of studies in multi-transfused and thalassemia patients were conducted among children. High HCV prevalence rates were observed with averages of about 42% among multi-transfused children and about 58% among children with thalassemia [156].

As regard circumcision, a widely practiced community activity in boys during the first 6 months of age, our study resulted no statistically significance difference in the risk of acquisition of HCV was found between circumcised and uncircumcised child. An explanation of our results is that most of our participants circumcised in health care facilities rather than informal health care providers. Most Egyptian studies confirmed that boys who were circumcised by informal health care providers were more likely to be infected with HCV than those circumcised by physicians or nurses

[128]. In a recent Egyptian study among adults one third (35.2%) of circumcised subjects by traditional healer were positive to HCV antibody [137].

Our study confirmed the results of previous studies as regard association of surgery and other medical procedures with increasing risk of HCV infection. In our work studied children with history of surgical procedures or diagnostic biopsy were 2 times more risky for HCV acquisition than that with negative history and also children with history of sutures were 4 times more risky for HCV acquisition than that with negative history. The previous risk is strongly associated with improper sterilization of surgical equipments. In a multivariate logistic regression analysis in a previous Egyptian study in which HCV acquisition is the dependent variable; surgical interventions seemed to be the most significant risk factor [157]. Many Egyptian community studies have found strong correlations between HCV infection and surgical procedures [19, 34, 128, 142, 158, 159]. A previous Egyptian study among children reported that, surgical intervention was almost five times a common to have occurred among anti-HCV positive children than normal children [128].

Even minor medical care procedures e.g. abscess drainage, endoscopic use whether therapeutic or diagnostic and urinary catheterization, within and beyond established health care facilities [143], were associated with increasing risk of HCV acquisition. Our studied children with history of endoscopy or urinary catheter were 5 times more risk for HCV acquisition than those with negative history. However our study did not identify abscess drainage as a risk factor for HCV acquisition.

Frequent diagnosis of HCV infection in patients without any parenteral risk factor suggests the existence of other transmission routes [52]. At respect, HCV RNA has been detected in the saliva of HCV-infected patients [160-163], and epidemiological studies proposed dental procedures are another probable risk factor. We searched the effect of dental procedures as a risk factor for HCV acquisition in our wok, however, the risk of acquisition of HCV was not found to be increased among children who exposed to dental procedures, what was consistent with most studies which have not found dental procedures to be a risk for HCV in Egypt or elsewhere [19,138,164]. An Egyptian study among adults found that HCV infection was not significantly associated with receiving of dental procedures [165]. However there is evidence that they may pose a risk [13]. A recent Egyptian study in children reported that dental procedures are a common potential risk factor for HCV infection, and in circumstances of prevalent infection and suboptimal sterile technique, they are a logical target for prevention programs [128].

The role of oral cavity fluids in the transmission of HCV is controversial. Several studies reported the presence of HCV RNA in saliva, but the infectivity of salivary HCV particles has not been confirmed. Then, there is no solid data supporting that the presence of HCV in saliva is an effective HCV transmission route [141]. In a study done in Mexico 2014 HCV RNA was detected in the saliva of 29 (64.4%) patients and was not detected in 16 (35.6%) patients [141]. Many previous studies reported the detection of HCV RNA on instruments after dental treatment in HCV infected patients [41]. In a study done in Egypt 2013 dental treatment was 16.9 times more likely to have occurred among children with chronic persistent HCV infection compared to anti-HCV negative children. This strong association with dental treatment persisted even after adjusting for all other variables including socioeconomic status, age, gender, and residence. However, although the route of infection is biologically plausible, it can still be a proxy for another unknown confounder (such as a common risk factor) shared by those who attended the dental clinic [157]. A contributing factor for transmission of HCV in saliva is the presence of periodontal disease [141], severe gingivitis [160] and salivary glands excreting the virus [166-168]. In the previous study which done in Mexico according to univariate analysis, three independent variables were associated with the detection of HCV RNA in saliva: gender, viral load and acceptable level of dental plaque [141].

Another possible form of transmission is contact with perforating/cutting objects, such as nail clippers and beard razors, in either professional (e.g., beauty salons and barber shops) [24,169-171] or domiciliary environments [172-173]. In our study history of shaving at a barber was a probable risk factor for HCV acquisition. Studied children with history of shaving at a barber were 2 times more risk for HCV acquisition than those with negative history. But none of our participant children were exposed to Manicure and pedicure.

Other possible mechanisms of *non-conventional* transmission of HCV through the percutaneous route include the practices of acupuncture [174,177,178], piercing[24], tattooing[175,176,179] and sharing cottons[131,180].In our work we found both tattooing and ear piercing were not associated with increasing risk for HCV acquisition and none of our studied children was exposed to folk medicine, a traditional maneuvers in which skin piercing takes place. That was inconsistent with the results of most studies from different countries. In a previous study done in Brazil, history of tattooing was a risk factor for HCV infection where those with history of tattoos were 8.5 times more frequent in infected subjects than others, they did not discriminate based on the locality of a practice or the number of tattoos, whereas a history of acupuncture and piercing was less frequent, demonstrating no comparative difference between the case and control groups [155].

In our study only 1 case of the 75 positive index cases was caused by vertical transmission. Although in other studies high RNA prevalence was documented among

infants of HCV positive mothers, ranging between 3.8% and 11.1% [181-184], but most studies documented that only 3%-5% of infants born to HCV-positive mothers have been infected by intrauterine or perinatal transmission. Maternal viral load, human immunodeficiency virus co-infection, prolonged rupture of membranes, fetal exposure to maternal infected blood consequent to vaginal or perineal lacerations and invasive monitoring of fetus increase the risk of viral transmission[185].

In our study, the variables found to be associated with increasing risk of hepatitis C virus infection by using the univariate analysis, tweleve risk factors, were subjected to logistic regression models, resulting in the identification of five indepandant predictors for acquisition of HCV infection which were: age, previous hospitalization, blood transfusion, sutures, invasive procedures.

In our work, as a result of multivariate logistic regression, invasive procedures, which were; history of previous hospital admission, including NICU, history of intravenous access, history of parenteral injection, history of blood transfusion or any of its derivatives, history of albumin transfusion, history of sutures, history of surgical procedures, diagnostic biopsy, dental procedures, history of endoscopy or urinary catheter and history of abscess drainage, were collectively associated with 9 times increase in HCV acquisition risk.

The results of our study was in part consistent with the results of the most recent Egyptian studies accomplished among adult population which attempted to identify the various HCV risk factors in Egypt responsible for the high incidence and prevalence rates of HCV. An Egyptian study in 2014 found that the most examined risk factors during their review analysis were surgery, transfusion, age, and hospitalization, Familial transmission, dental procedure, and schistosomiasis treatment and mother's HCV status. Other risk factors include illiteracy, number of pregnancies, gum treatment, stitches, and catheter use also associated with increasing risk [186].

In a case-control study done at another developing country as Brazil, in 2014[155] they found that an association with HCV infection was observed for the following variables: history blood transfusion, accidents with syringe and/or needle, tattoo, and past use of injectable medications with non-disposable syringes and/or needles [155]. Many other studies have found strong correlations between HCV infection and different medical exposures such as injections, blood transfusions, surgical procedures, perinatal care, and dental procedures [19, 34, 128, 142, 158, 159].

As there has been much debate regarding other HCV potential modes of transmission particularly as a substantial proportion (more than one third) of acute HCV cases do not have a defined parenteral exposure [18,187,188]. We studied intrafamilial transmission as we found that those with history of living with HCV

infected persons were 5 times more risk for HCV acquisition than those with negative history.

We conducted investigation of the intrafamilial co-infection of HCV among the HCV positive children, and their family members. Seroprevalence of HCV intrafamilial trasmission in our study was 46.7%.Where results by using PCR were only 31%. The seroprevalence was distributed as that 42% for mother-child co-infection, 34% for child-child co-infection and 24% for father-child co-infection. However results by PCR were distributed as that 52% for mother-child co-infection, 26% for child-child co-infection and 22% for father-child co-infection.

Our results as regard intrafamilial transmission were consistent with the results of many previous studies done in different countries in the world like Pakistan, New Mexico and others [189-190]. Most of these studies revealed that intrahousehold spread of HCV infections is possible[191-192].In a study done in Egypt for the family contacts of index patients, the prevalence was only 5.7%[193] . Also, in a village surveyed by the team of El-Hoseiny et al, children with infected siblings had a 20-fold increase in risk of infection [194].

Epidemiological evidence of intra-familial transmission relies on 5- to 10-fold higher prevalence of anti-HCV positivity among household contacts compared with general population [49, 76]. Possible explanations are (1) familial sharing of genes predisposing to HCV infection, (2) familial sharing of risk behaviors exposing to HCV infection, (3) intra-familial transmission, possibly sexual or domestic (ie, unapparent parenteral transmission through sharing of nail trimmers or other grooming items such as razors or toothbrushes), frequent contact of non intact skin or mucous membranes with blood-containing secretions or perhaps saliva are the most likely means of transmission, occurrences such as premastication of food and the presence of pyoderma or eczematous lesions could facilitate transmission in households[195].

We studied different risk factors accused for increasing intrafamilial transmission risk. We found that, the studied risk factors for intrafamilial co-infection, by using univariate models, which were; increasing contact times by hours, sleeping together, eating together, drinking together, sharing towels, sharing personal tools (combs, hairbrushes, toothbrushes, soap, cottons), sharing sharp instruments , sharing shaving instruments and direct exposure risk behaviors (accidental exposure to blood or any secretions from infected child to the skin or the eye and exposure to bite by infected persons were not associated with increasing risk for intrafamilial co-infection.

By using multivariate models or logistic regression technique we found that, 3 risk factors were independent predictors for HCV intrafamilial co- infection; drinking together, sharing towels and sharing shaving instruments.

Also, many previous studies searched risk factors for intrafamilial transmission. The habit of sharing perforating/cutting objects was found to be highly frequent among the studied subjects in the previous study of São Paulo which demonstrated a rate of 41% among HCV carriers and 35.2% among controls. However, their results were consistent with our results where no association with increasing HCV risk of intrafamilial transmission was found [155]. But by using multivariate models in the previous study they found that sharing of perforating/cutting objects are 1.2 times more frequent among the infected subjects than others [155].

Although accidental contact between contaminated blood and the skin or the ocular mucosa even in the absence of direct contact was not associated with increasing risk for intrafamilial transmission in our study, other studies revealed that, it is a probable risk factor for intrafmilial transmission [196-197]. In the case-control study done at State of São Paulo, Brazil, in 2014 [155], they found that those with history of contact with blood and/or secretion are 1.9 times more frequent among the infected subjects than others.

Being bitten by infected family member was considered a risk factor for HCV intrafamilal transmission, as acute hepatitis C cases had occurred in subjects bitten by HCV-infected individuals [43]. But our study revealed that being bitten by infected family member was not associated with increasing risk for intrafamilial HCV transmission.

Although sharing personal tools and grooming items was not a danger in transmission of HCV in our work, it is revealed to be a risk factor in other studies. Many Egyptian studies among adults searched the intrafamilial HCV transmission and the effects of sharing grooming items in increasing HCV risk of transmission. A previous study done in Greater Cairo, in which they examined the sequences of 100 acute hepatitis C cases and of their viraemic family members and a 262 controls in whom negative anti-HCV antibody. The previous study identified only three episodes of HCV intrafamilial transmission, the three cases of intrafamilial transmission had a history of sharing all grooming items [202].

Sharing of toothbrushes was not associated with increasing risk for intrafamilial transmission in our study which might be due to small sample size or irrelevant data. Transmission via sharing of toothbrushes is possible which was supported by the detection of HCV RNA on toothbrushes used by hepatitis C

patients[200], although it does not demonstrate the infectiousness of the corresponding virus.

Also, shared cottons was demonstrated in many studies as it was significantly associated with HCV infection independent of sharing needles and syringes [37,201] and HCV can persist as a dried sample for up to 1 week on cottons [148], but it yielded no association in our study.

By using multivariate models studied family members with history of sharing drink were 4 times more risk for HCV intrafamilial co- infection than those with no history which may be explaind by what mentioned before of that HCV RNA has been detected in the saliva of HCV-infected patients [44, 46,141].

In our study by using logistic regression technique or the multi-variate models those with history of sharing towels were14 times more risk for HCV intrafamilial co-infection than those with no history. That may be explained by detection of HCV RNA in semen [69], cervical smears [70], and saliva [44, 46,141] of infected patients; nevertheless, it remains to be proved that this RNA representing infectious HCV [71];which may contaminate the personal towels and entering skin through the skin acupuncture [144,174]. After percutaneous contact with contaminated blood or secretions, there is a risk rate of HCV infection ranging from 0% to 7%, with a mean of 1.8% [198]. Many studies searched the persistence of the virus in the environment or on any inanimate surfaces, which depends on the contact number, time, body parts, and how readily the virus is released from such surfaces. Some stated that dried virus in the presence of serum could survive for up to 5 days at room temperature or even to 3 weeks if present in suspension [199]. However, Kamili and colleagues demonstrated in a chimpanzee animal model that dried HCV derived from patient sera could survive for at least 16 hours but was not detectable after storage of 4 or 7 days [169]. Thus, HCV could remain viable for a prolonged time in the environment indicating that blood-contaminated surfaces can serve as HCV reservoirs.

Those with history of sharing shaving instruments, by logistic regression, had a 4 times more increase in the risk for HCV intrafamilial co- infection than those with no history. And sharing shaving instruments in our study was independent predictor for HCV intra-familial co-transmission which was consistent with results of many studies which showed that sharing beard razors is associated with increase HCV infection risk [169,170].

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, father-child co-infection had 23 times to occur in comparison to negative family for co-infection and child-child co-infection had 42 times to occur in comparison to negative family for co-infection.

As regard the parent-offspring correlation for HCV infection we found mother more risky for intrafamilial coinfection than fathers (65 times versus 23 times). These relations may be due to more contact time of the mothers with their infected children, more exposure to infectious blood or vomitus or other secretions of the infected child and the overall recruitments of the mothers in our study were more than fathers owing to refusal, travel or work.

We searched the effect of both the gender and the age of index case in intrafamilial co-infection rate. Female index case was more risky for intrafamilial co-infection than male. The previous relation may be owing to more caring for the boy child than girl in low socio-economic Egyptian societies. However there was a significant correlation between increasing age of index case and the co-infection rate in our study. As regard the 3 relations of intrafamilial co-infection (mother-child, father-child and sib-sib relations), co-infection rate increased in all of them but with varying degrees. The most obvious correlations affected by increasing the index age were father-child and sib-sib co-infection rate which were greater than mother-child co-infection. The exposure to various risk factors and to the intrafamilial high risk behaviors increased greatly with age which led to increase of co-infection rate. Surprisingly mother was the least to be affected by increasing age of index case in our work.

As regard a specific familial correlation which is child-child (sib-sib) in the context of a global familial study. We searched the effect of difference of the age of co-infected siblings in increasing the sib-sib co-infection rate. We found that with increasing difference in age of co-infected siblings the co-infection rate increased significantly and it was more obvious for whose difference in age 5 years or more where mean co-infection rate was 76 in comparison to 48 mean co-infection rate for siblings with less than 5 years difference in age. The previous correlation was observed in all families which had more than co-infected child, but it increased in families with 2 or 4 co-infected children than families with 3 co-infected children. These results are concordant with our previous results which revealed increasing risk of virus acquisition with increasing of age.

An Egyptian study accomplished by Arafa et al in which they studied the familial dependences in searching of intrafamilial transmission of HCV infection, they identified a significant relation between spouses, and a very strong mother-child and sib-sib correlations, in addition father-child correlation. They found that sib-sib co-infection had 9 times to occur and the sib-sib resemblance in terms of HCV serological status remained strongly significant (7 times risk) after adjustment for

mother-child and father-child HCV status. Also they explored the influence of age on estimates of sib-sib correlation. They found that the sib-sib OR was much higher when both siblings were under the age of 20 years than when both were aged 20 years or over, although it remained highly significant for the older pairs of siblings. For the younger sib pairs, the correlation was also stronger if the two siblings were born less than 5 years apart than if age difference was larger which differed than our results where co-infection rate increased with difference in age 5 years or more. In Arafa et al, for the older sib pairs, age difference had no effect on the strength of the association. So findings were consistent with a baseline sib-sib correlation, essentially independent of age, but increasing for young siblings of very similar age [194]. But the previous study did not determine the high similarity of sequences found in nine of the 29 sib-sib pairs with chronic infection whether it was due to close contacts and/or a common source of exposure. However, intrafamilial transmission of the virus could explain only part of the dependence observed among sib-sib pairs since most of the viral strains remained different between chronically infected siblings [194]. The previous observations could not be explained by a common source of exposure in a short period of time but it supported the role of host genetic factors in susceptibility/resistance to HCV infection as previously suggested by Yee [204]. The above mention relation between sib-sib co-infection could not be assessed in our study where no cases of sib-sib intrafamilial co-infection yielded results in sequencing of the NS5B gene.

We studied genetic concordance between co-infected family members in 2 families of positive family of co-infection (1 family with mother-child co-infection and the other with father-child co-infection) which yielded no genetic concordance. Although these results also support role of genetic factors in increasing risk of HCV transmission more than common source of infection, the samples were too small (about 5% of total families with positive intrafamilial co-infection) which was not conclusive. Arafa et al also studied the parent-offsprings correlation for HCV infection which was high, with very similar OR values (~3.5) for mother-child and father-child resemblance after adjustment for other risk factors (which differs to our results). In the previous study done by Arafa et al the similarity in OR values for these two relationships and the very low (0.8%) seroprevalence of HCV in children under the age of 10 years, provided strong evidence that perinatal HCV transmission plays no significant role in this settings as reported in other studies[24,55]. In the previous study presence of similar HCV strains in 18%of mother-child and father-child pairs provided further evidence of intrafamilial transmission of the virus in some cases, whereas, in others, it goes hand in hand with our results confirming that, host genetic factors controlling HCV infection[194].

Our results were in partially consistent with results of different studies done in Egypt among adults. Many recent Egyptian studies among adults searched the intrafamilial HCV transmission. The previous study which done in Greater Cairo, identified only three episodes of HCV intrafamilial transmission , as shown by (1) the

high degree of sequence homology of their viral isolates and (2) the absence of other reported risk exposures in the 1-6 months previous to symptoms onset, in a context of exhaustive assessment and precise identification of the timing of the events. The previous study documented less than 5% of all HCV transmission are caused by intra-familial transmission [202]. Meanwhile, the case control study based on the same HCV index cases showed that healthcare related exposures such as having had a catheter, an intravenous infusion, stitches or gum treatment accounted for 35% of new HCV infections. Thus, the results of the previous study was that, intra-familial transmission is of low relative importance compared to other HCV risk factors in urban Egypt, where control of nosocomial transmission of HCV should remain the priority[202].

Another cohort study performed in rural Egypt documented that two (11.7%) of 17 viraemic incident cases were infected by a viral strain identical to that of household member, suggesting that intrafamilial transmission may be limited in rural settings as well[203].

We found strong correlations in HCV seroprevalence between first-degree relatives and the infected child which can be explained by a combination of specific modes of intrafamilial viral transmission and genetic predisposition to HCV infection. The respective contribution of direct HCV transmission between relatives by close contacts or exposure to an unidentified common source of virus to the intrafamilial clustering of viral strains remains to be determined by an in depth community study. In conclusion the transmission of HCV preferably occurs via the blood route through hospital admission, intravenous access, parenteral injection, blood transfusion, albumin transfusion, sutures, surgical procedures, endoscopy and urinary catheterization. Other forms of contact with human blood and/or secretions may lead to HCV infection but likely with a lower frequency [155].

Lastly, our study and most of the previous studies tend to overestimate the proportion of HCV infections attributable to household contacts because (1) prevalence data reflect a cumulative incidence of infection over the years with no accurate relationship in time between viral exposure and the acquisition of infection, (2) of lack of exhaustive ascertainment of exposure to other potential sources of HCV, and (3)of the absence of phylogenetic analysis in all cases to confirm that anti-HCV concordant family members were infected with the same virus.

In summary, there remains considerable doubt regarding the form of virus acquisition for a significant proportion of HCV patients, a fact that can be minimized by carefully examining these patients and taking a strong epidemiological history.

SUMMARY

SUMMARY

Hepatitis C virus (HCV) affects about 3% of the world's population. The HCV-estimated prevalence in economically developed countries is relatively low with 1%-2% of the adult population whereas 5%-10% in less developed countries. The highest prevalence (15– 20%) has been reported from Egypt. In a previous Egyptian study accomplished among pediatric population, HCV seroprevalence between children was 5.8%.

Transmission of HCV preferably occurs via the blood route. Other forms of contact with human blood and/or secretions may lead to HCV infection but likely with a lower frequency. Major known modes of transmission include:

- blood transfusion or any of its derivatives
- organ transplant
- injectable treatment for schistosomiasis
- needle-stick injuries
- hospitalization
- medical and dental procedures
- injection drug use
- mother to child
- intra-familial transmission
- sharing contaminated materials like razors and shaving kits
- sharing of eating and drinking utensils.

In our work, we studied various risk factors for HCV infection among children. Also we searched the possibility of HCV intrafamilial co-infection and its contribution to HCV overall incidence.

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 clinic 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 will emphasize the following:

- Demographic data including: age, sex, the residence of the patient whether urban or rural, occupation and the socioeconomic class 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, sclerotherapy of varicose veins and endoscopy) and its site.

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

* Living with a household with HCV infection.

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

* Household practice:

- Contact time with the infected persons by hours.

- 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 food utensils as spoons or glasses, sharing towels, sharing personal tools as combs, hairbrushes, soap or sharing cottons, accidental exposure to blood or any of body fluid of infected persons and being bitten by HCV infected patients.

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 was screened for anti-HCV antibodies using ELISA test and all positive cases was confirmed by using HCV PCR.

4. In case of more than one family member were positive by HCV PCR, they were subjected to phylogenetic analyses.

The study showed that:

- Seroprevalence of HCV infection among families of index cases was 15.9% and 8.6% by using PCR.
- Seroprevalence of HCV infection among families of controls was 3.7% and 2.8% by using PCR.
- Each year increasing in the age of the child leads to increase risk of HCV infection acquisition by 1.3 times.

- Child with previous history of hospital admission was 6 times risk for acquisition of HCV than those with negative history of hospital admission.
- Those with history of intravenous access were 10 times more risk for HCV acquisition than those with negative history.
- Child with history of parenteral injection was 10 times more risk for HCV acquisition than that with negative history.
- Those with history of blood transfusion were 5 times more risk for HCV acquisition than that with negative history.
- Those with history of albumin transfusion were 16 times more risk for HCV acquisition than that with negative history.
- Those with history of sutures were 4 times more risk for HCV acquisition than that with negative history.
- Those with history of surgical procedures or diagnostic biopsy were 2 times more risk for HCV acquisition than that with negative history. And the overall risks of invasive procedures were 9 times more risk for HCV infection acquisition than those with no history.
- Those with history of endoscopy or urinary catheter were 5 times more risk for HCV acquisition than those with negative history.
- History of shaving at a barber was 2 times more risk for HCV acquisition than those with negative history.
- History of living with HCV infected persons were 5 times more risk for HCV acquisition than those with negative history.
- Serorevalence of HCV intrafamilial trasmission in our study was 46.7%, while by using PCR prevalence of HCV intrafamilial trasmission in our study was 31%.
- Mother was more risky for intrafamilial co-infection to HCV infection than father or sibling whether by ELISA or by PCR.
- By using logistic regression models mother-child co-infection had 65 times to occur in comparison to negative family for co-infection, father-child co-infection had 23 times to occur in comparison to negative family for co-infection and child-child co-infection had 42 times to occur in comparison to negative family for co-infection .
- Female index case was more risky for intrafamilial co-infection than male.
- There was a significant correlation between increasing age of index case and the co-infection rate.
- The sib-sib co-infection rate increased with increasing difference in age between infected siblings (higher if difference > 5 years).
- Sharing drinks, sharing towels and sharing shaving instruments were independent predictors for HCV intrafamilial transmission.
- Sharing drink were 4 times more risk for HCV intrafamilial co- infection than those with no history.
- Those with history of sharing towels were 14 times more risk for HCV intrafamilial co- infection than those with no history.

- Those with history of sharing shaving instruments were 4 times more risk for HCV intrafamilial co- infection than those with no history.
- Phylogenetic analysis was done on samples of 2 families with positive intra-familial co-infection which yielded no genetic concordance between co-infected family members.