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# Transfusion transmitted infections in frequently transfused thalassemic children living in Fayoum Governorate, Egypt: Current prevalence and risk factors

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### ABSTRACT

Regular blood transfusion therapy remains the primary treatment in thalassemia major (TM). Transfusion-transmitted infections (TTIs) and iron overload are considered to be the major drawbacks of this therapy. This cross-sectional study aimed to update the prevalence of the hepatitis C virus (HCV) antibody, PCR-confirmed HCV, hepatitis B surface antigen (HBsAg), and human immunodeficiency virus (HIV) antibody among TM children. Clinical and epidemiological factors that can affect HCV infection prevalence rate were studied. This study evaluated 121 children with  $\beta$ TM, including 61 males and 60 females with a mean age of  $7.99 \pm 3.57$  years. Patients were evaluated for the HCV, HBsAg, and HIV-1 & 2 antibodies. All tests were performed using ELISA. HCV positive cases were confirmed by RT-PCR. Twenty-five patients were positive for the HCV antibody (20.7%); 22 were confirmed positive by PCR. Six patients (5%) were HBsAg-positive. No patients were HIV-positive. Older age were associated with an increased frequency of HCV positive infection ( $P < 0.003$ ). More frequent transfusion,  $\geq 10$  times/year, and older age,  $\geq 10$  years, were reported as predictors of HCV infection ( $P = 0.018$  and  $0.011$ , respectively). A significant association of HCV between HBV infections was reported ( $P$  value =  $0.01$ ). There was no significant effect of the pre-transfusion or post-transfusion hemoglobin level on the frequency of HCV positive cases. HCV still represents a major health challenge for frequently transfused Egyptian patients. The prevalence rate of HBV infection remains relatively high. Therefore, it is necessary to implement measures to improve blood transfusion screening.

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### Introduction

Thalassemia is considered to be the most common monogenic disorder in the world [1]. In Egypt, the estimated carrier rate of thalassemia is 9–10%, which constitutes a major health problem [2].

Regular blood transfusion therapy remains the main line of treatment in beta-thalassemia major ( $\beta$ TM). Iron overload and transfusion-transmitted infections (TTIs) are considered to be the major drawbacks of this therapy and contribute to morbidity and mortality among patients [3]. Hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV-I/II) are the

main TTI viruses of clinical importance [4]. In developing countries where there are relatively lower blood safety standards, TTI, such as HCV, HBV and HIV, constitute a major problem [5].

Egypt has the highest hepatitis C virus (HCV) prevalence worldwide [6]. Within Egypt, Fayoum city (the city of the study) is considered to be one of the high HCV cluster cities [7]. HCV prevalence is even higher among those who have an increased risk of iatrogenic exposure, such as multi-transfused patients, thalassemia patients, and patients on hemodialysis [8]. HCV genotype 4 is the dominant HCV genotype in Egypt, which represents more than 90% of all HCV infections [9].

There is a high prevalence of HCV in Egypt, especially among the high risk stimulated Egyptian Ministry of Health (MOH), which makes it important to establish various control health strategies that are aimed at reducing the disease burden [10]. This warrants the need to investigate the current and future burden of HCV to assess the efficacy of these strategies.

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The aim of this study was to update the prevalence of HCV, HBV and HIV in thalassemia major patients with frequent transfusion and determine clinical and epidemiological factors that can affect the HCV infection prevalence rate. We aimed to detect the impending drivers of HCV transmission in TM patients.

## Methods

### Study design and setting

This cross-sectional study was performed from January 2016 to September 2016 on thalassemia major patients attending the hematology Unit of the Pediatric Department, Fayoum University Hospital (FUH), Egypt.

Fayoum is a large depression or basin in the southwest of Cairo, and Fayoum Governorate's population is 3.07 million people [11]. Most live in rural communities and work in agriculture and related industries. The average family size ranges from 4.1 to 4.5 from urban to rural communities, respectively [12].

### Inclusion criteria for this study

Patients diagnosed as  $\beta$ TM major according to their medical history, clinical picture, complete blood count (CBC) and hemoglobin electrophoresis. Patients were registered at the Hematology Unit, Pediatrics Department, and attended the unit for frequent safe packed cell transfusion. All of the studied cases had received a certified HBV vaccination, with all three doses as a compulsory vaccination consisting of recombinant Hep Vax vaccine. Out of 170 eligible registered TM patients (meeting the selection criteria), 159 were available during the study period and 121 agreed and gave verbal consent to participate in the study.

Patients were subjected to a full history evaluation (age, sex, residence, mother's and father's education), including a history of splenectomy or other previous surgical procedure (splenectomy and other surgeries), history of previous dental manipulation, and family history of HCV. Data regarding the type of chelation therapy and transfusion therapy parameters (onset, frequency and duration, pre- and post-transfusion hemoglobin level) were extracted from the patients' files and reported.

### Laboratory and virology examination

Five-milliliter venous blood samples were collected from each child participating in the study and divided into two aliquots. The first aliquot contained approximately 1 cm of blood with EDTA for complete blood count evaluation. The rest of the blood (second aliquot) was left to clot and centrifuged for 10 min at 3000 rpm to separate serum, which was divided into four Eppendorf tubes that were stored at  $-20^{\circ}\text{C}$  until analysis of serum ferritin, serum aminotransferases, HBV surface antigen (HBsAg), anti-HCV and HIV antibodies. A complete blood count estimation was performed using an Abbott Cell-Dyne 1600 (Abbott GmbH & Co., Wiesbaden-Delkenheim, Germany). The determination of serum ferritin and serum aminotransferases was performed on an Olympus autoanalyzer AU400 (Life Science, Hamburg, Germany).

Qualitative determination of HBsAg [13], anti-HCV [14] and HIV antibodies was performed using a third-generation ELISA kit [15]. All ELISA kits were supplied from Sinosource Biopharmaceutical, Inc., Sichuan, and China. Positive cases were confirmed by PCR to detect the presence of virus. Qualitative estimation of HCV-RNA in serum was detected using a nested RT-PCR assay (Amplicor HCV Monitor Test, version 2.0; Roche Diagnostics, Branchburg, NJ, USA). Interpretation of the results was performed according to manufacturer's instructions as follows: a titer of less than 10 IU/mL was

**Table 1**  
Sociodemographic characteristics of study participants.

Basal characteristics		N = 121 (%)
Sex	Female	60(49.6)
	Male	61(50.4)
Age in years	<10	78(74.5)
	$\geq 10$	43(35.5)
	Mean $\pm$ SD, Median(range)	7.99 $\pm$ 3.57 8(1–15)
Residence,	Rural	62(51.2)
	Urban	59(48.8)
Mother education	Illiterate & read write	88(72.3)
	Secondary & university education	33(27.3)
Father education	Illiterate & read write	80(66.1)
	Secondary & university education	41(33.9)

considered negative,  $10-10^2$  was repeated two months later, and more than  $10^2$  was interpreted as positive.

### Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) Version 16.0. Variables were presented using the number and percent for qualitative variables, mean, standard deviation, median, range and interquartile range for quantitative variables. Continuous and discrete variables were divided into categories based on the median reading of all of the study groups regarding this variable. A comparison between HCV-positive and HCV-negative patients were performed using the chi-square test for qualitative variables, Student's t-test for normally distributed quantitative variables and the Mann Whitney U test for not normally distributed variables. Forward stepwise logistic regression analysis was performed to assess the predictors and risk factors of HCV infection in TM patients. A P-value less than or equal to 0.05 was considered statistically significant.

### Ethical consideration

The study protocol was approved by the ethical committee of Faculty of medicine, Fayoum University. Informed oral consent was obtained from each child's parents before participation in the study.

## Results

The study enrolled 121 patients with B TM. Male sex represented nearly half of participants, 50.4%; the mean age was  $7.99 \pm 3.57$  years, which ranged from 1 to 15 years old; and the majority, 74.5%, were younger than 10 years. Rural children accounted for 51.2% of the participants. The percentages of educated mothers and fathers were 27.3% and 33.9%, respectively (Table 1).

A family history of HCV was encountered in 21 subjects (17.4%), and the median number of affected thalamic siblings 1 ranged from 0 to 10. The mean age, in months, of TM diagnosis was  $8.04 \pm 2.3$ , which ranged from 5 to 15 months. A history of dental procedure and surgery (splenectomy and others) were reported among 18 (14.9) and 43 (35.5) patients. Additionally, 33 (27.3%) patients underwent splenectomy, and most of the study group received chelation (91.7%) (Table 2).

HCV antibodies were detected in 20.7% of patients. All patients were HIV negative, and 6 (5%) were HBs Ag positive. HCV infection was confirmed by PCR in 22 out of 25 cases, representing 18.2% of all patients. By comparing HCV negative and positive cases, the mean age of confirmed cases was  $10.04 \pm 3.3$ , which was higher than that of negative cases  $7.5 \pm 3.47$ . An age  $\geq 10$  years was significantly

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