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Association of TGFB1 Gene Polymorphism with Congenital Heart Disease

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ABSTRACT

Background: One of the most prevalent congenital deformities in infants is congenital heart disease (CHD) that has high rates of morbidity and mortality. To enhance patient clinical outcomes, it is vital to investigate CHD pathophysiology. Cardiovascular illness has been linked to TGF-1 signaling disruptions.

Aim of the Study: The forecasting of CHD susceptibility in the Egyptian population is our aim.

Patients and methods: This case-control study was conducted at the Pediatric Cardiology Unit at the Zagazig Children Hospital, Faculty of Medicine, Zagazig University, with 60 CHD patients and 60 healthy controls of similar age and sex. Single nucleotide polymorphisms (SNPs) that are genotyped include: The TGFB1 rs1800471 and rs1982073 analysis were carried out using restriction fragment length polymorphism (RFLP PCR).

Results: The CT and TT genotypes of rs1982073 were significantly more prevalent in the CHD group compared to controls. The C allele was only identified in 65 (54%) of the CHD cases and the T allele in 55 (46%) of them, whereas the C allele was present in 94 (78%) of the control participants and the T allele in 26 (22%) of them. There was a significant difference between CHD cases and controls in terms of the T allele of rs1982073, which was higher in CHD cases compared to controls.

Conclusion: The current study shows a link between the TGFB1 gene variant rs1982073 and the incidence of congenital cardiac disease in Egyptian communities.

Keywords: TGFB1, Polymorphism, CHD.

INTRODUCTION

A set of structural heart defects or intrathoracic great vessels anomalies that are the result of improper cardiac development are referred to as congenital heart disease (CHD). Although surgical procedures and interventional therapies have advanced quickly over the past few decades, congenital heart disease (CHD) is still the leading non-infectious cause of infant mortality globally. Its incidence in newborns is estimated to be around 1% [(1)]. Furthermore, even after the efficient repair of cardiac irregularities, its related consequences such as heart failure, arrhythmia, and sudden cardiac death may still manifest. [(2)].

In recent years, CHD prevalence has increased among both infants and the general population. Before the age of five, only a tiny percentage of kids may recover on their own; the majority require surgery to address deformities. In general, late diagnosis of CHD leads to increased prenatal morbidity and mortality. Therefore, investigating CHD pathophysiology is essential to enhancing patient clinical outcomes. Heart defects may result from a variety of risk factors, including medication use, heavy alcohol consumption during pregnancy with measles (German) or rubella virus infection in the mother during the first trimester of pregnancy [(3,4)].

Despite substantial research, the precise cause of CHD is still mostly unknown. However, there is growing proof that genetic factors are a significant component in its development. First off, there is a larger risk of cardiac abnormalities emerging in the descendants of CHD patients than there is in the general population. Family clustering of CHD with varying morphologies is not unusual. Second, a number of genetic variations have been linked to a higher risk of CHD. Overall, our results show that CHD development and occurrence are strongly influenced by genetic predisposition to CHD [(5)].

Transforming growth factor- (TGF), a family of pluripotent cytokines that are ubiquitously produced, is connected to a wide range of physiological and pathological processes [(6)].

According to a previous study, TGF encouraged valve remodeling and differentiation throughout the development of the heart by promoting matrix organization and reducing cushion mesenchyme differentiation into cartilage cell lineage. [(7)].

TGF-1, one of TGF's isoforms, has been shown to participate in the vascular system's physiology, pathophysiology, and development, as well as the cell cycle, proliferation, differentiation, migration, maturation, and death. Numerous autoimmune, fibrotic, and cardiovascular disorders, as well as cancer, have all been linked to disruptions in TGF-1 signaling [(8)]. Additionally, it might cause organ fibrosis and malfunction when expressed too much. Chromosome 19q13.1-q13.3 contains the TGF-1 gene, which has six big introns and seven exons [(9)]. TGF1 gene polymorphisms may alter the expression and function of TGF1 protein, resulting in a variety of cardiovascular disorders [(10)].

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However, it is still unclear how CHD susceptibility and TGF-1 gene polymorphisms are related. Numerous polymorphisms in the TGF-1 gene, including rs1982073 and rs1800471, could affect the protein's expression or structure.

Aim of the Study: The forecasting of CHD susceptibility in the Egyptian population is our aim.

PATIENT AND METHODS

This case control research was conducted at Pediatric Cardiology Unit of Zagazig Children Hospital, Faculty of Medicine, Zagazig University on 60 CHD patients and 60 healthy controls of the same age and sex from January 2022 to January 2023.

Male and female CHD patients between the ages of 1 month and 12 years were chosen as the cases. Healthy volunteers who were matched for age and gender were chosen as the controls.

Exclusion criteria for the trial included patients with severe peripheral vascular disease, infection, severe kidney or liver failure, diabetes mellitus with secondary organ damage, and other systemic diseases.

All children were subjected to complete history taking, including information on age and sex, family history, drug use, consanguinity, detailed maternal history taking, which included information on maternal disease, maternal complications, mode and site of delivery, and radiation exposure, detailed clinical examinations, which included information on the heart, chest, pelvis, and abdomen, as well as the detection of dysmorphism and clinical syndrome, and radiological investigations (if applicable). Patients with CHD were confirmed with echocardiogram.

Echocardiography

According to the patient's age, an echocardiographic examination was performed in all cases when the patient was supine using Philips EPIQ cv system using S5-1 and S8-5 probe. A pediatric cardiologist with expertise in echocardiography performed the evaluation. To determine the underlying congenital heart disease and the expanded size and thickness of the right side of the right chamber (RV dilatation and hypertrophy), a two-dimensional transthoracic echocardiogram is performed.

Sample collection and DNA extraction

Each participant had a 10 ml of peripheral venous blood was drawn early in the morning and stored at -20 °C in a special collection container that contained EDTA. The Biospin Whole Blood The genomic DNA was extracted using the Genomic DNA Extraction Kit (Bioer technology CO., LTD., China).

PCR amplifications and genetic typing assay:

The PCR amplification required a total of 25 µl of 25 µl of 10 µl buffer, 2 µl of template DNA, 1 µl each of upstream and downstream primers (the primers used are listed in table 1). 0.5 µl of Taq DNA polymerase, 2 µl each of dNTP, and 13.5 µl of deionized sterile water. An initial denaturation at 94°C for 5 min was followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 65°C for 30 s, extension for 30 s at 72°C, and a final extension at 72°C for 10 min. The findings of the PCR were examined using agarose gel electrophoresis. SNPs in the TGF-1 gene were investigated using the restriction fragment length polymorphism (RFLP) method. The 25 µl combination needed for the enzyme digestion method included 0.5 µl MspAll, 2 µl 10 Buffer R, 10 µl PCR products, and 7.25 µl ddH2O. Electrophoresis on 2.5% agarose gel was used to separate the products.

Table 1. Primer sequences for TGFβ1 gene polymorphisms rs1982073 and rs1800471

SNP		Primer sequences
Rs 1982073	upstream	5′-TTCCCTCGAGGCCCTCCTA3- 3′
	downstream	5′-GCCGCAGCTTGGACAGGATC- 3′
Rs1800471	upstream	5′-GCTGC TACCGCTGCTGTGGC- 3′
	downstream	5′-TGTTGTACAGGGCGAGCACGG- 3′

Administrative design:

Ethical consideration:

A written informed consent was taken from caregivers of the patients with explanation of the procedure, possible hazards and Institutional Review Board (IRB) approval was attained (no. 9110/21/11/2021). This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The USA-based SPSS program version 18 was used to analyze the data. The quantitative data were presented as mean and standard deviation (SD) and were compared by the unpaired Student's t-test or one-way ANOVA. The qualitative data were presented as frequency and percentage and were compared by the Chi square test or Fischer exact test. At P0.05, the level of significance was indicated.

RESULTS

There was no significant difference between both groups regarding demographic data. There was significant difference between both groups regarding mode of delivery that 78% of CHD patients were delivered by CS while 55% of control group were delivered by CS and regarding DM and anemia, their percents were higher in CHD group compared to control group (Table 2).

Table 2: Baseline data of the studied groups

Variables	Control (N=60)	CHD (N=60)	P Value
Age	3.7 ± 0.6	3.6 ± 0.5	0.32
Sex **Male** **Female**	 31 (52%) 29 (48%)	 22 (37%) 38 (63%)	0.10
Mode of delivery **CS** **NVD**	 33 (55%) 27 (45%)	 47 (78%) 13 (22%)	0.007*
Maternal risk factor Hypertension DM Anemia UTI	 4 (6.7%) 1 (1.7%) 0 (0%) 0 (0%)	 7 (11.7%) 9 (15%) 4 (6.7%) 3 (5%)	 0.34 0.0080.017* 0.04*0.119 0.070.24

CS: Cesarean section, NVD: Normal vaginal delivery, DM: diabetes mellitus, UTI: Urinary tract infection, **Data are represented as mean ± SD or Number (%), *: Significant.**

Regarding history data, recurrent chest infection was the most common, while the most common clinical presentation was cough (Table 3).

Table 3: History data and clinical presentation of the studied groups

Variables	CHD (N=60)
Radiation exposure	3 (5%)
Drug therapy	12 (20%)
Consanguinity	24 (40%)
Family history	15 (25%)
NICU /PICU admission	27 (45%)
Recurrent chest infection	40 (67%)
Choking	37 (62)
Clinical presentation	
Coldness **Edema/ascites** **Dyspnea** **Cyanosis** **Cough**	3 (5%) 4 (7%) 41 (68%) 4 (7%) 47 (78%)

Data are represented as Number (%)

33% of CHD group had ASD+PFO, 25% had ASD alone, 15% had PDA alone and 15% had VSD alone (Table 4).

Table 4: Analysis/classification of the CHD group

Variables	CHD (N=60)	
	N	%
Diagnosis		
ASD alone	15	25
PDA alone	9	15
VSD alone	9	15
ASD+PFO	20	33
ASD+VSD	1	2
ASD+PDA	2	3
PDA+VSD	0	0
PDA+pFO	2	3
VSD+PFO	0	0
Tetralogy of Fallot (F4)	1	2
Aortic COA	1	2
ASD+PFO+VSD	0	0
ASD+PFO+PDA	0	0
ASD+VSD+PDA	0	0
VSD+PDA+PFO	0	0
ASD+VSD+PDA+PFO	0	0

Data are represented as Number (%)

There was no significant difference between both groups regarding genotypes and alleles of rs1800471 (Table 5).

Table 5: Genotypes and alleles distribution of rs1800471 on the studied groups

rs1800471	Control (N=60)	CHD (N=60)	Odds Ratio	95% CI	P Value
GG	**22 (37%)**	**18 (30%)**	**1**	**ref**	
GC	**25 (42%)**	**22 (37%)**	**1.07**	**0.46-2.5**	**0.99**
CC	**13 (21%)**	**20 (33%)**	**1.88**	**0.77-4.88**	**0.24**
G allele	**69 (58%)**	**58 (48%)**	**1**	**ref**	
C allele	**51 (42%)**	**62 (52%)**	**1.44**	**0.87-2.41**	**0.19**

Data are represented as Number (%)

Regarding rs1982073 genotyping in studied groups; CT and TT genotypes of rs1982073 were significantly higher in CHD group compared to controls. There was significant difference between CHD cases and controls regarding to T allele of rs1982073 that was higher in CHD cases than control (Table 6).

Table 6: Genotypes and alleles distribution of rs1982073 on the studied groups

rs1982073	Control (N=60)	CHD (N=60)	Odd Ratio	95% CI	P Value
CC	**42 (70%)**	**20 (33%)**	**1**	**ref**	
CT	**10 (17%)**	**25 (42%)**	**5.25**	**2.12-12.3**	**0.0002***
TT	**8 (13%)**	**15 (25%)**	**3.9**	**1.4-11.3**	**0.006***
C allele	**94 (78%)**	**65 (54%)**	**1**	**ref**	
T allele	**26 (22%)**	**55 (46%)**	**3.1**	**1.75-5.4**	**<0.0001***

Data are represented as Number (%), *: Significant

There was significant difference between different rs1800471 genotypes among CHD patients regarding dyspnea and cough that were more common in CC and GC than GG (Table 7).

Table 7: Demographic and clinical findings among CHD patients with different rs1800471 genotypes

rs1800471	GG (N=18)		GC (N=22)		CC (N=20)		P Value
Age	3.2 ± 0.7		2.1 ± 0.6		2.6 ± 0.5		0.5
	N	%	N	%	N	%	
Sex							
Male	6	33%	9	41%	7	35%	0.87
Female	12	67%	13	59%	13	65%	
Mode of delivery							
CS	14	78%	16	73%	17	85%	0.62
NVD	4	22%	6	27%	3	15%	
Maternal risk factor	4	22%	9	41%	10	50%	0.2
Radiation exposure	1	6%	1	4%	1	5%	0.99
Drug therapy	2	11%	3	14%	7	35%	0.12
Consanguinity	6	33%	9	41%	9	45%	0.76
Family history	4	22%	7	32%	4	20%	0.64
NICU /PICU admission	7	39%	12	55%	8	40%	0.52
Recurrent chest infection	10	55%	18	89%	12	60%	0.16
Choking	9	50%	17	77%	11	55%	0.14
Coldness	0	0%	1	4%	2	10%	0.62
Edema /ascites	0	0%	1	4%	3	15%	0.19
Dyspnea	8	44%	18	89%	15	75%	**0.03***
Cyanosis	1	6%	1	4%	2	10%	0.75
Cough	10	55%	19	86%	18	90%	**0.02***
Diagnosis							
ASD alone	6	33%	4	17%	5	25%	0.54
PDA alone	4	22%	4	17%	1	5%	0.28
VSD alone	5	28%	1	5%	3	15%	0.12
ASD+VSD	0	0	1	5%	0	0	0.4
ASD+PDA	0	0	1	5%	1	5%	0.63
PDA+pFO	0	0	1	5%	1	5%	0.64
Tetralogy of Fallot (F4)	0	0	1	5%	0	0	0.4
Aortic COA	0	0	0	0	1	5%	0.36
ASD+PFO	3	17%	9	41%	8	40%	0.2

Data are represented as mean ± SD or Number (%), *: Significant.

There was significant difference between different rs1982073 genotypes among CHD patients regarding dyspnea and cough that were more frequent in TT and CT than CC (Table 8).

Table 8: Demographic and clinical findings among CHD patients with different rs1982073 genotypes

rs1982073	CC (N=20)		CT (N=25)		TT (N=15)		P Value
Age	3.6 ± 3.5		2.07 ± 2.8		2.3 ± 2.4		0.8
	N	%	N	%	N	%	
Sex **Male** **Female**	 6 14	 30% 70%	 10 15	 40% 60%	 6 9	 40% 60%	0.75
Mode of delivery **CS** **NVD**	 16 4	 80% 20%	 19 6	 76% 24%	 12 3	 80% 20%	0.93
Maternal risk factor	5	25%	10	40%	8	53%	0.23
Radiation exposure	1	5%	1	4%	1	7%	0.93
Drug therapy	2	10%	5	20%	5	33%	0.23
Consanguinity	8	40%	9	36%	7	47%	0.8
Family history	5	25%	8	32%	2	13%	0.42
NICU /PICU admission	9	45%	12	48%	6	40%	0.88
Recurrent chest infection	12	60%	20	80%	8	53%	0.16
Choking	11	55%	17	68%	9	60%	0.66
Coldness	0	0%	1	4%	2	13%	0.19
Edema /ascites	0	0%	1	4%	3	20%	<0.05
Dyspnea	9	45%	22	88%	10	67%	**0.009***
Cyanosis	1	5%	2	8%	1	7%	0.92
Cough	11	55%	23	92%	13	87%	**0.008***
Diagnosis							
ASD alone	7	35%	4	16%	4	27%	0.34
PDA alone	5	25%	3	12%	1	6%	0.28
VSD alone	5	25%	2	8%	2	13%	0.28
ASD+VSD	0	0	1	4%	0	0	0.49
ASD+PDA	0	0	2	8%	0	0	0.24
PDA+pFO	0	0	2	8%	0	0	0.24
Tetralogy of Fallot (F4)	0	0	1	4%	0	0	0.49
Aortic COA	0	0	0	0	1	6%	0.22
ASD+PFO	3	15%	10	40%	7	48%	0.09

Data are represented as mean ± SD or Number (%), *: Significant

DISCUSSION

Our findings showed that there were no appreciable demographic differences between the two groups. This came in agreement with **Razzaghi *et al.* [11].** They included 420 children with CHD. Also, **Hassan *et al.* [12]** revealed that there were no appreciable variations in the demographic information between the patients and controls.

We demonstrated that there was a substantial difference in terms of delivery method, with 78% of CHD patients and 55% of the control group receiving their babies via CS, respectively. This came in agreement with **Bottega *et al.* [13].** They discovered that CHD patients had higher cesarean section rates than control patients. Cesarean sections were more frequently performed on CHD patients than on the general population.

Studies on normal pregnancies have shown that vaginal delivery is preferable than elective cesarean delivery in terms of the health of the mother, newborn, and future offspring [14].

Martin [15] discovered that the total cesarean delivery rate was 45% in their series between January 2004 and July 2009, which is higher than the general cesarean delivery rate of 31.8% in the United States in 2007. Despite a low rate of prenatal diagnosis, a large community-based study conducted in Sweden from 1992 to 2001 discovered that the probabilities of cesarean birth were around two times higher in CHD patients than in the general population **[16].**

In the current study, the CHD group (38%) had a higher maternal risk factor than the control group (8%). This came in agreement with **Ahmadi *et al.* [17]** who found that the history of anemia and DM were associated with an increased odd of CHD.

In the current study, 5% of the CHD group had received radiation exposure, 20% were on medication, 40% were consanguineous, 25% had a family history, 45% were admitted to the NICU/PICU, 67% had recurrent chest infections, and 62% had trouble eating. This came in agreement with **Ahmadi *et al.* [17].** In comparison to mothers in the control group, more mothers in the CHD group had a history of consanguineous marriage (32.5 vs. 18.6%, P 0.001), obesity before pregnancy (27.0% vs. 17.7%, P 0.001), and abortion history (14.6% vs. 47.0%). In comparison to the control group, more mothers in the case group were exposed to teratogens during the first trimester of pregnancy, including hair dye (9.7% vs. 4.1%, P = 0.001), canned food (17.3% vs. 5.3%, P = 0.001), detergents (21.7% vs. 10.8%, P 0.001), tobacco, alcohol, and opium (3.6% vs. 0.7%, P = 0.003).

We found that 78% of the CHD group experienced a cough, 68% had dyspnea, 7% had cyanosis and edema/ascites, and 5% had easy weariness. This came in agreement with **Askaryans and Xikmatov[18]** who discovered that the primary signs of CHD include breathing difficulties, cyanosis, syncope, underdeveloped limbs and muscles, poor eating or growth, or respiratory infections.

We demonstrated that 17% had PDA, 34% had ASD, 35% of the CHD group had PFO, and 14% had VSD. **Asghar [19]** revealed that the most common cardiac lesions in newborns were VSD followed by TGA with VSD. **Asghar [19]** showed that VSD was the commonest lesion. In agreement with our study, **Li *et al.* [20]** discovered that ventricular septal defect (VSD), secundum atrial septal defect (PDA), and patent ductus arteriosus (PDA), and multiple flaws were all present in 62.7%, 21.9%, 2.9%, and 12.6% of patients, respectively.

The genotypes and alleles of rs1800471 did not significantly differ between the two groups in the current investigation. In agreement with our study, **Shi *et al.* [21]** found that the frequency of the GC genotype for the polymorphism rs1800471 was 18.62% in the case group and 13.57% in the control group, respectively. While its C allele was more common in patients (9.31%) than in controls (6.79%). For the polymorphism rs1800471, there was no discernible change in genotype or allele frequencies between the case and control groups (P>0.05), indicating that there may not be any direct correlation between rs1800471 and CHD risk.

Lu *et al.* [22] demonstrated that for the gene rs1800471, when compared to the common GG genotype, the codominant GC genotype and the minor C allele in the dominant model both increased the risk of coronary heart disease (CHD) (OR = 1.15, 95% CI: 1.01-1.31; and OR = 1.16, 95% CI: 1.02-1.32, respectively). The CC genotype increased the risk by 1.25 times, but this was not statistically significant. Following Bonferroni correction for multiple testing, all significant correlations for rs1800469 and rs1982073 under the co-dominant and dominant models persisted. However, relationships were no longer statistically significant for rs1800471. Regarding the genotyping of rs1982073 in the examined groups, the healthy group had 42 (70%) controls with the CC genotype, while there were 10 (17%) and 8 (13%) controls with the CT and TT genotypes, respectively. 20 (33%) of the CHD group's cases had the CC genotype, whereas 15 (25%) and 25 (42) of the cases had the CT and TT genotypes. When compared to controls, the CHD group had considerably more of the CT and TT genotypes of rs1982073. In terms of the distribution of the rs1982073 allele, the C allele was present in 94 (78%) of the control subjects and the T allele in 26 (22%) of them, but the C allele was only found in 65 (54%) of the CHD cases and the T allele in 55 (46%) of them. Regarding the

T allele of rs1982073, which was greater in CHD cases than controls, there was a substantial difference between CHD cases and controls.

Shi *et al.* [21] demonstrated that the SNP rs1982073 has frequencies for the CT and TT genotypes of 41.38% and 20%, respectively than did the control group (51.43% and 23.57). The T allele was present in 40.69% of patients and 49.29 percent of controls, respectively. Additionally, there was a statistically significant difference between the two groups in the distributions of the CT genotype and T allele (P=0.021, P=0.043). All of the findings showed a clear correlation between the TGF-1 gene polymorphism rs1982073 with a decreased risk of CHD (OR = 0.521, 95%CI = 0.302-0.897; OR = 0.706, 95%CI = 0.507-0.983).

Lu *et al.* [22] demonstrated that among the included Caucasian groups, no significant variation for the ORs was found. With relation to both the CC and CC + TC vs. TT differences for rs1982073, however, there was some heterogeneity between Caucasian populations and non-Caucasian groups. For rs1982073, the TC genotype (OR = 1.18, 95% CI: 1.08-1.28) imparted a risk for CHD in the co-dominant model, whereas the CC genotype did not when compared to the typical TT genotype. According to a dominant model, having the minor C allele elevated the risk of CHD by 1.18 times. In genetic association research, it's possible that a positive association is fictitious, whilst a negative finding could be the consequence of a limited sample size.

With a heritability of 0.54, circulating TGF-1 levels are predominately genetically determined [23]. Gene expression, TGF-1 secretion, and plasma TGF-1 levels all rise in correlation with both of the minor risk alleles for CHD, rs1800469 and rs1982073 [24]. The significant LD between them may be the cause of these related observations. **Shah *et al.*** [25] found that AP1's recruitment to -509 C (the predominant non-risk allele of rs1800469), which results in transcriptional suppression of the TGFB1 gene, has only been proven in vivo and in vitro. However, more research is needed to identify the precise functional variant in this gene area. higher TGF1 levels were seen in various phases of plaque development in several histology studies, which lends support to the previously noted link between TGFB1 CHD risk alleles and higher TGF1 production [26].

Enhanced TGF-1 signaling has also been linked to increasing intima-media thickening and cartilaginous metaplasia of vascular media after vascular trauma [27].

CONCLUSION

The current study shows a link between the TGFB1 gene variant rs1982073 and the incidence of congenital cardiac disease in Egyptian communities. Therefore, increased TGF-1 signaling may contribute to the pathophysiology of congenital cardiac disease.

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