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Different outcomes of balloon atrial septostomy and the association of C677T polymorphism in MTHFR gene on TGA children

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ABSTRACT

Balloon atrial septostomy (BAS) remains an essential palliative interventional procedure in neonates with transposition of great arteries (TGA) and restrictive inter-circulatory mixing (low saturation) that does not respond to prostaglandin E1 therapy for maintaining ductal patency. The short- and intermediate-term outcomes of BAS were evaluated in patients with TGA, and the association of C677T polymorphism in the MTHFR gene was considered on these patients at Solemani Center for Heart Diseases, Dr. Jamal Ahmad Rasheed Pediatric Teaching Hospital, and Shar Hospital in Sulaymaniyah city. For this purpose, a retrospective study was used to evaluate short- and intermediate-term outcomes of BAS in 65 consecutive neonates who have been diagnosed with TGA and restrictive inter-circulatory mixing using echocardiography in three leading hospitals in Sulaymaniyah city, during the study period from June 2011 until January 2020. Also, for genetic polymorphism assessments, in addition to 65 patients, 65 healthy children were included in the study. 2ml of intravenous blood was collected and genomic DNA was purified from peripheral blood using the Blood genomic DNA extraction kit (Life Science, USA). PCR-RFLP method was used to determine the genotype of the subjects. All 65 of the neonates diagnosed with TGA were included in this study; 61 of the cases underwent the Rashkind procedure at a median age of 15 days, and four of the neonates died before the process. Prostaglandin E1 infusion was implemented on five of the cases before the procedure, three of whom remained hypoxic. All procedures were performed successfully under the echocardiography guide, but in 26 cases, the process was conducted with the aid of fluoroscopy with an improvement of mean SPO2 (52.59% ±7.84 to 86.57% ±3.39). An excellent short-term outcome was seen in 85.2% of the cases. Forty-three of the cases underwent the operation at the median age of six months. At the median age of 32 months, 60.7% of the cases had a good outcome on the follow-up. The results also showed a significant difference between the two groups in genotypes and alleles ($P<0.05$). This result confirms the relationship between C667T polymorphism of the MTHFR gene and transposition of great arteries disease. BAS is an effective palliative procedure in neonates with dextro-transposition of the great arteries (d-TGA) and can be safely performed at the bedside using echocardiography. It has an excellent short-term outcome and has a relatively good intermediate-term effect, but this depends on the later performed correction operation.

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Introduction

Transposition of the great arteries (TGA) is one of the most common and severe forms of congenital heart defects, in which the main arteries stem from morphologically inappropriate ventricles. Unpalliated TGA has a 90% mortality rate within the first year of life (1, 2) and a 30% mortality rate in the first week of life, without intervention (3). d-TGA accounts for about 5% and 7% of all congenital heart defects (4) and is regarded as one of the most congenital heart diseases, requiring intervention early in the neonatal period (3, 5).

In 1966, Rashkind and Miller performed balloon atrial septostomy (BAS) for the first time (6); it was the most critical factor influencing patients' survival with TGA (7). It remains an essential palliative interventional procedure in neonates with TGA who have severe pre-operative hypoxemia despite prostaglandin E1 infusion (5). Typically, it has been performed in cardiac catheterization laboratories

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under fluoroscopic guidance with hemodynamic monitoring (8).

TGA is a congenital heart anomaly with 0.45 cases per 1,000 live births, and the arterial switch operation (ASO) has become the treatment of choice for surgical correction of complete TGA (9). Since the first successful ASO was reported by Jatene et al. (10) in 1975, the mortality and morbidity have been reduced by variations in surgical techniques, such as that described by Lecompte et al. (1), which reduce the likelihood of pulmonary outflow obstruction. In contrast to the atrial switch procedures (Mustard and Senning operation), the ASO has the advantage of the maintenance of sinus rhythm, utilization of the left ventricle as the systemic ventricle and the mitral valve as the systemic atrioventricular valve. For patients with TGA, ventricular septal defect (VSD) and left ventricular outflow tract obstruction/ pulmonary stenosis (PS), the Rastelli operation, REV procedure and modified Nikaidoh procedure have been developed, and these procedures were observed favorable with long-term results and survival rate. Recently, many centers in South Korea reported satisfactory short-term and long-term postoperative results of complete TGA6 (1, 9, 10).

Methylenetetrahydrofolate reductase (MTHFR) increases the risk of cardiovascular disease (11). This enzyme catalyzes homocysteine to methionine through the methylation pathway (12). The most common genetic abnormality is the dysfunction of the MTHFR gene in the methylation pathway (13). The MTHFR gene is located at chromosomal position 1p36. The most common mutation in this gene occurs in exon 4 (nucleotide 677), which converts cytosine to thymine (14). The mutation also creates a site for HinfI cleavage, which replaces alanine with the amino acid at site 222 of the MTHFR enzyme structure (15). This phenomenon increases the instability of this enzyme against temperature, reduces its efficiency by about half, and increases homocysteine levels in people with low folate intake (16). The occurrence of vascular complications in patients with homocystinuria has led to the hypothesis that a slight to moderate increase in plasma homocysteine is associated with changes in the vessel wall (17).

This study aims to evaluate the short- and intermediate-term outcomes of the Rashkind balloon atrial septostomy (BAS), and to consider the association of C677T polymorphism in MTHFR on patients in the pediatric cardiology department in Sulaimani city.

Materials and methods

Patients and methods

The present study is a retrospective case study performed on 65 neonates at Sulaymaniyah Specialty Hospital for Heart Diseases and the neonatal department at Dr. Jamal Pediatric Teaching Hospital and the Shar Hospital Sulaymaniyah city, Iraq, from June 2011 to July 2020.

Transthoracic two-dimensional echocardiography with Doppler and color flow Doppler was performed on all cases, and d-TGA was diagnosed with or without septal defect or PDA (patent ductus arteriosus). After making a diagnosis, therapy began with Prostaglandin E1, at a dose of 0.05-0.1 mcg/kg/min to maintain ductal patency for five (7.7%) of the patients; the hypoxia did not improve in three of them despite a PgE1 infusion. BAS (balloon atrial septostomy) was performed on 61 (93.8%) of the patients, based on clinical hypoxia. It was confirmed that the communication between systemic and pulmonary circulations was either restricted or nonexistent by echocardiography. Four patients (6.2%) died during preparation for the procedure.

In the Cardiac Center, 26 (40%) of the cases atrial septostomy was done under echocardiography guidance and fluoroscopy guide; 11 (16.9%) of the cases were done in the Pediatric Teaching Hospital, and 24 (36.9%) were done in the neonatal intensive care unit department of Shar Hospital, under echocardiographic guidance alone. The cases were divided into two groups according to the age at which the Rashkind procedure was conducted. In the first group, the procedure was performed in the first month of life (n=35), and in the second group, the procedure was conducted after one month of age (n=26). A total of 43 cases underwent an operation at a median age of six months (0.5–12 months). The median age at the last follow-up is 32 months. Cases were followed up with at two points in time: the first month after the procedure (regarded as the short-term outcome) and one month after the procedure (considered the intermediate-term outcome). Patients with a good SPO2 and a stable hemodynamic state were regarded as a good outcome. Patients with a low SPO2 and an

unstable hemodynamic state were considered a bad outcome.

Inclusion and exclusion criteria: all included cases had TGA with either restrictive or nonexistent defects. There was no age limitation. Cases with large VSD (ventricular septal defect), ASD (atrial septal defect), and PDA were excluded.

Statistical analysis

Data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS 26) program. The quantitative variable's descriptive statistical analysis was carried out by calculating the median, mean, minimum, maximum, and standard deviations. By comparing oxygen values before and after the procedure, oxygen saturation was measured using a one-sample t-test. Bivariate correlation was used to analyze correlations between the age when the procedure was performed and the outcome. Statistical significance was achieved when $P < 0.01$.

Equipment

- Material (catheters, pressure transducers, oxymeters, needles, cannulae, introducers, dilators)
- Recording systems
- Imaging equipment
- Anesthesia equipment

Procedure technique

All procedures were conducted under continuous echocardiography monitoring, without general anesthesia, and using only intranasal midazolam sedation local anesthesia to the groin. The procedure started with positioning, using a technique invented by our department called the Angel position, in which a baby's legs are fixed together, and their hands are positioned. The procedure continues with the Seldinger technique and by inserting six French radial sheaths catheters into the femoral vein. Then through the sheath, the balloon was inserted up to RA and then across PFO, inflation was done with a screwed syringe, and septostomy was done with an inflated balloon and jerky movement twice on the septum (Figure 1). If the septum was thick, then a gradual dilatation septostomy (1mm followed by 2mm) is performed. Echocardiography is used to take a final look to assess the septostomy hole created during the procedure, the shunt, the function of the ventricles, and any related complications. In cases where fluoroscopy was used, looking for access was more comfortable using a fluoroscope.

After the procedure, all patients were observed for 48 hours to identify and manage any complications. Patients were frequently checked for bleeding within the first four hours. Two cases were kept on PGE1, as the septum was thick and the hole was unsatisfactory.

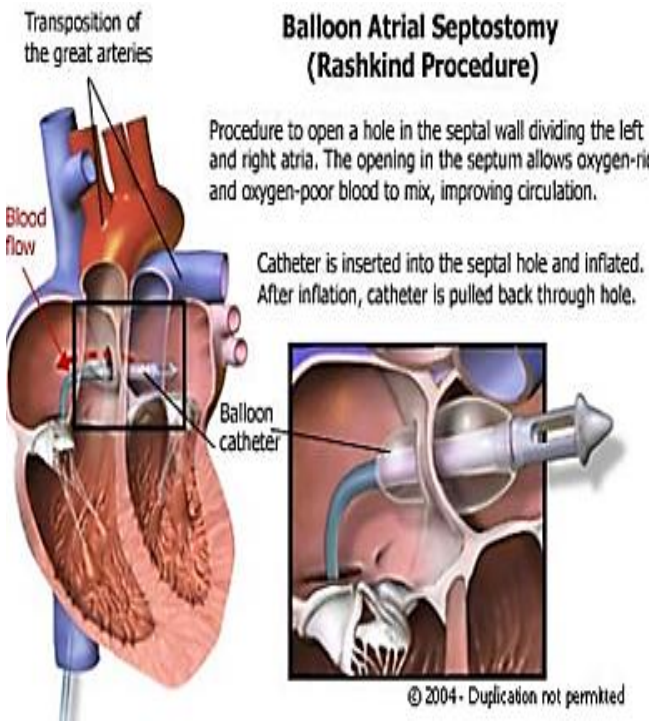

Figure 1. Rashkind- miller catheter use in atrial septostomy (18).

Genetic polymorphism assessments

In order to determine the genotype, in addition to 65 patients, 65 healthy children were included in the study for further evaluation. 2ml of intravenous blood was collected in tubes containing EDTA and stored at -4°C after transfer to the laboratory. Genomic DNA was purified from peripheral blood using the Blood genomic DNA extraction kit (Life Science, USA). PCR-RFLP method was used to determine the genotype of the subjects. Polymerase chain reaction components in a final volume of 25µl, including genomic DNA (1µl), 1µl forward primer and 1µl reverse primer, dNTP (0.5µl), Taq DNA Polymerase (0.2µl), MgCl2 (1.5µl), buffer (2.5µl), and distilled water (17.3µL). The sequences of the primers were (19):

Forward primer:
5'-TGTCTGCGGGATGAAGGAGAAGG-3'
Reveres primer:
5'-AGGACGGTGCGGTGAGAGTG-3'

BY PCR Thermal Cycler (Analytik Jena, Germany), a thermal program for primary denaturation was performed at 95°C for 5 minutes, then 28 consecutive PCR cycles including denaturation at 95°C (45 seconds), annealing at 67°C (one minute), extension at 72°C (one minute), and final extension at 72°C (5 minutes). The PCR product (198 bps) was examined by electrophoresis on 3% agarose gel and staining with ethidium bromide. Enzymatic digestion of the PCR product in a solution with a final volume of 20µl, including 10µl of PCR product, 2µl of buffer, 7µl of distilled water, and 1µl of Hinf1 enzyme at 37°C was performed at 20 h. After incubation at 65°C for 30 minutes, the digested products were electrophoresed on 3% agarose gel and stained with ethidium. The resulting fragments were 198bp C allele and 176bp mutant T allele. After determining the genotype, genotypic and allelic frequencies were calculated. Fisher's exact test was used to test the Hardy–Weinberg principle in control and patient groups because there were 5 pure mutated genotypes in these groups. This test was performed using the genetics package in R statistical software (20). A probability level of 0.05 was determined.

Results and discussion

The cases were from different cities in Iraq (63.1% from Sulaymaniyah, 10.8% from Kirkuk, 6.2% from Mosul, 6.2% from Diyala, 4.6% from Erbil, 4.6% from Duhok, 3.1% from Salahadeen, and 1.5% from Anbar). The babies' gestational age was 38.27 ± 1.37 weeks at the time of the procedure, the age of presentation was 1–48 days, and the median age was six days. Forty-eight (73.8%) were male, and 17 (26.2%) were female (Table 1).

The median age of the patients when the procedure was performed was 17 days—minimum one day and a maximum of 160 days (one case presented late and was associated with the PDA, one case at 150 days, four cases around 120 days, two cases around 90 days and all others below 60 days of age).

Sixty-five patients had TGA (with 12 [18.5%] of the cases having no associated anomaly, 24 [36.9%] PFO, six [9.2%] PDA-restrictive + PFO, five [7.7%] PDA-moderate + PFO, four [6.2%] PDA-moderate, three [4.6%] PDA-restrictive, three [4.6%] VSD-restrictive, three [4.6%] ASD-medium, two [3.1%] VSD-moderate, two [3.1%] VSD-restrictive + ASD-medium, one [1.5%] VSD-restrictive + PFO). Four (6.2%) of the cases died before undergoing the procedure, and 61 (93.8%) of the cases underwent BAS at a median age of 15 days (1–160 days). Thirty-five (57.4%) of the cases were done under the echocardiography guide, and 26 (42.6%) were done under echocardiography with the aid of a fluoroscopy guide. All were performed successfully, with saturation improving significantly from mean SPO2 (52.59% ± 7.84 to 86.57% ± 3.39) $P < 0.0001$ (Table 2)(Figure 2). The short-term outcome of the procedure was good in 52 (85.2%) of the cases; five (8.2%) of the cases were complicated by arrhythmia and were treated medically, and four (6.6%) of the cases passed within the first month of the procedure, before undergoing the correcting operation ($P < 0.0001$). Forty-three of the cases underwent the operation at a median age of six months (0.5–12 months), but the operation was not performed on 22` cases.

There was no significant difference in the outcome of the two groups, which were divided by procedure time, performed either before or after the patient had aged one month ($P > 0.01$).

The intermediate-term outcome about the short-term outcome: out of the 52 good outcome cases in the short-term follow up, six (9.84%) were not doing well, later on, 13 (21.31%) died during follow up period, and 33 (54.10%) of the cases remained in good health during follow up period. Whereas, out of the five cases with complications, four (6.54%) became well, and one (1.64%) died at the later follow-up (Table 3)(Figure 3 and Figure 4).

Finally, 37 (60.7%) of the cases had a good outcome without complications (their age at the follow-up time was between two and 84 months—median age was 32 months) ($P < 0.0001$). Six (9.8%) of the cases were not doing well, 18 (29.5%) died at the median age of two months (0.5–18 months), three (4.6%) had neurological complications, one had an intracranial hemorrhage and died one month after the procedure, and the other two patients suffered brain damage.

Table1. Gender, geographical distribution and associated anomalies predominance in TGA

Gender	Frequency	Percent
Male	48	73.8%
Female	17	26.2%
Total	65	100.0%
Geographical distribution		
Sulaimaniyah	41	63.08%
Kirkuk	7	10.77%
Diyala	4	6.15%
Mosul	4	6.15%
Duhok	3	4.62%
Erbil	3	4.62%
Salahadeen	2	3.07%
Anbar	1	1.54%
Total	65	100.00%
Co-anomaly		
PDA-mod.	4	6.2%
no associated anomaly	12	18.5%
PDA-res.+PFO	6	9.2%
PDA-res.	3	4.6%
VSD-mod.	2	3.1%
VSD-restrict.	3	4.6%
ASD-med.	3	4.6%
PFO	24	36.9%
PDA-mod.+PFO	5	7.7%
VSD-res.+PFO	1	1.5%
VSD-res.+ASD-med.	2	3.1%
Total	65	100.0%

Male: Female = 2.8:1

Table 2. variable statistics

Variables	Mean	Median	Std. Deviation	Minimum	Maximum
Gestational age/ weeks	38.27	38	1.3700	36.0	41.00
Presenting age/ days	13.79	6	14.894	1	48
Age of procedure/days	35.85	17	41.425	1	160
Operation age/ months	5.21	6	3.3249	.5	12.0
Death age/ months	4.29	2	4.2919	.5	18.0
Pre-SPO2 %	52.59	54	7.843	40	67
Post-SPO2%	86.57	87	3.398	78	92
Age at follow up/ months	36.05	32	22.976	2	84

Table 3. intermediate-term outcome in relation to short-term outcome

Short-outcome vs. intermediate-outcome			
Short outcome		Intermediate outcome	N
Good	2	Good	33
		not good	6
		Died	13
Complication		Good	4
		Died	1
Passed		Died	4
Total	1	Good	37
		not good	6
		Died	18
		Total	61

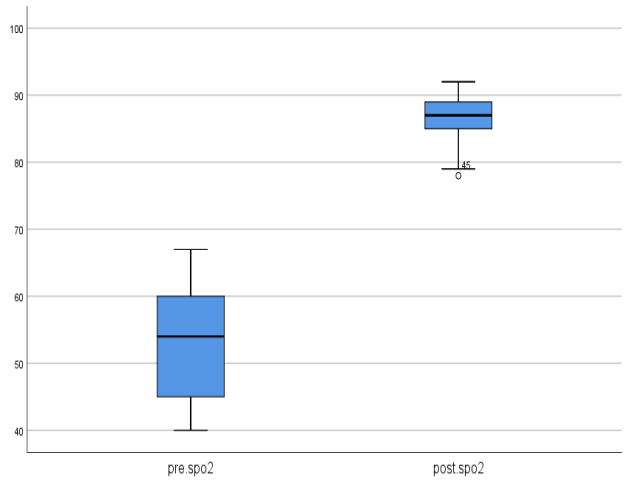

Figure 2. The increase in mean SPO2 before and after the Rashkind procedure.

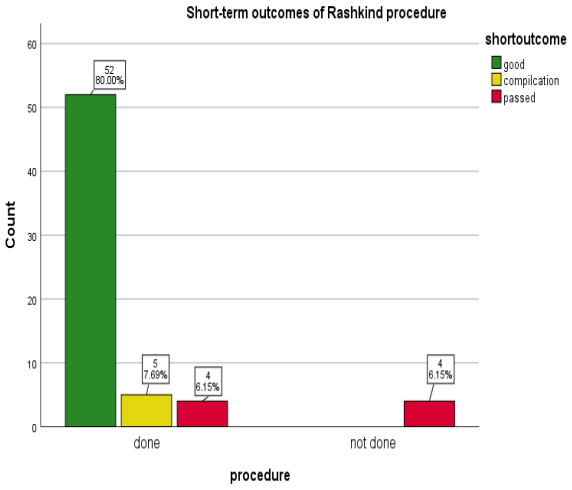

Figure 3. association between Rash kind procedure and short outcome.

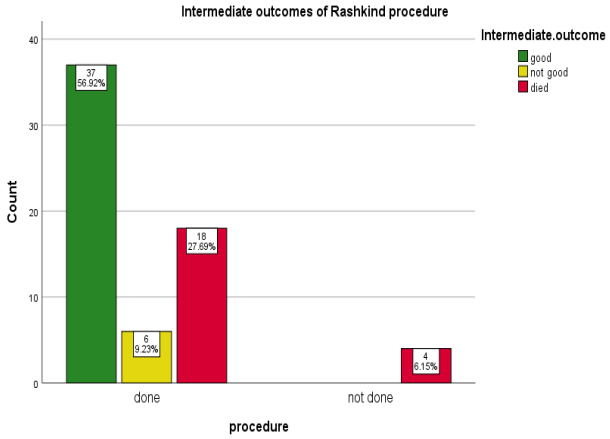

Figure 4. Association between the Rashkind procedure and the intermediate outcome.

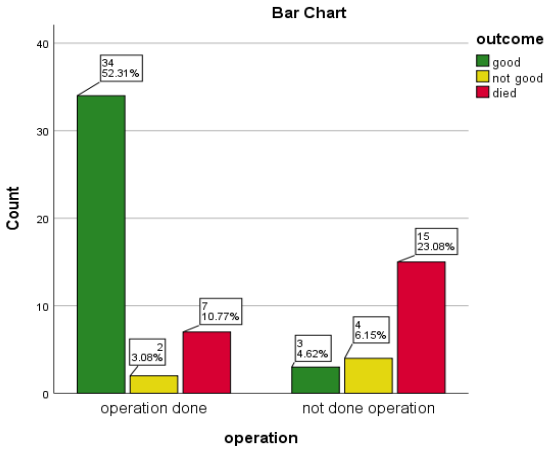

Figure 5. outcomes of corrective surgery.

Genetic polymorphism

The association of C677T polymorphism was evaluated in the MTHFR gene on TGA children. in the pediatric cardiology department in Sulaimani city. The results of genetic polymorphism showed that there was a significant difference between the two groups about genotypes and alleles (Table 4).

Table 4. Genotypic and allelic distribution in C677T polymorphism of MTHFR gene in the studied population

Genotype/Allele	Phenotypical Group		
	Control Group (n=65)	TGA Group (n=65)	*P*-Value
Genotype			
CC	42 (64.61%)	33 (50.76%)	0.0381
CT	22 (33.84%)	28 (43.08%)	0.0413
TT	1 (1.53%)	4 (6.16%)	0.0227
Allel			
C	51 (78.46%)	47 (72.30%)	0.0199
T	14 (21.54%)	18 (27.7%)	0.0284

This study, which was conducted using 65 neonates with TGA, demonstrates that BAS has an excellent immediate effect in stabilizing and increasing SPO2 in these patients before undergoing corrective surgery. This is compatible with a study conducted by Mansoura University Children's Hospital, which showed that BAS is a safe and effective palliative procedure with good immediate results (21). This agrees with a study carried out in New York where they found that BAS is a low-risk procedure with many benefits (7).

BAS had good short-term outcomes in 52 (85.2%) of the cases; five (8.2%) of the cases were complicated with arrhythmia and treated medically, and four (6.6%) of the cases passed in our study. This result corresponds with a study conducted at the Santa Maria Cardiovascular Clinic in Medellin. They found that BAS is a safe technique for the pre-operative stabilization of TGA patients (22).

The long-term outcome is significantly related to corrective surgery (Figure 5). The Rashkind balloon septostomy is a lifesaving procedure in early infancy. However, according to a study concerned with the palliative procedures for TGA (23), this procedure cannot replace an operation. This study is dated, but its results are still compatible with our study. There is a significant correlation between the Rashkind procedure and short- and intermediate-term outcomes. Furthermore, according to the same study, among all palliative procedures for TGA neonates, BAS is proven to be effective because of the high mortality rate among neonates, mostly below two weeks of age, when other palliative procedures were performed (24). Furthermore, our study showed that BAS could be safely performed as early as birth without a significant change in the outcome concerning age ($P > 0.01$ in 2 groups).

A nationwide study carried out at Johns Hopkins University School of Medicine that compared TGA patients undergoing the Rashkind procedure and those not undergoing the procedure showed that the latter had a higher mortality rate (25). This corresponds with our study, which has a 100% mortality rate. Moreover, the first group is not associated with necrotizing enterocolitis (this is also compatible with our study) but has a risk of stroke that is two times higher than the second group (our result is not consistent with that of this, but it is in agreement with that of a study done in New York where they found no association between BAS and brain injury (7.((

According to a Royal Hospital for Sick Children study, echocardiography is superior to the fluoroscopy guide in determining intracardiac anatomy during the procedure. It is helpful in patients with cardiac malposition (24). Furthermore, it can show the defect created by a balloon and can be measured accurately—this cannot be done with catheterization and angiography, which corresponds with our study. Although in our study, the primary guidance was echocardiography (with the occasional aid of fluoroscopy) there is no significant difference in the outcomes of echocardiographic guidance in comparison with those that included fluoroscopic guidance ($P > 0.01$). Another advantage of echocardiography guide BAS because it is less time-consuming and safer. It can be performed as a bedside procedure in a neonatal intensive care unit or other places when fluoroscopy is not available and avoids exposing the patient and operator to X-ray radiation (26-28).

In another study conducted at the University Medical Center Groningen, BAS improves cerebral oxygen saturation in neonates with TGA whose oxygenation may be impaired; our study also agrees with this because only three (4.6%) of the cases had a mental or neurological problem (5).

The prenatal diagnosis of TGA is crucial to detect severity, predict postnatal hemodynamic status, and determine the association between anomalies and the need for urgent and adequate therapy (3). However, in our study, we could not determine prenatal diagnosis's significance because all cases were diagnosed postnatally.

According to a study carried out at the American College of Cardiology, children with D-TGA diagnosed prenatally have better early complex cognitive skills, mainly executive function, compared with those diagnosed postnatally, in whom pre-operative acidosis and profound hypoxemia is more common (4).

About genetic polymorphism among TGA patients, results showed that The SNP C677T of the MTHFR gene was not in the Hardy–Weinberg principle in the Sulaimani population of Iraqi Kurdistan, and the frequency of the mutant (T) allele was 21.54% in the control group, and 27.7% in the TGA group. The results also showed a significant difference between the two groups in genotypes and alleles ($P<0.05$). This result confirms the relationship between C667T polymorphism of the MTHFR gene and transposition of great arteries disease.

Conclusion

Balloon atrial septostomy (BAS) is an effective palliative procedure in neonates with d-TGA, which can be safe and useful as cardiac catheterization at the bedside with echocardiography. Moreover, BAS is accepted as the best initial palliative procedure to stabilize infants with complete TGA before a corrective operation. Furthermore, BAS can be done under echocardiography or cine-angiography. The results also showed a significant difference between the two groups in genotypes and alleles ($P<0.05$). This result confirms the relationship between C667T polymorphism of the MTHFR gene and transposition of great arteries disease.

It is recommended that neonates with d-TGA be delivered in a center equipped to perform BAS. All patients with complete TGA did not require cardiac catheterization because echocardiography may be used reliably to diagnose complete TGA.

Conflict of Interest

The authors declare that don't have any conflict of interest in this study

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Non.

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