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Relation of Three-Dimensional Circumferential Strain Rate to Mediators of Intestinal Dysbiosis in Children with Chronic Systolic Dysfunction

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ABSTRACT

Background: Changes that may occur in intestinal microbiota have been shown to result in abnormal levels of intermediates, which can adversely affect the cardiovascular status.

Objectives: We aimed to study trimethylamine-N-oxide level (TMAO) in patients with chronic systolic dysfunction as a marker of intestinal dysbiosis and correlate it with cardiac status and echocardiographic findings.

Patients and methods: Twenty-five children with chronic cardiac dysfunction underwent sample collection for TMAO level. They have been compared to twenty-five age and sex-matched controls. Patients were assessed with conventional transthoracic echocardiography and three-dimensional speckle-derived strain imaging.

Results: Median age was 4.5 years. Patients had significantly lower ejection fraction (EF) and fractional shortening (FS) (P <0.001), and lower global, radial, circumferential and longitudinal 3-D strain rates (p<0.001). TMAO was significantly higher in patients (p<0.05). Best cut-off value was >6.2 um with sensitivity= 96% and specificity= 100%. TMAO correlated with Ross class, hospitalization rate as well as EF, FS, LV dimensions and volumes, and 3 D circumferential and radial strain rates. Linear regression analysis showed circumferential strain being an independent predictor of TMAO levels in the blood.

Conclusions: TMAO levels are increased in patients with chronic systolic dysfunction, which is related to their clinical status as well as their echocardiographic parameters. Three-dimensional circumferential strain rate seems to be an independent predictor of TMAO levels.

Keywords: Plasma trimethylamine-N-oxide, Three-dimensional strain rate, Dilated cardiomyopathy.

INTRODUCTION

Regardless to its cause, chronic cardiac dysfunction in children results in neurohormonal and molecular derangements as a consequence of the chronic congestive state and reduced cardiac output [1]. Cardiomyopathy is one of the commonest causes of cardiac dysfunction in the young age, with dilated cardiomyopathy being the most common identity in this group [2]. In contrast to heart failure (HF) secondary to structural heart disease, the outcome of the disease remains poor, and the estimated 5-year survival rate is around 50% [3].

Disruption of the normal gut microbiota is a common finding in patients with reduced ejection fraction heart failure (HFrEF) who suffer a prolonged state of immune dysfunction and inflammatory status which predispose to intestinal villi hypoxic atrophy leading to impaired functions and enhanced permeability [4]. The latter can significantly increase the passage of gut toxins into the blood stream, which in turn further contributes to the inflammatory state in the body [5].

The gut microbes metabolize dietary choline to trimethylamine, that is metabolized in the liver into trimethylamine-N-oxide (TMAO). Some reports have shown possible relations between TMAO and enhanced thrombosis and other life-threatening events including CNS thrombosis, myocardial ischemia, chronic kidney disease and overall mortality [6,7].

Speckle-tracking Imaging is an advanced echocardiography mode that analyzes left ventricular deformation. The three-dimensional STE has the advantage of being more accurate in assessing complex left ventricular mechanics from a single three-dimensional acquisition [8].

The main aim of the study was estimation of TMAO levels in children with chronic systolic dysfunction as a marker of altered gut microbiota and to correlate its levels with cardiovascular status in these patients.

PATIENTS AND METHODS

As a case-control study, twenty-five children and adolescents with dilated cardiomyopathy were compared to twenty-five sex and age-matched controls. We recruited patients from the Pediatric Cardiology Department during the period from December 2018 to March 2020.

Patients aged <18 years with the diagnosis of primary dilated cardiomyopathy with low ejection fraction heart failure were recruited in the study.

Primary dilated cardiomyopathy was defined as left ventricular ejection fraction < 55% and LVEDD exceeding 2 SD Z score excluding primary myocardial disease, non-dilated and secondary types of cardiomyopathy.

Patients with concomitant morbidities, especially liver and renal dysfunctions or overt intestinal failure, were excluded from the study.

Sample size: Using the Epi info program for sample size calculation, setting confidence interval at 95% and margin of error at 10 and based on the work by ***Sandek et al***, a sample size of 25 patients with heart failure was considered appropriate to achieve the study objectives [9].

Ethical considerations:

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Informed consent was obtained from the caregiver of the patients and controls prior to the study, along with the approval of Medical Science Ethical Committee of Ain Shams University (approval number M D 55 /2017). The work complied with the principles of the Declaration of Helsinki in 1975.

All patients and controls were assessed after history taking by general and detailed cardiac examination. Modified Ross classification was used to reflect the clinical status of patients [10].

We calculated the duration of illness as the time interval from the initial diagnosis of DCM till the time of sampling. Rate of hospitalization and the mortality rate were observed over a six months period.

2 D and 3D echocardiography:

Conventional transthoracic echocardiography was used to determine cardiac dimensions, and functions according to the recommendations of the American Society of Echocardiography. All patients under the age of three years were sedated to ensure good-quality image acquisition. A commercially available system (Vivid 9, GE Ultrasonography machine, Norway) was used with S5 or S6 transducers, according to the subject's age. A single expert echocardiographer performed all echocardiographic studies. Images were optimized with adjusted frame rate ≥30 Hz (30 frames/s). Breath holding technique was performed in cooperate children.

For the 3-D strain rate, offline data analysis was done using the software (EchoPAC BT12, 4D Auto LVQ; GE Vingmed Ultrasound AS, Germany). **(**Four-chamber, two-chamber, and three-chamber apical views and short-axis views were aligned. For the end-diastolic and end-systolic volumes, endocardial border was manually traced. Three-dimensional STE was used to determine end-systole global longitudinal strain (GLS), global circumferential strain (GCS), and global radial strain (GRS). The software automatically calculated global strain values.

The work of **Li *et al.*** was used as a reference for the average values of 3D speckle tracking in children with a mean global longitudinal strain of - 17.55 ± 2.40 (range: - 21.52 to -13.60), mean global circumferential strain -17.22 ± 3.41 (range: - 23.07 to -11.82), mean global radial strain 60.50 ± 13.58 (range: 41.85 to 83.34), and mean global strain rate - 30.22 ± 3.67 (range: 36.27 to - 24.25)[11]. Trimethylamine-N-oxide (TMAO) levels were determined by tandem mass spectrometry (AB SCIEX; Framingham MA). Patients were instructed to avoid intake of beef, eggs, or fish before sample withdrawal by 24-48 hours as it causes elevation of serum TMAO levels.

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing normally distributed data and Mann Whitney *U* test for independent samples when data were not normal. Within group comparison of numerical variables was done using repeated measures analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons in normal data and Freidman's test with posthoc multiple 2-group comparisons in not normal data. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-normal variables/non-linear monotonic relation. *p* values less than 0.05 was considered statistically significant.

A receiver operating characteristic (ROC) curve was generated using the values of TMAO to define best cut-off value with appropriate sensitivity and specificity. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

RESULTS

Among the cases, the median age of the involved patients was 4.5 years. The study included 18 females (72%) and seven males (28%). Median weight and mean BMI were significantly lower in the patient group (table 1).

Table (1): Demographic data and anthropometric measurements of cases and controls

		Controls (No. = 25)	Patients (No. = 25)	P-value	Sig.
Age (years)	Median (IQR)	4.5 (2.5 – 7)	4 (2.5 – 7.5)	0.854	NS
Sex	Females Males	18 (72.0%) 7 (28.0%)	18 (72.0%) 7 (28.0%)	1.000	NS
Weight (kg)	Median (IQR)	16 (13 – 25)	13 (11 – 21)	0.012	S
Height (cm)	Median (IQR)	105 (93 – 132)	96 (84 – 115)	0.225	NS
BMI	Mean±SD	17.04 ± 1.43	14.60 ± 2.92	<0.001	HS

BMI: Body mass index.

According to Ross classification, most of the patients were asymptomatic (class I), with around 24% belonging to class II HF and another 28 % suffering from grade III HF. The median illness duration was 9.7 months and all-cause mortality rate during follow-up was 8%.

Regarding echocardiographic parameters, patients had significantly higher cardiac dimensions and volumes and significantly lower EF and FS. Patients also had significantly lower values of 3D strain imaging (table 2).

Table (2): LV systolic functions using 2D M-mode and 3D speckle tracking in cases and controls

		Controls (No. = 25)	Patients (No. = 25)	P-value	Sig.
LV EDD (z-score)	Median (IQR)	0.01 (-0.44 –0.89)	5.6 (3.8 – 7.4)	<0.001	HS
LV ESD (z-score)	Median (IQR)	0.23 (-0.31 –0.62)	9.10 (5.6 – 11.1)	<0.001	HS
EDV (ml)	Mean±SD	50.44 ± 17.22	118.12 ± 41.76	<0.001	HS
ESV (ml)	Mean±SD	16.68 ± 7.86	78.04 ± 32.56	<0.001	HS
EF (%)	Mean±SD	68.46 ± 4.72	34.84 ± 9.56	<0.001	HS
FS (%)	Mean±SD	37.32 ± 3.41	16.76 ± 5.23	<0.001	HS
Global strain	Median (IQR)	-27 (-29 – -27)	-6 (-10 – -3)	<0.001	HS
Longitudinal strain	Median (IQR)	-15 (-16 – -15)	-3 (-10 – -2)	<0.001	HS
Circumferential strain	Median (IQR)	-14 (-16 – -14)	-4 (-10 – -2)	<0.001	HS
Radial strain	Median (IQR)	49 (48 – 51)	11 (4 – 29)	<0.001	HS

LVEDD: left ventricle end diastolic diameter, LVESD: left ventricle end systolic diameter ,EDV: end diastolic volume ,ESV: end systolic volume , EF: ejection fraction , FS: fractional shortening.

On assessment of TMAO level, patients had significantly higher levels (table 3). As expected, values of this marker are not determined yet in pediatrics. We established a ROC curve to determine best cut off value of abnormal TMAO, which was >6.2 µg/ml (sensitivity 96%, specificity 100%) (figure 1).

Table (3): Intestinal microbe-generated TMAO marker in cases and controls

TMAO marker (µg/ml)	Controls (No. = 25)	Patients (No. = 25)	P-value	Sig.
Mean±SD	2.69 ± 0.13	14.38 ± 2.23	<0.001	HS

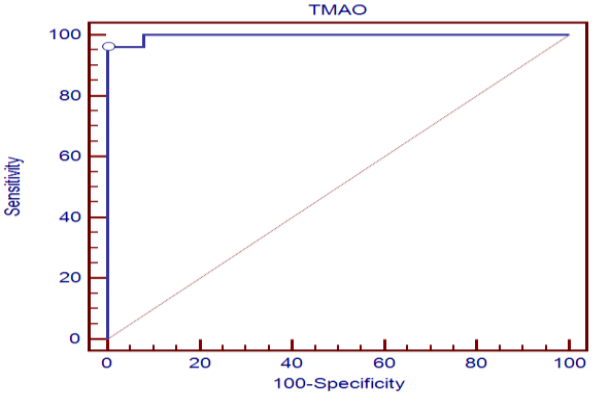

Fig (1): ROC curve for cut-off value of TMAO.

Measurement of TMAO levels in different patient groups showed a higher level in children with class III HF and with severely impaired systolic functions as well (table 5).

Table (5): Relation of TMAO with the other parameters in patients' group

		TMAO marker (µg/ml) No. = 25		P-value	Sig.
		Mean±SD	Range		
Sex	Females	13.84 ± 3.64	4.10 – 28.47	0.413	NS
	Males	15.79 ± 3.99	10.36 – 20.16		
BMI	Normal	14.78 ± 4.79	7.72 – 20.10	0.801	NS
	Low	14.20 ± 5.55	4.10 – 28.47		
Modified Ross	1	12.26 ± 3.94	7.83 – 18.16	0.025	S
	2	11.80 ± 5.67	4.10 – 19.23		
	3	15.96 ± 5.29	10.36 – 28.47		
EDV degree (ml/m^2)	Moderate	19.17 ± 0	19.17 – 19.17	0.361	NS
	Severe	14.18 ± 5.24	4.10 – 28.47		
EF impairment Degree (%)*	Mild	12.74 ± 4.37	4.10 – 20.16	0.014	S
	Moderate	12.77 ± 3.99	6.70 – 16.46		
	Severe	19.55 ± 5.16	12.50 – 28.47		
FS impairment degree (%)*	Mild	12.74 ± 4.37	4.10 – 20.16	0.014	S
	Moderate	12.77 ± 3.99	6.70 – 16.46		
	Severe	19.55 ± 5.16	12.50 – 28.47		

*Mild degree: EF=45-54%, Moderate degree: EF=30-44%, Severe degree: EF<30%
*Mild degree: FS=22-26%, Moderate degree: FS=17-21%, Severe degree: FS <17%

Positive correlations were found with Ross classification and frequency of hospitalization but not with other clinical parameters. TMAO levels also correlated with echocardiographic parameters, including left ventricular diameters, EF and FS, and circumferential and radial strain rates (table 6).

Table (6): Correlation of TMAO and the other studied parameters in patients' group

	TMAO marker	
	r	**P - value**
Age (years)	0.108	0.607
Weight (kg)	0.046	0.828
Height (cm)	0.260	0.209
BMI	-0.207	0.321
Ross Classification	**0.544****	**0.005**
Duration (months)	-0.149	0.499
Morbidity (Hospitalization frequency per year)	**0.504***	**0.012**
Mortality	-0.061	0.771
LV EDD z-score	**0.587****	**0.002**
LV ESD z-score	**0.681****	**0.000**
EDV (ml)	-0.160	0.446
EDV (ml/m2)	-0.280	0.176
ESV (ml)	-0.236	0.256
EF (%)	**-0.665****	**0.000**
FS (%)	**-0.564****	**0.003**
SV	-0.008	0.970
Global strain	-0.223	0.285
Longitudinal strain	-0.309	0.133
Circumferential strain	**-0.490***	**0.013**
Radial strain	**-0.522****	**0.007**

Linear regression analysis showed only circumferential strain being a possible predictor of TMAO levels in the blood with a standardized coefficient.

Table (7): Univariate analysis for predictors of TMAO marker

	Un-standardized Coefficients		**Standardized Coefficients**	**t**	**Sig.**
	B	**Std. Error**	**Beta**		
Circumferential strain	0.636	0.144	0.538	4.424	0.001

Linear regression analysis

DISCUSSION

Recently, there has been a growing interest in studying the effect of gut on the functioning of several organs, including the heart, the pancreas and other endocrinal glands[12]. The current study's main aim was to detect intestinal dysbiosis in children with HFrEF reflected by elevated serum TMAO levels and to correlate its level to their clinical and radiological parameters.

In his study, ***Trøseid et al.*** found that blood levels of TMAO were elevated in adult patients with heart failure (P = 0.01) [13]. Also, ***Tang et al*** cohort study on adults with stable heart failure showed elevated TMAO levels with a median level of 5 um (IQR: 3 to 8.5 um) in comparison to healthy controls with a median level of 3.5 um (IQR: 2.3 to 5.7 um) [14]. Similarly, our study showed that children with DCM and chronic heart failure had significantly higher TMAO levels when compared to controls (14.3±5.23 um versus 2.69 ± 1.03 um with a p-value <0.01) and a cut-off value of >6.2 µg/ml (sensitivity 96%, specificity 100%) confirming possible gut dysbiosis in these children as the amount of TMAO depends mainly on the gut microbiota, and the lining mucus layer integrity; both can be affected in children with heart failure [12].

We have tried to correlate the level of TMAO with clinical and echocardiographic parameters in these children. TMAO levels were not significantly different in male or female patients but were significantly different in patients with different Ross classifications being higher in patients with worse Ross class (p= 0.025), denoting a relationship to the severity of the condition. TMAO levels have also been shown to be related to the hospitalization rate but not to the mortality (p = 0.012, 0.771, respectively). These results come in concordance with those of ***Tang et al.*** who related TMAO levels to severity of HF and poorer clinical outcome [6]. Moreover, **Suzuki *et al.*** correlated high TMAO levels with unfavourable outcomes in their study [15].

On correlating TMAO level with the different echocardiographic parameters, the levels were higher in patients with worse systolic functions evidenced by lower ejection fraction and fractional shortening (mean of 12.7 µg/ml in a mild or moderate decrease in systolic functions versus a mean of 19.5 µg/ml in those with a severe reduction in functions, p=0.014). TMAO levels showed positive correlation with LVESD (P < 0.001) and LVEDD (p = 0.002) and negative correlation with EF (p < 0.001) and FS (p = 0.003) as well as circumferential strain (p = 0.013) and radial Strain (p = 0.007). These results contrast that of ***Trøseid et al***., who did not find any correlation between TMAO levels or its precursor with left ventricular volume or ejection fraction [13].

Also, ***Tang et al.*** found that Elevated plasma TMAO levels correlate with left ventricular diastolic but not systolic dysfunction and carry a poorer long-term outcomes in chronic systolic HF [6].

The correlation between TMAO levels and circumferential but not longitudinal strain may arise from the fact that the latter deteriorates early in the process of heart failure, unlike the former, which is preserved till advanced stages of the disease denoting a state of intestinal dysbiosis in severe cases of HFrEF only but not in milder stages. This fact has been studied by ***Yu et al.*** who found that longitudinal strain is affected early before appearance of any clinical symptoms [16]. In contrast, the circumferential strain is

affected late in a trial to compensate for longitudinal dysfunction [17, 18].

Also, ***Wang and his colleagues*** stated that longitudinal strain is an early sensitive indicator in patients with subclinical ventricular dysfunction and correlates with myocardial fibrosis [18] . On the other hand, ***Mizuguchi et al.*** found that circumferential fibers contraction maintains global cardiac functions in case of longitudinal function impairment [19].

The regression analysis has shown that circumferential strain is an independent predictor of TMAO level ($p>0.05$). A deteriorating 3D circumferential rather than longitudinal strain in children with DCM may point out a state of intestinal dysbiosis and elevated TMAO levels with possible adverse consequences in this patient population.

LIMITATIONS OF THE STUDY

Lack of large sample size prevented eliciting minor differences between cases and controls. Also, the fact that it is a single centre study limits the generalizability of the results. In addition, longer follow-ups of patients may better highlight the possible correlation of TMAO to mortality and morbidity in these patients. Furthermore, no dietary modifications or pharmacological intervention was done to ameliorate TMAO level and to monitor cardiac functions accordingly.

CONCLUSIONS

Intestinal dysbiosis occurs in children with chronic systolic dysfunction as evidenced by elevated TMAO levels, which adversely affect functional status in these children. A deteriorating circumferential is an independent predictor of TMAO levels and intestinal dysbiosis.

Conflict of interests: None.

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