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Methylglyoxal in Correlation with Persistent Pulmonary Hypertension of Neonates

Eman R. Youness [1], Mones M. Abu Shady [2], Hisham W. Bader [2], Mohamed EL-Sonbaty[2], Shaimaa A Hashem [2], WalaaAlsharanyAbuelhamd,[3]Hanan Hanna[4] *

[1] Medical Biochemistry Department and [2]Child Health Department, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt, [3] Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt, [4]Chemical Pathology, Faculty of Medicine, Delta University for Science and Technology, Gamassa, Egypt.

***Corresponding Author:** Hanan H Hanna**, Email:** hananagm@hotmail.com

Mobile: 01014510667**, ORCID NO.:**0000-0003-4796-8919

ABSTRACT

Background: Methylglyoxal has been documented to increase in circulation and at tissue level not only in diabetes but also in hypertension.

Objective: Our objective was to report the association between methylglyoxal (MG) levels in the blood and newborn persistent pulmonary hypertension.

Subjects and Methodology: Forty near-term and term neonates with evidence of persistent pulmonary hypertension (PPHN); were further alienated into two groups**,** Group (1):20 cases with PPHN with hypoxia and Group (2):20 cases with PPHN without hypoxia,40 healthy cross-matched controls with normal hearts were included in the study. Echocardiography was done to establish the diagnosis of PPHN, APGAR score, and methylglyoxal (MG) levels, random blood sugar,complete blood picture, serum creatinine, blood urea and oxygenation index were measured.

Results: A highly significant increase in the mean levels of MG was found in cases (whether with or without hypoxia) compared to controls (31.15± 19, 18.6 ± 10.01 ng/uL versus 12.8 ± 6.2respectively).

Conclusion: Methylglyoxal initiates pulmonary hypertension either by suppressing the production of nitric oxide (vasodilator) or by releasing vasoconstrictors. Furthermore, there was strong evidence that systolic blood pressure, hemoglobin, blood sugar, and pulmonary hypertension were the main predictors of MG

Keywords: Methylglyoxal, Pulmonary hypertension, Neonates, Hypoxia.

INTRODUCTION

Methylglyoxal (MG) is a reactive carbonyl species. It is generated endogenously during glycolysis in the cell. Furthermore, endogenous exposure to MG occurs as a result of glycated protein catabolism**(1)**. MG is produced exogenously during the Maillard reaction, sugar autoxidation, and liberation of lipids during processing and storage**(2)**.

MG is augmented three-fold to five-fold in diabetic patients due to leak out of cells **(3)** and circulating at a concentration as high as 8umol/L. At this level, MG significantly reinforces apoptosis caused by induction of DNA damage and oxidative stress[4]. Direct alteration of heat shock protein 27 (HSP 27) at amino acid Arg-188 permits HSP 27 to suppress cytochrome c-mediated caspase activation and may allow MG to act as an anti-apoptotic modulator[5].

Methylglyoxal has been documented to increase in circulation and at tissue level not only in diabetes but also in hypertension[6].

Persistent pulmonary hypertension of newborns (PPHN) is a comparatively usual state that occurs in 0.5 to 7 per 1000 live births causing mortality ranging from 4 to 33 %.[7] It is characterized clinically by hypoxemic respiratory failure owing to privation of transition of the pulmonary vasculature from a high-resistance fetal to a low-resistance extra uterine circuit. High pulmonary vascular resistance (PVR) results in right-to-left shunting across the patent foramen ovale and the patent ductus arteriosus, causing hypoxemia[8].

At the cellular level, it is characterized by noticeable endothelial dysfunction with a constrictor excess over vasodilator ingredients **(9)**.

As a result of chronic exposure to hypoxia, pulmonary vessel wall thickening with augmented depositionn of connective tissue and neomuscularization occur, which is termed pulmonary vascular remodeling. That remodeling affects right ventricle (RV) after load, reduces left ventricle (LV) preload (owing to the diminished pulmonary venous return), and compromises the function left and right ventricular in PPHN[10].

The effects of dilated right heart pressure loading included left ventricle compression and a shift in the interventricular septum, both of which caused diminished LV filling and thus LV cardiac output (LVO). This could lead to a reduction in blood pressure with PPHN, demanding the use of vasoactive inotropes as adrenaline and dopamine[11].

Numerous studies have revealed the relationship between low cardiac output in the setting of PPHN with mortality and morbidity [12,13].

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AIM OF THE STUDY

This work aimed to recognize relationship, if any; between the serum level of MG and PPHN in neonates.

PATIENTS AND METHODS

A cross-sectional study was conducted on 40 near-term and term neonates (35weeks of gestation or more) with evidence of persistent pulmonary hypertension (PPHN) and 40 healthy cross-matched controls with normal hearts, who were admitted to the neonatal intensive care unit of Children's Hospital, Cairo University, ***From February 2022- June2022.***

The cases were further alienated into two groups; Group (1):20 cases with PPHN with hypoxia and Group (2):20 cases with PPHN without hypoxia. Neonates less than 35 weeks of age, large or small for gestational age, were excluded from the study.

All cases and controls were subjected to comprehensive history taking and examination, including gestational age, gender, maternal illness and medications, and mode of delivery.

APGAR score at 1 and 5 min, birth weight, admission diagnosis, oxygen support, duration of admission, and outcome. PPHN was often related with symptoms and signs of perinatal distress, confirmed by echocardiography findings.

An **X-ray** was done to evaluate the occurrence of underlying parenchymal lung disease (e.g., pneumonia, surfactant, deficiency meconium aspiration syndrome) and/or to disregard underlying disorders (e.g.,congenital diaphragmatic hernia).

Echocardiography was done as it is considered the most reliable noninvasive test to establish the diagnosis of PPHN, and associated cardiac function and exclude associated structural heart disease.

Biochemical investigation: Five ml of blood was taken from the umbilical cord blood during delivery. A part was put on EDTA and the other part in plain tubes. For all patients and controls, complete blood picture, blood urea, serum creatinine, random blood sugar, and oxygenation indeindex were formed. The methylglyoxal (MG) concentrations in the serum were measured using a multiplex enzyme-linked immunosorbent assay (ELISA) (Millipore®, Merck KGaA, Darmstadt, Germany).

Ethical Approval

The current study was carried out in accordance with the principles and regulations of the Helsinki's declaration. Informed consent was taken from the patient's guardians, before enrollment with an explanation of the type of study.Approval from the Ethical Committee of the Faculty of Medicine, Delta University for Science and Technology, was obtained (no. FM2208006).

Statistical analysis

Statistical analysis was performed using statistical package for social sciences (SPSS) version 21 for windows (IBM Corp., Armonk, NY, USA). Continuous data were expressed as mean ± standard deviation, minimum, maximum. Kruskal-Wallis test was used to compare methylglyoxal between the 3 groups of subjects. Spearman correlation analysis was conducted to evaluate the association between continuous exposure and continuous covariates. Categorical data were expressed as frequencies and percentages, and were analyzed with the two-tailed chi square test. Multiple linear regression analysis was used to find the predictors of methylglyoxal in the studied subjects. $P < 0.05$ was accepted as statistically significant.

RESULTS

Eighty neonates (40 cases and 40 controls) were examined for the current study; **Group (1):** 20 cases of PPHN without hypoxia (12 males, 8 females) their mean gestational age was 37.3 ±1.17. **Group (2):** 20 cases of PPHN with hypoxia (8males, 12 females), their mean gestational age was 36.45±1-39. **In the control group** (20 males, 20 females), their mean gestational age was 36.9 ±l.29.

There was **positive consanguinity** in 33 cases and 32 in controls, while there was negative consanguinity in 7 cases and 8 in controls. 37 cases and 21 controls were delivered by CS, while 3 cases and 19 controls were delivered by vaginal delivery. As regards outcome, 25 lived while 15 died (two from group PPHN without hypoxia and 13 cases from group PPHN with hypoxia). The most common cause of death was pulmonary crisis (8 cases).

Table I shows the initial diagnosis of all cases, where transient tachypnea of the newborn (TTN) was the most common (14 cases). There was a highly significant difference between the 2 groups of cases as regards the initial diagnosis.

Table I: Initial diagnosis

Groups		Initial diagnosis												Total
		Unknown	RDS	IDM	RDS+ Tracheo-esophageal fistula	TTN	Meconium aspiration	perinatal asphyxia	CHD	HIE	Osteo-genesis-imperfecta	RDS+ IDM	Non-immune hydrops fetalis	
Pulmonary hypertension without hypoxia	Count	1	5	0	0	14	0	0	0	0	0	0	0	20
	% within Group	5.0%	25.0 %	0.0 %	0.0%	70. 0%	0.0%	0.0%	0.0 %	0.0 %	0.0%	0.0 %	0.0%	100%
Pulmonary hypertension With hypoxia	Count	0	2	2	1	0	3	5	2	2	1	1	1	20
	% within Group	0.0%	10.0 %	10.0 %	5.0%	0.0 %	15.0%	25.0%	10.0%	10.0%	5.0%	5.0 %	5.0%	100%

Chi-Square Tests= 34.286, P<0.001*, RDS=Respiratory distress syndrome IDM=Insulin dependent Diabetes mellitus
TTN= Transient tachypnea of the newborn CHD=Congenital heart disease Hypoxic-ischemic encephalopathy

There was a highly significant difference between cases (whether with or without hypoxia) and controls as regard the mean level of methylglyoxal (Table II).

Table II: Comparison of methylglyoxal between all groups

	Methylglyoxal (ng/ml)					
Groups	No.	Mean	±SD	Mean Rank	Chi-Square	P
Pulmonary hypertension without hypoxia	20	18.610	10.015	40.50	33.122	<0.001* a and c b and c
Pulmonary hypertension with hypoxia	20	31.155	19.313	64.90		
Control	40	12.833	6.271	28.30		

*p is significant, a= Pulmonary hypertension without hypoxia, b= pulmonary hypertension with hypoxia, c= control

Table III shows the correlations between MG and various variables, including Apgar score, random blood sugar, systolic blood pressure, blood urea, serum creatinine and hemoglobin.

Table III: Pearson correlation among methylglyoxal and different variables

Methylglyoxal (ng/ml)	
Gestational age (weeks)	r = -0.152
	p= 0.179
Down score	r = -0.008
	p= 0.959
Apgar 1 min	r = -0.547**
	p= <0.001
Apgar 5 min	r = -0.526**
	p= <0.001
Birth weight (kg)	r = -0.158
	p= 0.161
Head circumference (cm)	r = -0.077
	p= 0.499
Heart rate /minute	r = 0.130
	p= 0.249
Systolic Bp(mm HG)	r = 0.293**
	p= 0.008
Diastolic Bp(mm HG)	r = -0.090
	p= 0.428
Hemoglobin (g/dL)	r = -0.383**
	p= <0.001
Hematocrit (%)	r = -0.209
	p= 0.063
Total leucocytic count (x103/cmm)	r = -0.023
	p= 0.838
Platelets (x103/cmm)	r = 0.097
	p= 0.391
Random blood sugar (mg/dL)	r = 0.451**
	p= <0.001
Blood urea (mg/dL)	r = 0.354**
	p= 0.001
Serum creatinine (mg/dL)	r = 0.387**
	p= <0.001
RDW (%)	r = 0.391**
	p= <0.001
Oxygenation index	r = 0.035
	p= 0.832

**** Correlation is highly significant** RDW: Red Cell Distribution Width
BP: blood pressure

Table IV shows predictors of MG in the studied subjects, where systolic BP, hemoglobin, random blood sugar, and pulmonary hypertension were the main predictors of MG.

Table IV: Predictors of methylglyoxal in the studied subjects (Linear regression analysis)

Parameters	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	-15.196	31.058		-0.489	0.626	-77.140	46.749
Apgar 1 min	-1.944	2.127	-0.295	-0.914	0.364	-6.185	2.298
Apgar 5 min	0.744	2.625	0.087	0.284	0.778	-4.490	5.979
Systolic blood pressure (mm Hg)	0.553	0.244	0.223	2.265	0.027*	0.066	1.041
Hemoglobin (g/dL)	-2.813	1.021	-0.273	-2.755	0.007*	-4.849	-0.777
Random blood sugar (mg/dL)	0.218	0.080	0.298	2.733	0.008*	0.059	0.378
Blood urea (mg/dL)	0.239	0.191	0.161	1.249	0.216	-0.142	0.620
Serum creatinine (mg/dL)	-6.198	11.211	-0.073	-0.553	0.582	-28.558	16.162
RDW (%)	1.214	1.015	0.181	1.196	0.236	-0.811	3.239
Group of subjects	4.189	2.044	0.254	2.049	0.044*	0.112	8.266

*p is significant APGAR: Appearance, Pulse, Grimace, Activity and Respiration
RDW: Red Cell Distribution Width

DISCUSSION

Methylglyoxal($C3H_4O_2$), which is also known as 2-oxo-propanal or pyruvaldehyde, consists of fatty acids, carbohydrates, and proteins by enzymatic and non-enzymatic metabolic pathways[14].

Methylglyoxal is a dicarbonyl molecule having both irreversible protein damage by the creation of advanced glycation end products and aldehyde and ketone moieties, which are extremely reactive with diverse amino acid residues in proteins, resulting in oxidative stress[6,15,17]. Augmented levels of MG in tissue have been correlated with vascular remodeling and endothelial damage sharing in the development of hypertension in animals and humans.[18,19]

Yet the potential role of MG in the onset of pulmonary hypertension in humans has not been estimated till now.

The current study looked at the relationship between MG levels and persistent pulmonary hypertension in newborns. We observed a highly significant increase in MG in cases with PPHN compared to controls. Furthermore, MG levels increased non-significantly in hypoxia cases compared to non-hypoxic cases.

Different etiological causes, such as respiratory distress, neonatal sepsis, and TTN, alter glucose metabolism due to insulin resistance and increase the production of the reactive aldehyde methylglyoxal, we believe. Excess MG binds sulfhydryl groups of membrane proteins, modifying calcium channels and augmenting free Ca^{2+} in the cytosol, which has been related to endothelial destruction and vascular remodeling, causing the development of hypertension.[20,21]

Similar to our results, **Khajali** found that two factors contribute to the pathogenesis of pulmonary hypertension in broiler chickens[14]. First, the primary damage of arginine, which is the nitric oxide precursor and the key vasodilator that prevents pulmonary hypertension occurrence. Second, high MG concentration harms the vascular endothelium, causing systemic vasoconstriction and hypertension, potentially by diminishing the release of nitric oxide or by vasoconstrictors liberation.

Our study, also revealed, that systolic blood pressure, hemoglobin, random blood sugar, and pulmonary hypertension were the main predictors of MG. Similar to our results, **Braun and Sweazea** found that elevating blood sugar leads to enhanced MG production[22].

Based on the present findings, oxidative stress conditions lead to insulin resistance and abnormal

glycolysis with increased production of MG. Methylglyoxal initiates pulmonary hypertension either by causing the release of vasoconstrictors or by prohibiting the release of vasodilator nitric oxide. Additional studies are required to demonstrate the impact of MG scavengers as an adjuvant therapy to reduce pulmonary hypertension.

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REFERENCES

1. **Botta M (2018):** Neglected diseases: Extensive space for modern drug discovery. AnnualReports in Medicinal Chemistry.https://www.bookdepository.com/author/Maurizio+Botta
2. **Maruf A, O'Brien P,Naserzadeh P *et al.* (2018):** Methotrexate induced mitochondrial injury and cytochrome c release in rat liver hepatocytes. Drug and Chemical Toxicology, 41(1):51-61, Doi.org/10.1080/01480545.2017.1289221
3. **Brownlee M, Aiello L, Cooper M *et al.* (2011):** Complications of diabetes mellitus.Doi.1417-1501. 10.1016/B978-1-4377-0324-5.00033-X.
4. **Zheng J, Guo H, Ou J *et al.* (2021):** Benefits, deleterious effects and mitigation of methylglyoxal in food. A criticalreview. Trends in Food Science and Technology, 107:201-212. DOI. 107. 10.1016/j.tifs.2020.10.031
5. **De Bari L, Scire A, Christina M *et al.* (2021):** Interplay among oxidative stress, methylglyoxal pathway and glutathionylation. Antioxidants, 10(1):19.DOI:10.3390/antiox10010019
6. **Wu L (2006):** Is methylglyoxal a causative factor for hypertension development? Can J PhysiolPharmacol; 84(1):129-39. DOI: 10.1139/Y05-137.
7. **Lipkin P, Davidson D, Spivak L *et al.*(2002**): Neurodevelopmental and medical outcomes of persistent pulmonary hypertension in term newborns treated with nitric oxide. J Pediatr.,140:306-10.DOI: 10.1067/mpd.2002.122730
8. **Gao Y, Raj J (2010**): Regulation of pulmonary circulation in fetus and new born. Physiol Rev., 90: 1291-335. DOI: 10.1152/physrev.00032.2009
9. **Sehgal A, Athikarisamy, Adamopoulos M (2012):** Global myocardial function is compromised in infants with pulmonary hypertension. Acta Paediatr.,101: 410-3. DOI: 10.1111/j.1651-2227.2011.02572.x
10. **Peterson A, Deatsman S, Frommelt M *et al.*(2009):** Correlation of echocardiographic markers and therapy in persistent pulmonary hypertension of newborn. PediatrCardiol., 30: 160-5. DOI: 10.1007/s00246-008-9303-3
11. **Wei Z, Yue- E, Xiao-Yan Y *et al.* (2020):** Oral drugs used to treat persistent pulmonary hypertension of newborn - Expert. Review of Clinical Pharmacology, 13:1295-1308. DOI: 10.1080/17512433.2020.1850257
12. **EL-Khuffash A, Patrick J, Colm B *et al.*(2018):**The use of milrinone in neonates with persistent pulmonary hypertension of newborn. Maternal Health, Neonatology, and Perinatology, 4:24, 2-12. Doi.org/10.1186/s40748-018-0093-1
13. **Siefkes M, Lakshminrusimha S (2021):** Management of systemic hypotension in term infants with persistent pulmonary hypertension of the newborn: an illustratereview. Archives of Disease in Childhood,106:446 -55. DOI: 10.1136/archdischild-2020-319705
14. **Khajali F, Liyanage R, Wideman R (2011):** Methylglyoxal and pulmonary hypertension in broiler chickens. Poult Sci.,90(6):1287-94. Doi:10.3382/ps.2010-01120
15. **Wang X, Chang T, Jiang B *et al.*(2007):** Attenuation of hypertension development by aminoguanidine in spontaneously hypertensive rats: Role of methylglyoxal. Am J. Hypertens., 20: 629-36. DOI: 10.1016/j.amjhyper.2006.12.003
16. **Wang X, Desai K, Wu L (2005):** Vascular methylglyaxal metabolism and the development of hypertension. J. Hypertens., 23: 1565-73. DOI: 10.1097/01.hjh.0000173778.85233.1b
17. **Wang X, Jia X, Chang T *et al.*(2008):** Attenuation of hypertension development by scavenging methylglyoxal in Fructose treated rats. J. Hypertons.,26: 765-72.DOI: 10.1097/HJH.0b013e3282f4a13c
18. **Chang T, Wu L (2006):** Methylglyoxal, oxidative stress, and hypertension. Can. J. Physiol. Pharmaco.L, 84 :1229-38.DOI: 10.1139/y06-077
19. **Sankaralingam S, Xu H, Jiang Y *et al.*(2009):** Evidence for increased methylglyoxal in the vasculature of women with preeclampsia: role in upregulation of LOX-1 and arginase. Hypertension,54(4):897-904. doi:10.1161/HYPERTENSIONAHA.109.135228
20. **Vasdev S, Stuckless J (2010**): Role of methylglyoxal in essential hypertension. Int. J. Angiol., 19 (2):58 -65. DOI: 10.1055/s-0031-1278375
21. **Myrthe M, Spronck B, Delhaas T *et al.* (2021):** The putative role of methylglyoxal in arterial stiffening: A review, Heart, Lung and Circulation, 30: 1681- 93. DOI: 10.1016/j.hlc.2021.06.527
22. **Braun E, Sweazea K (2008):** Glucose regulation in birds. Comp BiochemPhysiol B Biochem Mol Biol., 151(1):1-9. Doi: 10.1016/j.cbpb.2008.05.007.