Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (9) Issue (1) (2024), (55-59) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg

The Role of serum of Interleukin-6 in Predicting the Development of Meconium Aspiration Syndrome in Infants Born with Meconium-Stained Amniotic Fluid

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Abstract

Background: When a baby breaths in a combination of amniotic fluid and meconium shortly after birth, it may cause meconium aspiration syndrome (MAS). Aim and objectives: Evaluation of Interleukin-6 (IL-6) serum levels in infants whose amniotic fluid has been tainted with meconium. Patient and methods: The subjects of this comparative cross-sectional research were 120 full-term infants whose mothers had MSAF. They were split into One group, called the MAS group, consisted of sixty newborns who had meconium aspiration syndrome, whereas the other, called the control group, consisted of sixty neonates who did not have the condition. Results: For Il-6, there was a positive correlation with trauma, newborn pulse, stuff cells, AST, and ALT, and a negative correlation with haemoglobin and PLT. However, for recurrent infection, PROM, foetal distress, labour complications, white blood cells, CRP, urea, creatinine, bilirubin, sodium, potassium, sulphur dioxide, pH, bicarbonate, and carbon dioxide, there was no statistically significant correlation. Conclusion: There was a strong negative link between Il-6 and Haemoglobin and PLT, but a strong positive correlation with neonatal pulse, stuff cells, AST, and ALT.

Key words: IL-6, Meconium maternal chronic illness, Congenital malformations

1. Introduction

Amniotic fluid stained with meconium is seen in around 8-25% of live deliveries, and about 5% of those cases develop meconium aspiration syndrome. Intubation is necessary for around 30–50% of newborns with meconium aspiration syndrome [1,6].

Serious complications including as pneumothorax, atelectasis, chemical and secondary bacterial pneumonia, surfactant suppression, pulmonary hypertension, and complications-related morbidity and death may occur as a result of tracheal aspiration of meconium. [5].

While typical findings like generalised, rough, homogeneous opacities due to pneumonia and interstitial edoema may not be visible until 48-72 hours into the course of meconium aspiration syndrome, non-specific findings like increased ventilation in radiography, flattening of the diaphragm, and irregular linear or patchy areas of atelectasis are noticeable in the early stages. Consequently, indicators that could identify patients at risk of developing meconium aspiration syndrome are required. [7].

We recognise inflammation's critical involvement in MAS pathogenesis. Both in vitro and in vivo investigations have shown that MAS levels of cytokines rise. [8,9].

Serum Interleukin-6 (IL-6) levels in newborns whose amniotic fluid had meconium staining were the focus of this investigation.

2. Patient and methods

This comparative cross sectional study conducted on 120 term newborns, who were born from mothers who had Meconium-Stained Amniotic Fluid (MSAF), Patients were divided according to outcome into two groups: **Group 1 (MAS group):** included 60 neonates who developed meconium aspiration syndrome and **Group 2(Control group):** included 60 neonates who not developed meconium aspiration syndrome.

Inclusion criteria: Both sexes (Males and females), and those who born from mothers who have Meconium-Stained Amniotic Fluid (MSAF)

Exclusion criteria: The presence of major congenital malformations: (i.e., congenital heart, cerebral, lung, abdominal malformations), newborns with severe perinatal asphyxia (Stage 2 or stage 3 hypoxic-ischemic encephalopathy findings according to Sarnat and Sarnat (6)), those with positive blood culture and lack of parental consent.

Method:

All patients were subjected to: Complete history taking, Physical examinations included: (general examination included vital sign, anthropometric measurements (weight and height), APGAR score in the 1st and 5th minutes (7) and Downs Respiratory Distress Syndrome (RDS) Scoring System (8) and systematic examination included cardiovascular system, gastrointestinal Tract (GIT) and abdomen, central nervous system (CNS), musculoskeletal System and chest examination) and Investigational Studies included radiological: X-ray and ECHO and laboratory investigations.

Serum of IL-6 (interleukin-6): The quantity of the target bound between a matched antibody pair was measured by the Human IL-6 solid-phase sandwich ELISA (enzyme-linked immunosorbent assay). The provided microplate contained wells that were pre-coated with a target-specific antibody. Following the addition of samples, standards, or controls to these wells, the immobilised (capture) antibody was bound. After the enzyme, antibody, and target complex were assembled into a sandwich with the second (detector) antibody, a substrate solution was introduced to generate a detectable signal. The original specimen's target concentration was inversely related to the signal intensity.

Steps: Take out as many strips as you'd like, and then let them cool to room temperature. The unsold desiccant and strips were returned to the sealed aluminium foil container and kept at 2-8℃. Reserve blank wells; they may be disregarded when measuring at two different wavelengths. Fill each well with 100µL of the matching standard or sample. The 0pg/mL well should contain 100µL of Standard Diluent, so please keep that in mind. Incubate at 37℃ for 90 minutes after sealing the wells/plate with the adhesive tape strip.At least 30 minutes before use, prepare the necessary amount of biotinylated antibody. Repeatedly wash the ELISA plate. Fill up every well with 100µL of the produced biotinylated antibody. Incubate at 37℃ for 60 minutes after sealing the reaction wells with the adhesive tape strip.Half an hour before you need it, have the enzyme conjugate ready. Rinse the ELISA plate three times. Except for the blank wells, add 100µl of produced Enzyme Conjugate to every other well. Use the adhesive tape strip to seal the wells, and then incubate at 37 for 30 minutes.Five times, wash the ELISA plate. Before incubating at 37℃ in a darkened incubator, add 100µL of the Colour Reagent that has been made to each well (including the blank well). It is possible to terminate incubation after the top standards' colouring has darkened and a colour gradient has emerged. Keep the chromogenic process under control for at least 30 minutes. Include 100µL of Colour Reagent C in each well, including the blank well. Blend well. Within 10 minutes, read the optical density (OD) at 450 nm.

Ethical Consideration: Parents were asked for their informed permission before their children could be included in the research. We got the go-ahead from the Benha Faculty of Medicine's Research Ethics Committee.

3. Results

Table 1 showed that there was no statistically significant difference between MAS and control groups regarding to residence, while there was statistically significant difference according to gestational age and sex.

Table 2 showed that IL-6 was statistically higher in maternal chronic illness and trauma. While there was no statistical difference in IL- 6 level as regards to sex, residence, consanguinity, recurrent infection, polyhydramnios, PROM, multiple gestation, erythroblastosis fetalis or mode of delivery , fetal distress and in patients with history of complications during labor.

Table 3 showed that there was a significant positive correlation between IL-6 and pulse, while there was no significant correlation between IL-6 and Apgar score, and systolic blood pressure, gestational age, Capillary refill time (sec.), down score and Hospital stay duration (days).

Table 4 showed that there was a significant positive correlation between IL-6 and staff, ALT, AST, and a significant negative correlation between IL-6 and Hemoglobin, platelets., while there was no significant correlation between IL-6 and WBCs, segmented, c-reactive protein, urea, creatinine, bilirubin, sodium, potassium, SaO2, PH, CO2, or HCO3.

Table (1) Sociodemographic data of the studied groups.

		MAS group (N=40)		Control group (N=40)		Test	P value
		N.	%	N.	%		
Gestational age (weeks)	**Mean ±SD**	36.3±1.5		34.6±1.12		t=7.03	< 0.001
	Range	32-39		32-36			
Sex	**Male**	30	75%	12	30%	X^2=16.2	< 0.001
	Female	10	25%	28	70%		
Residence	**Urban**	15	37.5%	17	42.5%	X^2=0.2	0.64
	Rural	25	62.5%	23	57.5%		

t: student t-test, X^2: Chi-square test, MAS: meconium aspiration syndrome

Table (2) Interleukin-6 according to perinatal data in the studied neonates.

		Serum of IL-6 (ng/ml)			Test	P value
		Mean±SD	Min.	Max.		
Sex	**Male**	48.5±30.1	20.0	112.5	t=1.19	0.84
	Female	47.3±22.6	20.0	100.0		
Residence	**Urban**	45.9±26.3	20.0	109.9	t=0.04	0.96
	Rural	46.2±32.0	20.0	112.5		
Consanguinity	**+ve**	46.0±29.2	20.0	110.0	t=0.07	0.93
	-ve	45.5±23.4	20.0	112.5		
Maternal	**Yes**	59.7±30.9	20.0	110.0	t=2.65	0.009
chronic illness	**No**	43.6±24.2	20.0	112.5		
Recurrent	**Yes**	57.1±26.8	20.7	112.5	t=1.89	0.0613
infection	**No**	52.0±23.5	20.0	110.0		
Polyhydramnios	**Yes**	52.1±26.1	20.0	109.9	t=1.7	0.077
	No	41.2±24.3	20.0	112.5		
PROM	**Yes**	55.1±28.5	20.0	112.5	t= 1.80	0.075
	No	44.0±26.5	20.0	110.0		
Trauma	**Yes**	59.5±30.8	20.0	100.0	t=2.5	0.01
	No	43.0±25.5	20.0	112.5		
Multiple	**Yes**	51.9±28.4	20.0	112.5	t=1.8	0.069
gestation	**No**	40.9±22.7	20.0	109.9		
Fetal distress	**Yes**	59.1±27.2	20.0	109.9	t=1.61	0.110
	No	49.7±25.7	20.0	112.5		
Erythroplastosis	**Yes**	81.4±31.6	54.3	112.5	t=1.5	0.122
fetalis	**No**	70.8±24.7	20.0	110.0		
Mode of	**CS**	48.7±26.5	20.0	112.5	t=0.25	0.79
delivery	**NVD**	50.3±26.9	20.0	110.0		
Complications	**Yes**	71.5±24.8	29.8	112.5	t=1.19	0.237
during labor	**No**	65.8±17.4	20.0	99.5		

t: student t-test, *: significant, IL: interleukins, PROM: premature rupture of membrane.

Table (3) Correlation between Interleukin-6 and some clinical dataof the studied neonates.

	Serum of IL-6 (ng/ml)	
	r	P value
Gestational age (weeks)	-0.046	0.423
Apgar score	-0.189	0.068
Pulse (min.)	0.047	0.016*
Systolic blood pressure (mmHg)	-0.086	0.655
Diastolic blood pressure (mmHg)	0.423	0.098
Capillary refill time (sec.)	0.177	0.323
Temperature (c)	0.378	0.152
Down score	0.486	0.103
Duration of O2 support	0.586	0.652
Hospital stay duration (days)	0.656	0.09

r: Correlation coefficient, *: significant, IL: interleukin

Table (4) Correlation between Interleukin-6 and laboratory investigations of the studied neonates

	Serum of IL-6 (ng/ml)	
	r	**P value**
Hemoglobin	-0.152	0.026*
Platelets	-0.165	0.013*
WBCs	0.113	0.092
Staff	0.038	0.012*
Segmented	0.004	0.863
C-reactive protein	0.573	0.053
Urea	-0.074	0.405
Creatinine	0.083	0.389
AST	0.028	0.009*
ALT	0.069	0.025*
Bilirubin total	-0.056	0.738
Sodium	-0.048	0.596
Potassium	-0.063	0.479
RBS	-0.049	0.836
SaO2	-0.638	0.235
PH	-0.536	0.069
HCO3	-0.456	0.073
CO2	0.423	0.145

r: Correlation coefficient, *: significant, IL: interleukin WBCs: white blood cells: AST: Aspartate aminotransferase, ALT: Alanine transaminase, RBS: random bloodsugar.

4.Discussion

There was a statistically significant difference in terms of gestational age and sex, but no difference in terms of residency between the MAS and control groups, according to our data.

Ekmen et al. found no statistically significant difference in gestational age or sex between the groups they analysed, which contradicts our findings [4].

Hirani et al. who presented evidence linking longer gestational periods, higher birth weights (>2.5 kg), and the use of caesarean sections to an increased risk of MAS. Mortality and morbidity rates associated with MAS are high. [10].

Trauma was associated with significantly elevated levels of IL-6, according to the present investigation.

Sex, place of residency, consanguinity, recurrent infection, polyhydramnios, polyhydramniosis, multiple gestation, erythroblastosis fetalis, delivery style, foetal distress, and a history of labour problems did not significantly affect IL-6 levels.

However, the aforementioned risk factors—such as low birth weight, gender, postdates, and nulliparity—did not show any association with MAS in these studies [11,12].

Our findings revealed a positive correlation between IL-6 and pulse, but no such correlation with Apgar score, systolic blood pressure, gestational age, capillary refill time (sec.), down score, or hospital stay duration (days).

Ekmen, et al., He said that IL-6 levels were compared between ventilated MAS positive and MAS negative infants. infants ventilated with MAS (-) had a mean IL-6 of 60 whereas those ventilated with MAS (+) had a mean of 541. four, with a p-value less than 0.05.

Lindenskov et al. findings in animal models corroborate the hypothesis that complement activation is associated with meconium aspiration syndrome and reduced pulmonary function [13].

The results demonstrated that IL-6 was positively correlated with staff, ALT, and AST and negatively correlated with haemoglobin and platelets. In contrast, IL-6 did not show any significant correlation with white blood cells, segmented, c-reactive protein, urea, creatinine, bilirubin, sodium, potassium, SaO2, PH, CO2, or HCO3.

As a result of a systemic review and meta-analysis conducted by **Hou, et al.,** the authors emphasized that IL-6 and CRP frequently increase in noninfectious diseases and may function as useful prognostic tools in these infants, consisting of both infected and non-infected patients [14].

Hofer, et al., found that increased C-reactive protein, low leukocyte count, and low absolute neutrophil count are associated with severe MAS, and they also stated that these findings may be used in clinical practice to ascertain the trajectory of MAS patients [15].

Ekmen et al. found no association between IL-6, CRP, or white blood cell levels (p = 0.58 and 0.869, respectively) [4], which contradicts our findings.

Hsieh, et al., They found that levels of interleukin 6 were greater in neonates whose births were stained with meconium [16].

5.Conclusion

There was a statistically significant negative link between Il-6 and Haemoglobin, PLT, and AST and ALT, but a positively correlated relationship with neonatal pulse, stuff cells, AST, and trauma.

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