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ORIGINAL ARTICLE

Study of Serum Transforming Growth Factor-β1 and Bone Morphogenetic Protein-7 in Preterm with Respiratory Distress Syndrome

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ABSTRACT

Background: Neonatal RDS is important cause of mortality and morbidity in preterm newborn and occurs exclusively in preterm and its incidence is inversely related to gestational age. TGF β1and BMP 7 are cytokines released In preterm with RDS on mechanical ventilator. The study aimed To study role of both TGF β1and BMP 7 in preterm with RDS managed with mechanical ventilation either receiving or not receiving surfactant thereby. **Methods:**The study was conducted on 47 preterm with RDS receiving mechanical ventilation. 22 received surfactant and 25 did not received. Antenatal. Natal history was taken, complete clinical examination, ABGAR score was done. CBC, blood gas analysis, CRP, blood culture, chest x ray was done... TGF β1and BMP 7 was estimated at 2nd and 7th day by ELIZA kits.**Results:**Both TGF β1 and BMP 7 did not show any significant difference between PS and NPS cases at 2nd day of RDS while showed significant higher value at 7th day in NPS cases compared to PS cases. At 7th day Both cytokines decreased significantly in PS group, In NPS group TGF β1 raised significantly while BMP 7 did not changed significantly compared to 2nd day. No significant relation between both cytokines at 2nd day while showed positive correlation at 7th day in RDS cases (PS+NPS) with no relation in NPS cases at 7th day.**Conclusions:** Both TGF β1and BMP 7 significantly rises in RDS cases and decrease with surfactant therapy. Both cytokines may have value in monitoring and management of preterm with RDS.

Keywords: Preterm; Respiratory distress syndrome (RDS) ;Transforming growth factor TGF (TGF β1);Bone morphogenesis protein 7 (BMP 7)

INTRODUCTION

Neonatal respiratory distress syndrome "NRDS"is important cause of neonatal mortality and morbidity and occurs almost exclusively in preterm new born and its incidence is inversely related to gestational age [1]. Despite great improvement in management of "NRDS "including mechanical ventilation and surfactant therapy, still high incidence of complications are there. Among important complication of NRDs is broncho-pulmonery dysplasia "BPD" [2]

Recent studies showed that inflammatory mediators, cytokines plays important role in development of BPD. [3], these mediators can increase the permeability of alveolar capillary membrane and cause alveolar edema, which can reduce pulmonary surfactant (PS) and decrease lung compliance resulting in formation of extensive atelectasis and intrapulmonary shunt eventually lead to hypoxemia. [4]

Transforming growth factors β1 "TGF β1" and bone "body" morphogenetic protein -7 are members of TGF β superfamily. TGF β1 is up regulated in lung fibroblast and involved in development of acute and chronic lung injury and fibrosis which possibly results in BPD and chronic lung disease during NRDs (5format). TGF- β signaling can negatively regulate the branching and septation [6] phases of lung development. Overexpression of TGF-β1 between postnatal days (P) P7 and P14 in the mouse [7] both induced histological changes analogous to those seen in BPD.

BMP 7 is expressed in airway epithelium and is essential for lung development and prevention of RDS. Also it is involved in allergic inflammation of the lung and supposed to antagonize the effect of TGF β1 including inflammatory effect [8,9].Very little reports available about the role of TGF β1 and BMP-7 in preterm with NRDs,

Because they are expressed in neonatal RDS, and have role in pathogenesis, of the disease and development of complication we estimated level of

both cytokine in neonates with RDS with and without surfactant thereby.

METHODS

Patients groups: Forty seven **(**47) preterm meet the following criteria was selected: 1-gestational age between 28-32 weeks 2-has diagnostic criteria of RDS: a- progressive dyspnea, retraction. b- x- ray changes of air Broncho-gram. 3-blood gas changes of impending respiratory failure, hypoxemia, and respiratory acidosis 4-patient needs mechanical ventilator (10). Patients group were divided into two groups: a-preterm treated with surfactant (PS) group "22" b-preterm did not treated with surfactant (NPS) group"25".

***Laboratory investigations*:** About 2 ml of blood was extracted. Routine investigations was done including: CBC, and CRP, blood gas analysis was done for all cases. Blood sample for routine investigations, TGF β1 and BMP 7 were taken on 2nd day. Another sample was taken for TGF β1 and BMP 7 on 7th day.

***Estimation of TGF β1 and BMP 7*:** Serum sample were used **the TGF** β1 using DRGTGF. β1 ELIZA kits which solid phase enzyme linked sorbent assay (ELIZA) bases on sandwich principle (11).

***Radiology*:** Chest x ray was done for all cases in the study to confirm diagnosis and follow up of cases of RDS**.**

***Exclusion criterion* cases:** showing other causes of respiratory distress other than RDS as sepsis**.** Congenital anomalies, cardiovascular, CNS diseases and metabolic diseases ware excluded from the study**.** The study was approved from Ethical Committee of General Organization for Institutes and Teaching Hospitals in Cairo.

The study was done according to Code of Ethics of the World Medical Association (Declaration of Helsinki). Written consent was taken from eligible person "parents" of the cases of the study with explanation of any possible effect of the procedure taken in the study.

STATISTICAL ANALYSIS

Data were analyzed using SPSS 20 computer program (IBM, Endicott, Broome County, New York, United States). Data were expressed as mean ± SD for categorized variables. Tests of significance "Chi-square and T tests" and correlation study were done where appropriate. The correlation coefficient method was used to correlate different parameter .Regression Model was used to find out the most significant independent predictors affecting outcome. P value > 0.05 = statistically insignificant, P ≤ 0.05* = statistically significant, P < 0.01**= highly significant

RESULTS

This study included 47 cases with RDS: 22 cases treated with surfactant (PS) in 1st 24 hours according to slandered protocol, 25 cases did not receive surfactant (NPS) because of financial cost or lack of insurance coverage.. PS and NPS cases were matched regarding gestational age, sex and weight (P was 0.065 & 0.51 and 0.059 respectively) **(Table1).**

Among PS cases 3 of 22 cases died while in NPS cases 13 of 25 cases died (P was 0.044) " Death was significantly lower in PS cases compared to NPS cases " . PS cases showed non-significant less number of complicated cases compared to NPS cases (5 out of 22 versus 12 out of 25 respectively. P was 0.067).Also, the ventilation duration was lower in PS cases compared to NPS cases (135.067±101.98 hours versus 218.86±92.8 hours respectively, P was 0.029). **(Table 2)**.

PS cases showed higher values of WBCs , RBCs (but still in normal range) , and lower values of CRP compared to NPS cases (6.53±4.4 ,4.5±0.53 ,21.82±2.63 versus 4.5±14 ,4.12±0.77 ,35.48±26.22, P was 0.047 ,0.031 and 0.026 respectively) , while no significant differences regarding platelets and Hb were found in both groups (162.36±66.86 , 15.8±1.2 versus 157.4± 43.5 ,14.99 ±1.9 , P was 0.768 , 0.093 respectively) . **(Table 3).**

TGF β1 was not significantly different between PS and NPS cases at *2nd day* (43.67±20.53 & 46.8±13.28 , P was 0.533) while it was higher at *7th day* in NPS compared to PS cases (49.44±12.36 &37.86±17.45 , P was 0.011) .It was significantly lower at *7th than 2nd day* in PS cases (P was 0.00) while it was higher at 7th day than *2nd day* in NPS cases .(P was 0.00). **(Table 4).**

BMP 7 was not significantly different at *2nd day* between PS and NPS cases (48.43±14.71, 52.31±16.31 P was 0.398) while it was higher in NPS cases than PS cases at *7th day* (42.64±12.28, 53.48±18.6, P was 0.025) . I At *7th day* it was lower in PS cases than *2nd day*, while in NPS did not show any significant differences between *2nd and 7th day* (P was 0.00, 0.519 respectively). **(Table 5).**

TGF β1 did not show any significant relation to weight , gestational age ,WBCs , Hb or BMP 7 at *2nd day* of age (P was 0.21 ,0.593 ,0.074 and 0.763 and 0.146 respectively) , while it showed positive correlation with CRP at 2nd day of age (P was 0.02) **(Table 6).**

BMP 7 did not show any relation to weight, gestational age WBCs, Hb , CRP or TGF β1 (P was 0.774 ,0.535 ,0.273 ,0.699 , 0.306 and 0.146 respectively) **(Table 5).**

At 7th day of age both TGF β1 and BMP 7 showed positive correlation with each other in RDS cases (PS & NPS, P was 0.025), while they did not show any relation in NPS cases alone (P was 0.61,)

Table 1: RDS Cases Regarding Clinical Parameters

	PS (22)	NPS (25)	P
Death (chi square test) Died Survived	3 (13.6%) 19 (86.4%)	10 (40%) 15 (60 %)	0.044
Complication (chi square +ve test) -ve	5 (22.7%) 17 (77.3. %)	12 (48 %) 13 (52 %)	0.067
Ventilation Duration(hs) Survived) T-test (t= -2.316)	218.86±92.8	135.067±101.98	0.029

Table 2: Laboratory Data among RDS Cases (T –test)

	PS (22)	NPS (25)	T	P
WBCs	$6.63\times10^3\pm4.4$	4.12±1.4	2.58	0.022
Platelets	$162.36 \times 10^3\pm66.86$	157.4±43.5	0.297	0.768
Hb (gm)	15.8±1.2	14.99±1.9	1.715	0.093
RBCs	$4.5 \times 10^6\pm0.53$	4.12±0.77	2.175	0.031
CRP(mg)	21.82±12.63	35.48±26..22	2.318	0.026

Table 3: TGF 1β at 2nd and 7th day of RDS Cases

Group	Number of cases	2nd day	7th day	P value (paired t test)
PS	22	43.67±20.53	37.86±17.45	0.00
NPS	25	46.8±13.28	49.44±12.36	0.00
P value	T test independent sample	0.533	0.011	

Table 4: BMP-7 at 2nd and 7th day in RDS Cases

Group	Number of cases	2nd day	7th day	P value(paired t test)
PS	22	48.43±14.71	42.64±12.28	0.00
NPS	25	52.31±16.31	53.48±18.6	0.519
P value	T test (independent sample)	0.398	0.025	

Table 5: Correlation of TGF 1β , BMP 7 with Laboratory Data ,Gestational age and Weight in RDS Cases

	TGF 1β		BMP 7	
	R 2nd day	P 2nd day	R 2nd day	P 2nd day
Gestational age	0.186	0.21	0.049	0.77
Weight	0.08	0.593	0.093	0.54
WBCs	-0.263	0.074	-0.163	0.273
Hb	0.051	-0.763	0.58	0.699
CRP	0.152	0.02	0.152	0.306

Table 6: Correlation between both TGF 1β and BMP 7 at 2nd and 7th Day of age

	BMP 7	
	R	P
TGF 1β	2nd day 7th day	2nd day 7th day
	0.215 0.325	0.146 0.025

DISCUSSION

Neonatal RDS still the leading cause of neonatal mortality and morbidity despite great advances in its management [12,13] .One of the most pathophysiologic event is surfactant deficiency by type II alveolar cells which results from prematurity[14,15], acidosis, cold stress, hypovolemia, and hypoxemia , invasive mechanical ventilation [16]. Surfactant deficiency results in the alveoli collapse and then atelectasis,

pulmonary edema and lung membrane [17]. The aim of this study was to evaluate two variants of inflammatory cytokines in preterm neonates with RDS received mechanical ventilation with and without surfactant thereby. Most of our patient were extreme low birth weight and has gestational age < 32 week and need ventilator support and surfactant therapy. Healthy or simple preterm cannot be included in this gestational age, or weight range and cannot be taken as a control group. Cases did not receive surfactant therapy were accidentally because of lack of availability of surfactant therapy. because of financial reason and lack of insurance.

PS cases showed significant lower mortality, non-significant lower morbidity and significant lower ventilator support duration compared with NPS cases (P was 0.044, 0.067 and 0.029 respectively). These
results reveal the value and importance of surfactant in management of neonatal RDS. Surfactant therapy is considered one of major advances in preterm with RDS in last decades [18] Wang L.P. et al [19] found similar to our results that preterm with mechanical ventilation received surfactant "PS" had lower mortality rate, shorter ventilator duration than preterm did not receive it "NPS" (P was < 0.05 for both) . The most frequent complications found were : intracranial hemorrhage " 3 in PS , 5 in NPS" , pneumothorax "1 in PS ,3 in NPS" , sepsis "1 in PS , 1 in NPS" and PDA "3 in NPS" . These complications were lower in PS group compared to NPS group "P was < 0.05" The difference from our study in apparent less percentage of death and shorter duration of ventilation duration from our study may be due to higher gestational age and weight compared with our cases. Surfactant can re-expand the atrophic alveoli by reducing the surface tension, improve ventilation and gas exchange, enhance oxygenation functions and remarkably shorten the mechanical ventilation duration and oxygenation time [19]

NPS cases showed higher value of CRP than PS cases (P was. 0.026). This may be explained that CRP is an acute phase reactant. It increases with respiratory distress and inflammation which accompany RDS [20]

No significant difference regarding TGF β1 at 2nd day between PS and NPS cases (P was 0.533) while it was lower in PS cases than NPS cases at 7th day (P was 0.011). At 7th day it was significantly lower in PS cases than 2nd day while in NPS cases it was significantly higher (P; was 0.00 in both groups). High level of TGF β1 in 2nd day in neonates with RDS (PS & NPS) is explained by inflammatory process associated with RDS and lung injury caused by mechanical ventilation. TGF β1 is a cytokine secreted by macrophages, epithelial, endothelial cells and fibroblasts, it regulates cell growth and differentiation [21] and is involved in acute and chronic lung injury occurrence. High expression was found in pulmonary fibrosis and BPD [22]. Decrease level of TGF β1 at 7th day in PS group is explained by decease inflammatory response and respiratory distress. Higher level at 7th day in NPS reflects continuing inflammation and lung injury. Absence of significant difference at 2nd indicates same degree of pathological process and lung injury before surfactant pathological effect to be apparent. It is possible that clinical effect of surfactant occurs earlier than pathological effect. Similar result was found by Wang et al [19] . They found similar values for both PS and NPS groups at 1st day. The values increase significantly for both groups at 3rd day, decreased significantly at 7th day in PS group and stay high for NPS group. At 3rd day the value is higher in NPS group than PS group. Several explanations were suggested regarding decrease level of TGF β1 in response to surfactant thereby. Surfactant thereby improve lung compliance, decrease risk of lung injury and inflammation in response to mechanical ventilation [18] and consequently decrease release of cytokines including TGF β1 and BMP 7 [23] . Rise in TGF β1 level in NPS group at 7th day compared to 2nd day is explained possibly by progression of respiratory distress and increased lung injury and inflammation due to absence of surfactant and progressive decreased lung compliance. TGF β1 inhibited surfactant component expression and epithelial cell maturation in cultured human fetal lung [24].It is expected in NPS cases decreased lung compliance as result of surfactant deficiency increase lung injury and inflammation and release of mediators including TGF β1 which inhibits surfactant synthesis increasing inflammation and decreasing compliance and ventilation damage . In PS group surfactant administration cuts this vicious circle by improving lung compliance and decreasing inflammation and barotrauma resulting from ventilator support.

BMP 7 showed similar results to TGF β1. No significant differences between PS and NPS cases in 2nd day while it was significantly lower in PS cases compared to NPS cases at 7th day
(P was 0.398 ,0.025 respectively) . Also at 7th day it decreased significantly in PS cases while no significant changes regarding NPS cases compared to 2nd day (P was 0.519 , 0.00 respectively) . Similar results were found by Xiao-Qing L et al [10]. BMP 7 produced in airway epithelium and has role in allergic inflammation and antagonize fibrotic effect of TGF β1, decreases its level in

broncho-alveolar lavage in dose dependent manner [5] and produced to down regulate its secretion [19]. Absence of correlation between both cytokines at 2nd day and positive correlation at 7th day indicate its role in RDS. In PS cases it decreased at 7th day in response to decrease level of TGF β1 while remain high in NPS group in trial to down regulate TGF β1 which remain high .Positive correlation at 7th day support this suggestion

This study indicates the value of surfactant therapy in treating preterm with RDS. Also it reveals the importance of both TGF β1 and BMP 7 in monitoring RDS cases receiving mechanical ventilation and their prognostic value. Also the study has therapeutic implication. administration of exogenous BMP-7 to preterm with RDS may have reversing the condition and decreasing its complication [10]

The study has limitation because of limited number of cases and detailed complications not statistically significant for evaluation of both TGF β1 and BMP 7 in different complication. Also no follow up made for development of sequels of NICU admission after periods of time. Because of low gestational age and birth weight we could not take healthy control for comparison.

Further study is required with various ranges of gestational ages and weights and larger number of cases with statically significant complications after follow up for period of time.

Conflict of interest: no conflict of interest

Financial Disclosure*:* no financial disclosure

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