CHAPTER IV

DISCUSSION

This study was performed with the 40 pediatric patients, aged 5.3±0.62 years old in 20 patients of group A and 5.4±0.63 years old in 20 patients of group B. All subjects were admitted in the acute care or intensive care unit with the lung problems or pneumonia. From the illness data of patients showed various primary diseases, but the condition of patients were stable. The main problems for inclusion criteria of patients in this study were lung secretion and atelectasis, and those patients were transferred to physical therapy under supervision of doctors (see appendix B). The characteristic of pediatric patients in group A and B showed not different (Table 4); for example, CBC laboratory (sodium, potassium, and chloride), including hematocrits, lymphocytes, neutrophils, and white blood cells from the hematological data. All values showed within normal range. The severity of the lung damage in group A and B were not different before intervention. (Table 4)

OXIDATIVE STRESS IN PEDIATRIC PATIENTS WITH LUNG INFECTION

The evidence of the oxidative stress situation in 40 pediatric patients were found with high of MDA and HA concentrations and show low antioxidants with the mean value of Vit E level in the blood in the 1st day. The MDA level in the 1st day of group A and B showed high concentration in serum (21.58±1.3 μmol/L and 21.43±1.6 μmol/L) and in tracheal aspirates (34.9±3.7, 31.6±3.2 μmol/L) (Table 5, Figure 15). In the normal value of serum MDA level had recorded that in serum should be less than 10 μmol/L (Yagi, 1998). The mean value of MDA in this study

was higher than in the previous report that studied in the 32 patients with lower airway infection (3.5±1.4 μmol/L) (Nowak, et al., 1996).

Hyaluronic acid (HA) can be produced in the situation of inflammation and remolding of the tissue, including lung tissue and its rapid turnover in most tissue with a t1/2 in the order of 1 day. (Johnsson, et al., 1998) The increasing HA concentration in a few experiments showed direct effect of oxygen-induced stimulation of inflammatory mediators (Elias, et al., 1988), or of oxygen free radicals (Tate, et al., 1982). HA is used to be a marker in arthritis (RA) and osteoarthritis (OA), and the levels in the serum about 130.1±82.5 ng/ml and 160±135.66 ng/ml respectively (Pothacharoen, 2000). In inflammatory response showed increasing HA concentrations in 24 patients with ankylosing spondylitis (AS) (30.3±3.0 mg/L), and in RA (171±13 mg/l) higher than controls (22.4±1.6 mg/L). However, the concentrations of hyaluronic acid in some tissue and fluid showed various, 0.1-0.01 mg/l in human serum, 3600-1420 mg/l in human synovial fluid (Laurent, et al., 1989). HA levels are elevated 9-18 folds, suggesting a role for HA in tumor progression and metastasis (Knudson, et al., 1989; Toole, et al., 1989). In this study showed HA level was very high values in group A and group B in the first day, 3545.3±733.3 ng/ml and 3402.1±720.9 ng/ml in group A and group B respectively (Table 8, Figure 18). These higher value had no report in the past.

Gutathione (GSH) in the blood is one of antioxidant for evaluation of oxidative stress in various diseases. GSH can be extra-cellular and intracellular fraction. In the lung disease, there is detection of GSH which is at the lung epithelial lining fluid more than in the blood, because it can prevent hydroxyl formation in the extra-cellular space. (Cantin, et al., 1987). The glutathione can be detected in the whole blood and erythrocytes. In this study showed the significantly correlation between the concentration of GSH in mg per deciliter of whole blood and pack red cell. (Table 10-12). Although many papers represented a low

concentration of GSH; for examples, the study in premature infants showed relatively high plasma concentration of GSSG from the oxidation of GSH or the decrease of effectiveness of reduction of GSSG by cellular GSH reductase. (Smith, et al., 2000), and the preliminary studies showed that plasma GSH level in preterm with respiratory distress were markedly lower than in normal preterm neonates (Jain, et al., 1995). But the GSH concentration in this study on the 1st day had same amounts as in the study of Metscvasht et al (1999) (Table 6). The early postnatal life, days 2-4 of newborn babies has differ significantly of GSH concentrations from healthy adult (66.4-12.5 mg/dl red blood cells).

Vit E is located in the membrane bilayer of the cells and its distribution depends on the presence of extra-cellular lipids such as surfactant or lipoproteins; Indeed, Vit E is secreted by alveolar type II cells together with surfactant lipid (Rustow, et al., 1993). It can inhibit of lipid oxidation by trapping the chain-carrying $LOO^{\bullet}$ and its structure change to unreactive alpha-tocopheroxyl radicals (α-$TO^{\bullet}$) (Niki, et al., 1996). From the values of Vit E concentrations in the 1st day of both groups showed the low concentrations of Vit E in the serum and tracheal aspirates (6.3±1.1 and 2.4±.7 mg/L respectively) (Table 7). The values of Vit E in sera of group A in our patients showed higher than in the previous study that performed in 16 adult patients with septic shock (3.6±2.0 mg/L) (Goode, et al., 1995), but less in group B. The value of normal healthy infants age under one year, should be more than 9 mg/L (Halliwell, et al., 1989).

All substances were showed that low of antioxidant concentrations and the high activity of free radicals. In this study found that all patients represented chronic lung disease, such as respiratory distress syndrome (RDS), brochopulmonary dysphasia (BPD), pneumonia, and respiratory failure (Table 15). There are some studies of the oxidative stress in the lung, especially in neonate or infants similar to this works (Russell et al (1994).

THE EFFECTS OF PHYSICAL THERAPY

From the six days of physical therapy with two methods of treatments A and B, the values of MDA, GSH, Vit E and HA levels between group A and B showed no statistically different.

Both methods of physical therapy could reduce in the mean values of MDA concentrations in serum and tracheal aspirates which produced from lipid peroxidation on the 6th day when compared with the 1st day. (Figure 15).

General observation showed the mean value of GSH concentration in serum and tracheal aspirates increased in the 6th day of treatment. The changes of GSH concentration in the TA were found significance reduced in both groups. But in the blood, GSH concentration reduced significantly only in group B treatment. Although GSH concentration in TA of group B on the 1st day was lower than in the group A significantly, but the mean value of GSH concentration in the 6th day was increased as same as in the group A. (Figure 16)

The changes of Vit E in the blood and TA showed an improvement with higher concentrations in the 6th day compared with the 1st day. Although in the group B, the plasma Vit E concentrations in the 1st day were lower than group A, but in the 6th day were also increased. The Vit E levels in TA samples in both groups showed the same increasing after treatment, but the values showed much higher in TA than serum. (Figure 17)

Some surprising, the HA concentrations in the TA were found very high compared with in serum. In the 1st day, HA concentration in blood and TA in both groups were not statistically significant. Two methods of physical therapy could reduce the HA concentration in the serum. HA concentrations in TA were significantly reduced in the 6th day in only group B. (Figure 18)

In the lung injury data showed reduced in the lung injury score and improved in the oxygenation index (PaO_2/FiO_2 ratio) in both groups, but significantly only in group A. (Table 9, Figure 19)

All values in the 6th day compared with the 1st day, the changes in the group A patients who were treated with postural drainage, percussion, vibration and group B patients who were treated as same as in group A, and were received aerosol humidity supplement, showed values were not different.

In the group B treatment showed non-significantly reduced in lung injury score and increased in PaO_2/FiO_2 ratio, but in the group A showed positively. In group B showed significantly increased in GSH concentration and also reduced significantly in HA concentrations in blood and tracheal aspirates with p value less than 0.05. In group A showed also increased GSH levels in tracheal aspirates, and also increased in Vit E concentrations in the blood. The HA levels showed strong significantly reduced in the serum

From the changes of GSH, MDA, Vit E, and HA concentrations in TA in both groups within six days of physical therapy showed very surprising. In the result showed the declined trend of the MDA, HA and lung injury score, whereas inclined trend of the GSH, Vit E and PaO_2/FiO_2 (Table 10, Figures 20-25).

In conclusion of improvement, group B treatment showed improve the GSH concentration in blood and TA, as well as reduced of HA in the blood, In the same results of group A that showed increase of GSH and Vit E concentrations and HA in the blood. Group A treatment seemed to regain the antioxidants in the blood, GSH and Vit E so that it might be a reason of improved in lung injury score and oxygenation index than in group B that improved only in GSH concentration.

CORRELATION

The correlation of MDA, GSH, Vit E, and HA concentrations in blood and tracheal aspirates, lung injury score, and PaO_2/FiO_2 ratio were analyzed. A few evidences showed closed correlation between all substances that reflects to oxidative stress in the lung. There are many works that supporte this study i.e.. Studying the serum MDA had shown to be correlated with many substances in the blood; for example, studying in the 32 patients with lower airway infection showed that MDA was positive correlation with erythrocytes sedimentation rate (ESR) (Nowak, et al., 1996), and it showed a weak negative correlated with antioxidant in the 25 infants. (Drury, et al., 1998)

Alpha-tocopherol (Vit E) is an breaking-chain of lipid peroxidation. It showed a closed correlated with MDA in the blood such as in the experiment with premature baboon model of hyperoxia which was induced BPD (Berger, et al., 1998) and the previous study showed the Vit E closely negative correlated with MDA in cystic fibrosis (Goode, et al., 1995). Including, studying in 8 patients with ARDS showed high levels of MDA and low levels of Vit E (Metnitz, et al., 1999). Interestingly in vivo experiment in rat tissue, the Vit E-deficient rat showed high level of lipid hydroperoxides (Tokumaru, et al., 1997)

High HA concentrations in peripheral bronchi and alveoli were possibly response to the high local of coal dust. There is also evidence that GAGs were associated with lipid deposition in arterial walls. (Grundboeck-Jusco, et al., 1992)

Conclusion that many studies showed the correlation between MDA, HA, and Vit E and lipid peroxidation in various condition. However, there were not shown the relationship among HA, MDA, Vit E with GSH.

In this study showed the significant correlation between the erythrocytes GSH and whole blood glutathione concentrations in the blood in both groups.

Group A showed the significantly correlation in blood, MDA and HA, whereas in TA showed the correlation between MDA and Vit E; HA and MDA; HA and Vit E. (Table 11). But in group B showed the positive correlation between HA and Vit E levels in TA (Table 12).

The summary of 40 cases, (Table 13) showed three significantly correlated couples; (i) erythrocytes GSH and whole blood GSH; (ii) serum MDA and serum HA; (iii) serum MDA and erythrocytes GSH. In the TA showed significantly correlated couples; (i) Vit E and GSH; (ii) HA and MDA; (iii) HA and PaO_2/FiO_2 ratio; (iv) MDA and PaO_2/FiO_2 ratio, and (v) VitE and PaO_2/FiO_2 ratio.

A few studies have shown the highly correlation between GSH and MDA in the blood. This study supported the previous study of Nowak D et al (1998) and Drury TA et al (1998). GSH is one of antioxidant that can detoxifing lipid peroxides from peroxidation, therefore the low GSH concentration reflects to the high level of MDA in blood.

High MDA concentration in blood showed positive correlated with HA. In the lung damage, MDA products showed high level as same as the HA products from glycosaminoglycan degradation or remolding of tissue. HA can diffuse into the blood so that it can be detected in serum as same as the MDA found in serum.

The correlation between HA and MDA concentrations were not only found in the blood, but dramatically observed significantly correlation in the TA. The higher levels of HA and MDA cocentrations in the TA than in blood should be better marker and easily detected for predict the lung condition. Therefore the determination of MDA, HA, and Vit E concentrations in the TA may be a new choice of analysis for lung diseases.

BIOCHEMICAL SUBSTANCES IN TRACHEAL ASPIRATES

From the correlation results showed the possible markers for the evaluation and the progression of treatment. The levels of HA, MDA and Vit E in tracheal aspirates are higher concentrations than in serum (Table 5-8)

MDA may be a good marker for detection of the lung injury with lipid peroxidation. In many studies had demonstrated the correlation between MDA levels and lung infection. For examples; studying in 144 Tracheal aspirates from 86 preterm infants with oxygen dependent was found the strong positive correlation between MPO activity and MDA (Buss, 2000). Lipid peroxidation is a degraded process that occur from destruction of the lung tissue that found in the previous clinical study in 65 ventilated preterm infants with BPD. The results showed higher activity of elastase activity in tracheal aspirates, which were a risk for proteolytic destruction of lung parenchymal elastic fibers. (Bruce, et al., 1992) Changes of the MDA concentrations in the TA might be response of lung injury and the amount of MDA concentrations were found three fold greater than in plasma, Higher MDA level in fluid may be explained by the fact that the direct affect of damage to lung tissue such as in the study of Demling R et al (1994) in 18 adult sheep that showed the lung injury with smoke and inhalation promoted threefold higher MDA concentration in the airway fluid than in plasma.

HA may be a potential marker that found very high concentration in the TA in this study. Many papers reported the HA as a marker of joint diseases mostly. There are a few studies that show the correlation between HA and lung diseases; such as, studying in the patients with interstitial disease and sarcoidosis (Hallgren, et al., 1985), patients with acute extrinsic alveolitis (Bjermer, et al., 1987), COPD-induced horses (Tulamo, et al., 1997).

The mean value of GSH concentration in this study found in normal range (more than 47 mg/dl erythrocytes). The GSH concentration in the tracheal aspirates

was found similarly to the other studies, For examples, studying in preterm infants with chronic lung disease showed low level of GSH in BALF, 1.1 (0.5-3.1 μmol/L) compared with infants without chronic lung disease 4.7 (0.2-16.8 μmol/L) (Grigg, et al., 1993) and the level of total glutathione concentration in 19 healthy nonsmoking and 12 healthy smoking, ages 31±2 yrs, that found in epithelial lung fluid (ELF) showed higher than in plasma (429±34 μM ,3.0±0.6 μM, respectively) (Cantin, 1987). In this study found that there was no correlation between GSH in tracheal aspirates and lung injury as same as in the previous studying in BAL fluid or ELF concentration of GSH and the lung injury score.

The Vit E concentration in this study which detected by HPLC were much higher than in the plasma. It is opposite to Shock BC, et al (2001) that studied in 124 children (ages 1.6-12.6 yr) found the low concentration of VitE in BAL than in adults, 0.026 μM (mean) and 0.7 μM (mean) respectively. But the Vit E levels in serum in this study were same value found in the studying of Schok et al, ranges 6.83-34.65 μM (Shock, et al., 2001).

In conclusion, MDA, HA and Vit E concentrations in the TA were significantly closed correlation with PaO_2/FiO_2 ratio, except GSH concentration. From the report of Kelly FJ, et al (1995), the alveolar lining fluid showed high MDA concentration and low of Vit E concentration, in opposite to this study that found significance high levels of Vit E and low levels of GSH in tracheal aspirates. It can be explained that the high levels of Vit E in the tracheal aspirated may be the production from the lung-degraded tissue or may be one of antioxidants in the lung. Whereas the reducing of GSH concentration reflects to high free radicals in the lung. Whether or not all subjects of this study showed the remolding in lung tissue and improve antioxidant (Vit E) in the lung tissue from lipid peroxidation.

BIOCHEMICAL MARKERS AND LUNG INJURY

From many studies found the correlation between the lung injury severity with biochemical markers. For examples, the correlation between oxidative measures and myeloperoxidase concentrations in the lung indicated that neutrophil oxidants could be responsible for the injury (Winterbourin, et al., 2000).

Some studies found a good correlation between MDA and lung injury, such as studying in the 21 preterm babies with RDS (Moison, et al., 1998). The serum MDA level was higher in infants requiring mechanical ventilation compared to those breathing spontaneously, but MDA levels were no correlated with FiO_2 level and arterial oxygen tension. (Yigit, et al., 1998). In addition that results in this study showed a correlation between MDA and HA in tracheal aspirates significantly.

Previous study in 12 patients with ARDS showed higher levels of HA in serum (619 μg/L) than in normal subjects (353 μg/L). HA in BAL fluids showed inverse correlation with oxygenation index (PaO_2/FiO_2) as same as in this study (Yoder, et al., 1991).

However, in this study, the effects of various drugs were not included and analysed. All subjects were received only bronchodilator drugs; berodual and ventoline every day, and no evidences showed the relationship between drug effects on these biochemical markers. Most patients received paracetamol when they only showed fever. But there are some reports about antibiotic drug, dexamethasone that showed the effect of drug on neutrophils and elastase in tracheobroncheal lavage fluid (Haworth, 2000) and also reduced other potentially injurious mediators in the lung of BPD. Interestingly the results of mechanical ventilator or dexamethasone treatment does not induce activity of superoxide dismutase (SOD), catalase, or glutathione peroxidase (GPX) in the lungs of newborm piglets. (Davis, et al., 1995).

CLINICAL RESPONSE OF PHYSICAL THERAPY IN THIS STUDY

In the general view of patients who received the different treatments of physical therapy showed improved in antioxidant and reduced in free radicals. In 40 cases patients, there were some cases showed clinical improvement, but some cases showed non-improvement. The markers for that a clinical improve of the treatment was PaO_2/FiO_2 ratio and the ventilator setting in six days.

All cases could be classified into two groups, improved group (21 from 40 cases) and non-improved group (19 from 40 cases). Surprisingly the results of MDA, GSH, Vit E, and HA in blood and tracheal aspirates (TA) were found as following (Table 14);

In the improved group, the levels in blood and tracheal aspirates showed significantly increased in GSH concentration as well as significantly reduced in MDA and HA in the 6^{th} day compared with the 1^{st} day. The lung injury showed significance decreasing dramatically as well as the increasing the PaO_2/FiO_2 ratio on the 6^{th} day. But Vit E showed increasing only in the TA. For in the non-improved group showed the only increasing in GSH in TA, and reducing in HA in the blood. Lung injury and oxygenation index in this group were not shown improvement. Therefore, clinical improvement of physical therapy depended on the changes of antioxidant compounds and free radical action in the lung.

For both groups were classified as the improved and non-improved groups. There are the significant correlation between biochemical substances and lung injury score; (i) between MDA and HA in TA (Figures 29-30), and (ii) between MDA in TA and PaO_2/FiO_2 ratio (Figures 31-32).

Table 14. The concentrations of GSH, MDA, Vit E, HA, lung injury score and PaO_2/FiO_2 ratio between improved and non-improved groups from all patients.

	Day	**Improved group (n=21)**		**Non-improved group (n=20)**	
		Blood	TA	Blood	TA
GSH (mg/dl)	1	53.0±3.4	2.7±0.4	65.5±3.3	2.3±3.7
	6	71.9±3.2**	6.4±0.5**	63.4±3.3	3.8±.61*
MDA (umol/L)	1	21.9±0.9	33.1±2.9	12.4±1.1	33.3±4.0
	6	18.5±1.2**	24.5±1.7*	22.4±1.3	34.3±3.6
Vit E (mg/L)	1	5.6±1.1	16.6±3.5	3.5±8.4	24.0±3.3
	6	7.5±1.6	37.3±4.2**	3.9±1.1	19.2±4.8
HA (X100 ng/ml)	1	270.0±5.4	931.8±206.7	447.8±8.2	585.0±209.6
	6	121.9±2.6**	153.6±50.2**	286.3±4.8*	577.3±231.2
Lung injury Score	1	1.1±0.2		1.1±0.2	
	6	0.9±0.2*		1.0±0.2	
PaO_2/FiO_2	1	121.5±13.9		143.5±15	
	6	186.7±11.6**		132.3±9.6	

TA = tracheal aspirates, GSH = glutathione, MDA= malondialdehyde, HA = hyaluronic acid, Vit E = alpha-tocopherol. Data represent mean±SE in each groups. Significantly difference in the 6^{th} day compared with in the 1^{st} day. * $P<0.05$, ** $P<0.01$

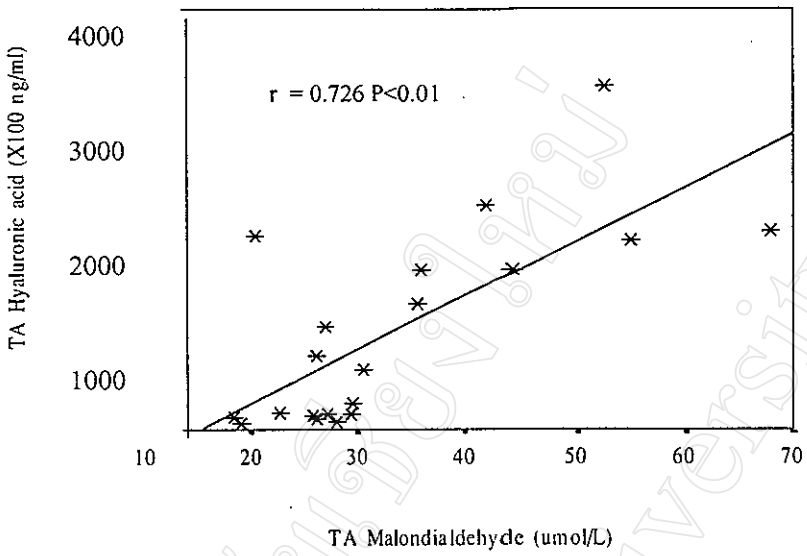

Figure 29. Scatter plots showing relationship between malondialdehye and hyaluronic acid in tracheal aspirate in improved group. Each point represents data from a single case. Solid line represents the correlation line.

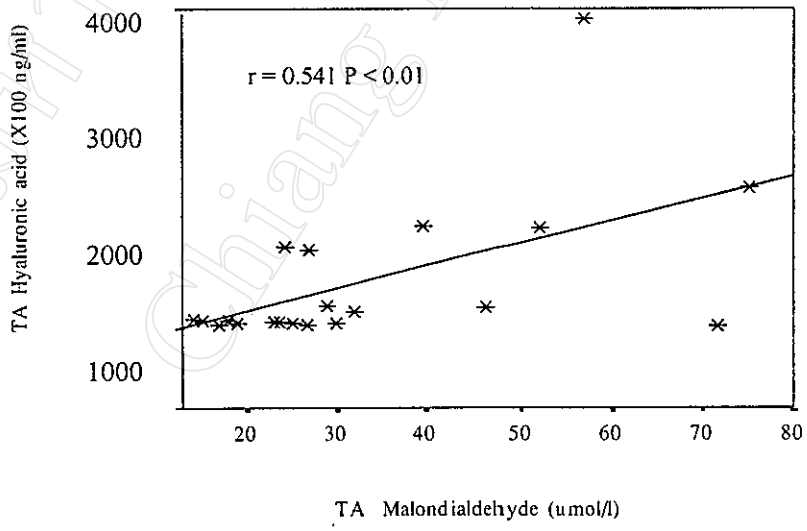

Figure 30. Scatter plots showing relationship between malondialdehye and hyaluronic acid in tracheal aspirate in non-improved group. Each point represents data from a single case. Solid line represents the correlation line.

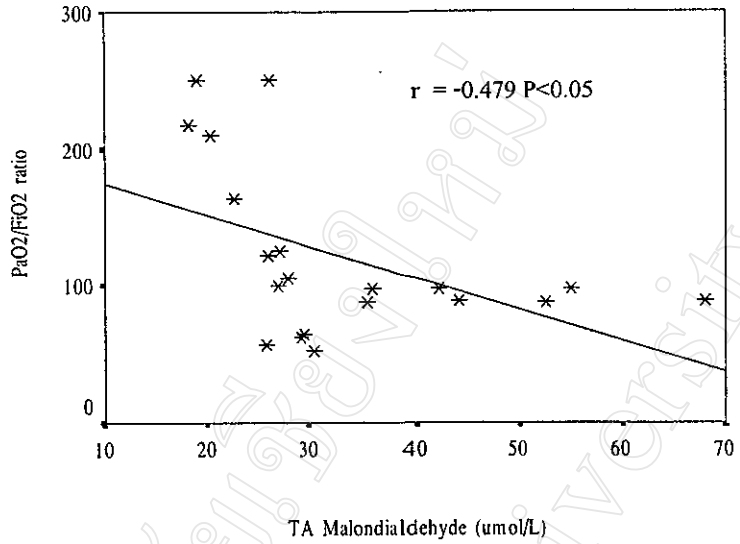

Figure 31. Scatter plots showing relationship between malondialdehye in tracheal aspirates and PaO_2/FiO_2 ratio in improved group. Each point represents data from a single case. Solid line represent the correlation line.

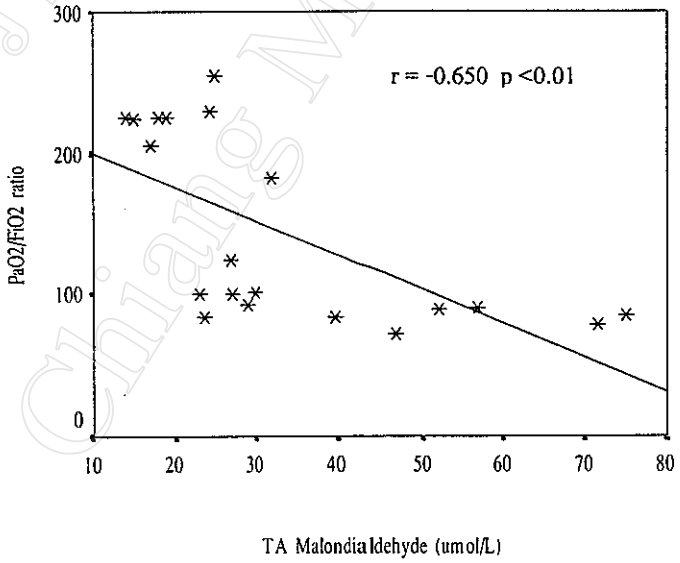

Figure 32. Scatter plots showing relationship between malondialdehye in tracheal aspirates and PaO_2/FiO_2 ratio in non-improved group. Each point represents data from a single case. Solid line represents the correlation line.

SUMMARY

The results of this study showed the improvement in the oxygenation index and reduced in lung injury after physical therapy for six days by removing secretion and lung atelectasis. The results also showed the changes of biochemical markers of oxidative stress by increasing GSH and Vit E concentrations, as well as reducing HA and MDA concentrations in the blood and TA.

This study also found the correlation of biochemical markers in the tracheal aspirate fluid, which TA were easy and comfortable to collect, in the incubated padiatric patients from the routine daily suction. In the blood showed a good significance positive correlation between serum MDA and HA, and showed inverse correlation between serum MDA and erythrocyte GSH. Although no correlations were found among GSH, MDA, HA, and Vit E in the blood and trahceal aspirates.

For the potential markers in this study, i.e. HA, MDA and Vit E levels which detected in tracheal aspirates (TA), were significantly correlation with PaO_2/FiO_2 ratio that was directly affect on lung injury.

From the study showed the clinical responsiveness from physical therapy showed improved in GSH, and PaO_2/FiO_2 ratio, including reduced in MDA, HA and lung injury score in the improved group more than non-improved group. In addition they found the correlation between HA and MDA in TA, as well as the correlation between MDA in TA with PaO_2/FiO_2.