

# Pulmonary Parenchymal Alveolar Histological Study in Experimental Tracheo-oesophageal Malformations

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## ABSTRACT

**Objective:** Children operated on for tracheo-oesophageal malformation (TOM) often suffer from postoperative respiratory system difficulties. There is little current literature about this subject. This study aimed to investigate the causes of these problems in rats with experimental TOM by evaluating the lung alveolar histology.

**Subjects and methods:** Twenty Wistar albino rats were used for the experiment. Twelve rats with a sperm positive vaginal smear received 1.75 mg/kg intraperitoneal adriamycin on days six to nine of gestation. A sham group was infused with saline instead of adriamycin. A control group was not subjected to any additional procedure. Their fetuses were dissected under surgical microscope. After examining the trachea and oesophagus, the lungs were dissected and fixed in 10% formalin. The groups were compared with respect to alveolar flat cell (Type-1), capillary density and air space percentage in the samples obtained under light microscopy. Statistical evaluation was performed through Mann-Whitney-U tests and Pearson Chi-squared tests.

**Results:** Type-1 cell ratio and air space percentage were the highest for the control and sham groups. However, the group that received adriamycin and developed TOM had the lowest values. There were no statistically significant differences between the groups with respect to capillary density.

**Conclusion:** In rats with experimentally produced TOM, the pulmonary parenchyma showed delayed maturation. This could be the cause of the frequently seen respiratory system pathologies in children suffering from TOM. Further studies should be done to elucidate this.

# Estudio Histológico Alveolar y Parenquimal Pulmonar en Malformaciones Tráqueo-Esofágicas Experimentales

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## RESUMEN

**Objetivo:** Los niños operados por malformaciones tráqueo-esofágicas (MTE) sufren a menudo de dificultades postoperatorias en el sistema respiratorio. En el presente, existe poca literatura sobre este problema. Este estudio va encaminado a investigar las causas de estos problemas en ratas con MTE experimentales, mediante la evaluación de la histología alveolar del pulmón.

**Sujetos y métodos:** Veinte ratas albinas Wistar fueron usadas en el experimento. Doce ratas con froto vaginal positivo de esperma recibieron 1.75 mg/kg de adriamicina intraperitoneal en los días seis a nueve de gestación. A un grupo de simulación (sham group) se le dio una solución salina en lugar de adriamicina. El grupo de control no fue sometido a ningún procedimiento adicional. Sus fetos fueron disecados bajo microscopio quirúrgico. Luego de examinar la tráquea y el esófago, los pulmones fueron disecados y puestos en formaldehído al 10%. Recurriendo a la microscopía lumínica, los grupos fueron comparados con respecto a células alveolares planas (tipo 1), densidad capilar y porcentaje de espacio de aire en las muestras obtenidas. La evaluación estadística se realizó mediante tests U de Mann-Whitney y tests de Chi-cuadrado Pearson.

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**Resultados:** La proporción de células de tipo I y el porcentaje de espacio de aire, fueron los más altos para los grupos de simulación y control. Sin embargo, el grupo que recibió adriamicina y desarrolló MTE, tuvo los valores más bajos. Con respecto a la densidad capilar, no hubo diferencias estadísticamente significativas entre los grupos.

**Conclusión:** En ratas con malformación MTE producida experimentalmente, el parénquima pulmonar mostró retardo en su maduración. Esto podría ser la causa de las patologías del sistema respiratorio observadas con frecuencia en niños que padecen de MTE. Se requieren estudios ulteriores a fin de dilucidar esta cuestión.

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## INTRODUCTION

The frequency of Tracheo-oesophageal malformations (TOMs) varies between 1/3000 and 1/5000 births (1). The early disturbance of organogenesis that results in oesophageal atresia (OA), regardless of the exact cause, also affects other systems. Numerous reports have claimed that between 50% and 70% of infants with OA have associated anomalies (2). In 1973, Quan and Smith suggested a broad spectrum of associated malformations that might or might not appear together and are associated with TOM (3). These associated malformations affect the survival of the patient. Respiratory symptoms occur even after effective repair of TOM. These complaints have been attributed to aspiration due to gastro-oesophageal reflux (GOR), motility disorders of the oesophagus and morphological and histological disorders in the tracheo-bronchial tree (4, 5).

Various reports have shown that children with TOM often suffer from asthma and bronchitis associated symptoms which persist until adolescence. Half of these children have a history of hospitalization (6, 7).

In a series of 334 cases with OA, while 31% of those under five years of age were reported to suffer from pneumonia, 74% had bronchitic attacks (7). Spirometric studies revealed both obstructive and restrictive abnormalities in half of these patients, a third of whom were shown by bronchoscopy to have narrowing of the tracheo-bronchial tree and histologically, to have inflammation (8, 9). These findings were observed in the postoperative period. No pre-operative observations exist concerning pulmonary pathologies in patients with TOM.

There are four overlapping stages of lung development spanning the embryonic to childhood periods. In the pseudoglandular period, the lung resembles a gland. In the canalicular period, the lung tissue becomes highly vascularized and respiratory bronchioles, alveolar ducts and some primitive alveoli (terminal sacs) are seen. Towards the end of the canalicular period, respiration becomes possible because a part of the "terminal sacs" with thin walls (alveoli) on the ends of respiratory bronchioles is formed. In the terminal sac period, the terminal sacs are initially covered with cubic epithelium. Later on, cubic epithelia is thinned, turning into a flattened epithelium. In this period, the epithelia of terminal sacs are highly thinned. In the alveolar period, however, the epithelia of terminal sacs alter in shape to very thin flat epithelia. Type-I alveolar cells are so thin that adjacent

capillary vessels protrude into the terminal sacs, facilitating gas exchange. Throughout alveolar maturation, the thin cubic epithelium lining the air sacs which later become a single layered flat epithelium with increased density of capillary veins covering these sacs provide alveolar maturation which facilitates gas exchange (10, 11).

This study was planned to investigate whether pulmonary complaints of patients with OA were not only associated with GOR-related aspiration, motility disorders and morphological changes in the tracheo-bronchial tree but also associated with disorders in alveolar pulmonary histology. This study aimed to find out whether a difference in alveolar histology can be responsible for respiratory symptoms after TOM operations occur.

## SUBJECTS AND METHODS

Twenty female Wistar albino rats with mean weight of 210 g were time mated. A sperm positive vaginal smear received in the morning after mating represented day 0 of gestation. Twelve rats received an intraperitoneal injection of 1.75 mg/kg of adriamycin dissolved in 2 ml of normal saline on days six to nine of gestation (12). A sham group, four rats, received 2 ml intraperitoneal normal saline injection on days six to nine of gestation. Four rats in the control group were not subjected to any procedure.

The fetuses were harvested by laparotomy on the 21<sup>st</sup> embryonic day. Thoracotomy was performed on the newborns under a binocular surgical microscope. After examining the trachea and oesophagus for TOM, fetal lungs were dissected, removed and fixed in 10% formalin. The lung samples were dyed with haematoxylin-eosin, periodic acid Schiff and trichrome, and evaluated under light microscopy. Type-I cell ratio, capillary density and air space percentage of all the groups were determined and compared. The numerical data were described as mean  $\pm$  SD and compared when appropriate. Mann-Whitney U and Pearson Chi-square (for capillary density) tests were used in statistical comparisons. This study was approved by the Local Hospital Experimental Committee.

The study involved 33 newborn rats, six rats in each of the control and sham groups (n = 12), nine rats in the group that received adriamycin and developed no (TOM-) and 12 rats in the group that received adriamycin and developed (TOM+) (Table).

Table: Mean  $\pm$  SD of Type 1 cell ratio, capillary density and air space percentage of fetal rat lung.

Group	Type 1 cell ratio	Capillary density	Air space percentage
Control (n = 6)	68.33 $\pm$ 10.32	1.50 $\pm$ 0.54	75.92 $\pm$ 4.98
Sham (n = 6)	67.98 $\pm$ 9.89	1.52 $\pm$ 0.48	75.83 $\pm$ 4.91
Adriamycin, TOM (-) (n = 9)	57.77 $\pm$ 13.94	1.66 $\pm$ 0.5	75.55 $\pm$ 12.61
Adriamycin, TOM (+) (n = 12)	3.33 $\pm$ 6.5*	1.83 $\pm$ 0.36	54.58 $\pm$ 4.50*

Mann-Whitney U test was used for Type 1 cell ratio and air space percentage and Pearson chi-square test was used for capillary density.\*  $p < 0.05$

## RESULTS

Type-1 cell ratio was highest in the control group (68.33%; Table; Fig. 1). It was the lowest in the group that received

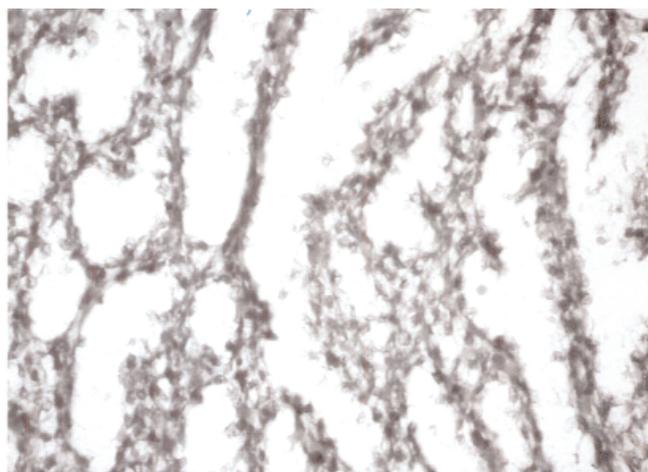


Fig. 1: Pulmonary tissue air space ratio was high and alveoli are covered with Type 1 flat epithelium in control group (H&E x 100).

adriamycin and developed TOM (3.3%, Fig. 2, Table). The difference between the groups was statistically significant ( $p = 0.001$ ). There were no statistically significant differences in Type-1 cell ratio between the control and sham groups ( $p > 0.05$ ). Furthermore, no statistically significant difference in Type-1 cell ratio was detected between the group that developed no TOM (Fig. 3) and control and sham groups ( $p = 0.081$ ). In the group that received adriamycin and developed TOM, almost all of the primordial alveolar were formed by cubic epithelia. There were no flat alveolar epithelium upon maturation of the alveoli. The air-space ratio in the tissues was 50%–60% and most of the alveoli had a collapsed appearance. In other words, there was delayed alveolar development (Fig. 2). There were also sporadic mitotic activity in the lymphocytes and epithelial cells observed in the capillaries.

The inter-group comparison of capillary density (Table) revealed no statistically significant differences ( $p >$

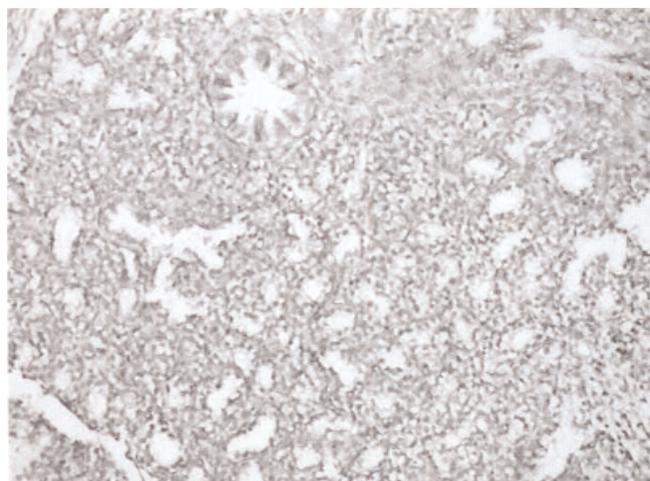


Fig. 2: Pulmonary tissue air space was low and most alveoli were collapsed. Alveoli were covered with cubic epithelium in adriamycin plus TOM (+) (H&E x 100).

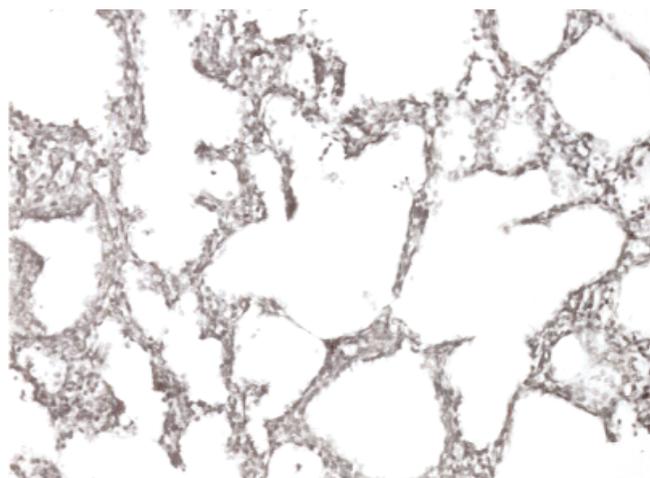


Fig. 3: Pulmonary tissue air space ratio was high and alveoli are covered with Type 1 flat epithelium in adriamycin plus TOM (-) (H&E x 50).

0.05, all comparisons). The highest value was detected in the group that received adriamycin and developed TOM (1.83) and the lowest value was detected in the control group (1.50). The capillary density of the sham group was 1.52 and 1.66 in the group that did not develop TOM.

The highest ratio of air to space was in the control group (75.92%). The air space ratio for the sham group was 75.83%, while the lowest ratio was 54.83% for the group that developed TOM (Table). The air space ratio for the group that developed no TOM was 75.55%. The comparisons of the air space ratios of the groups revealed that there were no statistically significant differences between the control, sham and TOM (-) groups ( $p > 0.05$ ) whereas there was a statistically significant difference between the control, sham versus TOM (+) groups and TOM (+) and TOM (-) groups respectively ( $p = 0.001$ ,  $p = 0.002$  and  $p = 0.001$ ).

## DISCUSSION

Pulmonary complications are among the most frequent causes of morbidity and mortality in TOM and after repair of TOM. These complaints have been attributed to GOR, motility disorders of the oesophagus and morphological and histological disorders in the tracheo-bronchial tree (4, 5). However, no clinical observations exist concerning pre-operative alveolar structure in children with TOM and neither are there many experimental studies. In the present study, Type-1 cell ratio, capillary density and air space percentage were considered bases for evaluation of pulmonary alveolar histology in TOM model.

A previous study on respiratory complaints experienced by patients with OA aimed to show that the complaints were not necessarily due to GOR and motility disorders but associated with the lung parenchyma. To this end, in addition to oesophageal histology, alveolar size and septal wall thickness have been studied. The highest mean alveolar size and the lowest mean alveolar septal wall thickness were reported to be in the group with OA. Thus, lung maturation of the group with OA was more advanced, and no association between pulmonary problems and parenchyma was established (13). Contrary to the findings of that study, in our study the lowest air space percentage, the lowest Type-1 cell ratio and the highest capillary density were detected in the group that received adriamycin and developed TOM.

In the light of the findings of the present study, it might be said that the patients who are given adriamycin and develop TOM have delayed pulmonary maturation and disordered endoderm and mesoderm interaction (low Type-1 cell ratio, disordered capillary density and type cell ratio).

Pulmonary complaints developing in patients with OA and persisting until adolescence may be attributed to delayed maturation of pulmonary parenchyma. After adolescence, fewer pulmonary complaints may be associated with completion of maturation.

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