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# Increasing of Caspase-1 mRNA in the Lung and Heart of Hyperthyroid Broiler Chickens

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**Abstract:** To clarify the effect of T3-induced hyperthyroidism on Caspase-1 mRNA expression in the lung and ventricles of the heart, semi quantitative reverse transcription-PCR was performed on total RNAs isolated from broiler chicken lung and heart after feeding supplementary T3 (1.5 mg T3 $kg^{-1}$) for 2 weeks. The *Caspase-1* genes were expressed in the lung and heart (right and left ventricles) of control and $T_3$-treated broilers at 14th day of ages. The relative amount of Caspase-1 mRNA expression in the lung, right ventricle and left ventricle of heart was higher ($p<0.05$) in T3-treated broilers than in control broilers at 14th day of age. Among T3-treated groups, the relative amount of Caspase-1 mRNA expression in the lung was also significantly ($p<0.05$) higher than left and right ventricles of heart while the variations among control groups were not significant. In conclusion, *Caspase-1* gene was constitutively expressed in the lung and heart of broiler chickens. It is probable that increased *Caspase-1* gene expression in the lung and ventricles of heart are involved in the pathophysiology of cardiovascular function in broilers with hyperthyroidism. Further studies are required to find the pathways involved in the apoptosis.

**Key words:** Broiler chicken, hyperthyroidism, caspase, apoptosis, heart, cardiovascular, Iran

## INTRODUCTION

Apoptosis, programmed cell death is a regulating mechanism enabling the removal of superabundantly produced and unnecessary at the certain moment cells (Bernecker *et al*., 2003). Disturbances of the apoptosis regulation contribute to the pathogenesis of many diseases. Apoptosis is triggered by a variety of intracellular and extracellular signals. The molecular events involved in apoptosis are largely mediated by specific cysteine proteases called caspases (Nicholson and Thornberry, 1997; Wencker *et al*., 2003). Generally, the apoptotic process is initiated by the activation of ≥1 initiator caspases (Caspase-1, -2, -8, -9 and -10).

The initiator caspases activate ≥1 executioner or effector caspases (Caspase-3, -6 and -7) by cleavage of their proenzymes. The effector caspases lead to the successive dismantling of the cell by cleaving intracellular substrates such as poly (ADPribose) polymerase, specific cytoskeletal proteins and nuclear lamins (Lincz, 1998; Nunez *et al*., 1998; Sundaresan *et al*., 2008). Thyroid hormone affects all tissues and modulates the rate of metabolic activity. Thyroid hormones increase metabolism, oxygen consumption and in certain molecular pathways in the heart and vasculature cause cardiovascular derangement. It is well established that hyperthyroidism induces a hyperdynamic cardiovascular state (high cardiac output with low systemic vascular resistance) which is associated with a faster heart rate, enhanced ventricular systolic and diastolic function and cardiac hypertrophy (Fazio *et al*., 2004; Hassanpour *et al*., 2010).

The pathogenesis of tissue dysfunction in severe hyperthyroidism is unknown. Ultrastructural and functional changes in mitochondria such as enlargement, a mass increase and formation of megamitochondria have been reported in the tissues of hyperthyroid patients and in a rat model of hyperthyroidism. Decrease in mitochondrial transmembrane potential and proton motive force and altered cellular oxidation-reduction occur during mitochondrial-mediated apoptosis (Kalderon *et al*., 1995;

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Upadhyay *et al*., 2004). The objective of this study was to determine concentrations of Caspase-1 mRNA expression in the lung and right and left ventricles of heart in broiler chickens with hyperthyroidism induced experimentally by 3, 5, 30-ltriiodothyronine (T3).

## MATERIALS AND METHODS

**Animals:** Twenty four, 1 day old fast growing chickens (Ross 308) were divided at random into two equal groups with three replicates per group. Chicks were reared for 2 weeks in floor pens on wood shaving litter under standard conditions with *ad libitum* access to water and a standard ration (Starter: 13 MJ ME $kg^{-1}$ of diet, 230 g $kg^{-1}$ Crude Protein (CP) formulated to meet requirements for broilers. In the treatment group, T3 was added to the ration (1.5 mg T3 $kg^{-1}$) after 5 days of rearing (Hassanpour *et al*., 2009). At the end of rearing, 6 chicks from each group were randomly selected and then were killed by decapitation. The lung and heart immediately removed, frozen in liquid nitrogen and stored at -70°C for subsequent RNA analysis.

**RNA extraction of lung and heart (right and left ventricles) tissue:** Single-step, acid guanidinium thiocyanate/phenol/chloroform extraction was used for total RNA extraction of right and left ventricular tissues (Chomczynski and Sacchi, 1987). Homogenised lung tissue (100 mg) was prepared in a denaturing solution containing 4 M guanidinium thiocyanate. The homogenate was mixed sequentially with 2 M sodium acetate (pH 4), water-saturated phenol and chloroform/isoamyl alcohol (49:1). The resulting mixture was centrifuged, yielding an upper aqueous phase containing total RNA. Following 100% isopropanol precipitation, the RNA pellet was redissolved in denaturing solution, reprecipitated with isopropanol and washed with 75% ethanol. The RNA samples were resuspended in DEPC-treated water. Amount and quality of RNA were determined by spectrophotometry. Only RNA of sufficient purity having an absorbance ratio (A260/280) >1.9 was considered for synthesis of cDNA. It was analysed by electrophoresis on a 1.5% agarose gel, stained with 0.5 mg $mL^{-1}$ ethidium bromide.

**Semiquantitative reverse-transcription PCR:** The extracted RNA was reverse-transcribed to cDNA in a 20 mL volume containing 1 mg of extracted RNA, 200 ng random hexamer, 0.5 mM dNTP. This mixture was heated to 65°C for 5 min and 40 u of RNase inhibitor, RT buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$), 10 mM DTT and 200 uM-MLV reverse transcriptase (Invitrogen, Karlsruhe, Germany) were added. This mixture was incubated for 10 min at 25°C followed by 50 min at 37°C. The prepared cDNAs were heated at 75°C for 15 min to denature the M-MLV reverse transcriptase and stored at -20°C. The primer sequences for the PCR reactions are following as: Caspase-1, 5'- CGGCCAGCGCCATCTTCATT-3' and 5'-AGGGAGCTGTCACAGTGCGT-3'; β-actin (used as a housekeepinggene),5'-ACTGGATTTCGAGCAGGAGAT-3' and 5'-TTAGAAGCATTTGCGGTGGACAA-3'. Normalisation of the samples was accomplished using RT-PCR for the housekeeping gene *β-actin* to control the efficacy of the RNA extraction, integrity and amount of Caspase-1 mRNA present in the samples. PCR reaction conditions were optimised for each of the primer pairs to obtain a linear relationship between input RNA and final PCR product.

The PCR was performed in a total volume of 25 mL containing 5 mM Tris-HCl; 10 mM NaCl; 0.01 mM EDTA; 1.5 mM $MgCl_2$; 0.1 mM of each dNTP; 0.1 mM of each primer; 2 μL cDNA and 1.25 u Taq polymerase (Promega, Germany). The PCR program for Caspase-1 consisted of 2 min at 94°C, 25 cycles of amplification (50 sec at 94°C, 50 sec at 66°C and 45 sec at 72°C). The PCR program for β-actin consisted of 2 min at 94°C, 20 cycles of amplification (30 sec at 94°C, 30 sec at 60°C and 60 sec at 72°C).

An aliquot of each reaction mixture was subjected to electrophoresis in 2% agarose gel and stained with 0.5 mg $mL^{-1}$ ethidium bromide. Density of bands was determined using Photo-capt V.99 image software and relative densities were expressed as Caspase-1/β-actin density.

**Statistical analysis:** All results are represented as mean±SEM. Comparisons were made between control and T3-treated groups at the same age for each lung and left and right ventricle of heart using Independent-sample t-test (SPSS-14.0 package). Comparisons were also made among lung, right ventricle and left ventricle of heart at the same group using ANOVA with $p<0.05$ accepted as significant.

## RESULTS

**Effect of T3 on Caspase-1 mRNA expression in the lung and heart (right and left ventricles):** Expression of *Caspase-1* gene was studied using semi-quantitative RT-PCR in the lung and heart (right and left ventricles) of broiler chickens after 2 weeks of rearing period. Reverse transcription-PCR results are shown in Table 1 and Fig. 1 and 2. The expression of β-actin was detected in all

Table 1: Relative density of Caspase-1/β-actin PCR products in the lung and heart (ventricles) of chickens

| Groups | n | Lung | Right ventricle | Left ventricle |
|---|---|---|---|---|
| Control | 6 | 2.396±0.083[a] | 1.956±0.139[a] | 2.024±0.084[a] |
| Treatment | 6 | 3.556±0.066[a] | 2.702±0.117[b] | 2.664±0.081[b] |

Values are means±SE; n: number of chickens; [a, b]Means with the different indices at each row are significantly different for $p<0.05$

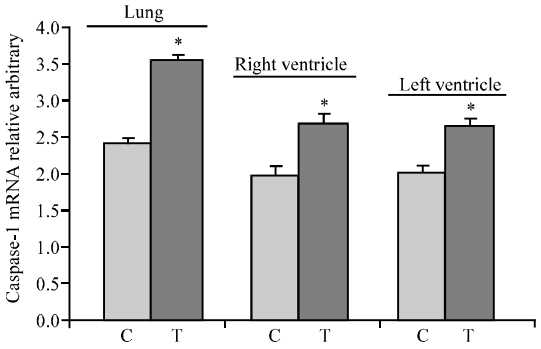


Fig. 1: Comparison of relative density of Caspase-1/β-actin PCR products in the lung and heart (ventricles) of Chickens in the intact (C) and T3-induced hyperthyroid (T) chickens at 14th day of age. Values are means±SE. *$p<0.05$ from corresponding control

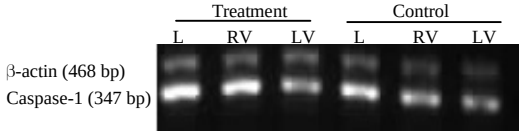


Fig. 2: Gel electrophoresis of semi-quantitative RT-PCR for determination of Caspase-1 mRNA levels of Left (LV) and Right (RV) ventricles of heart and Lung (L) in the intact (control) and T3-induced hyperthyroid (Treatment) chickens

samples. The *Caspase-1* genes were expressed in the lung and heart (right and left ventricles) of control and T3-treated broilers at 14th day of ages. The relative amount of Caspase-1 mRNA expression in the lung, right ventricle and left ventricle of heart was higher ($p<0.05$) in T3-treated broilers than in control broilers at 14th day of age. These increasings in the lung, right ventricle and left ventricle of heart were 48.4, 38.1 and 31.6%, respectively. Among T3-treated groups, the relative amount of Caspase-1 mRNA expression in the lung was also significantly ($p<0.05$) higher than left and right ventricles of heart while the variations among control groups were not significant (Table 1).

## DISCUSSION

This research was designed to investigate *Caspase-1* gene expression in the lung and heart of broiler chickens in both intact and T3-induced hyperthyroid chickens. The primary function of Caspase-1 is processing of inflammatory cytokines like Interleukin (IL)-1β (Slee *et al*., 1999). However, Caspase-1 is involved in the ceramide-induced apoptosis in chicken oviductal cells (Kim *et al*., 2005). Further in mammals upregulation of the Caspase-1 mRNA in the corpus luteum is coincident with the onset of luteolysis (Rueda *et al*., 1997). Caspase-1 is a key enzyme in converting IL-18 and IL-1 to their active forms (Kondo *et al*., 1996).

In the current study, constitutive expression of Caspase-1 was noted in the lung and heart of chickens. Sundaresan *et al*. (2008) also concluded that Caspase-1 is distributed in postovulatory follicles. Thyroid hormone has relevant effects on the pulmonary and cardiovascular system (Klein and Ojamaa, 2001). Most of the molecular and cellular mechanisms of thyroid hormone have been clarified but it is not well known in the hyperthyroidism especially in broiler chickens. Thyroid hormone may exert both genomic and nongenomic effects. Different studies reported that excessive thyroid hormone increase apoptosis. Upadhyay *et al*. (2004) found an increased DNA fragmentation in the liver of hyperthyroid rats which suggested increased apoptosis. They also studied Caspase-3 activation and observed significant increases in Caspase-3 activity in hyperthyroid rats. Excessive thyroid hormone induces cardiac hypertrophy and promotes heart failure in patients with hyperthyroidism but the mechanism remains elusive. Wang *et al*. (2010) confirmed that Apoptotic rates increased and DNA laddering was detectable in T4-treated rat hearts. They suggested that this enhanced apoptosis may lead to heart failure. In the study, the researchers found that *Caspase-1* gene is expressed in the lung and heart of hyperthyroid chickens more than intact chickens which is evidence of increased apoptosis in the hyperthyroidism. Of course, these data showed that apoptosis in the lung of hyperthyroid chickens were higher than heart.

## CONCLUSION

This study shows that *Caspase-1* gene was constitutively expressed in the lung and heart of broiler chickens. It is probable that increased *Caspase-1* gene expression in the lung and ventricles of heart are involved in the pathophysiology of cardiovascular function in broilers with hyperthyroidism. Further studies are required to find the pathways involved in the apoptosis.

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