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Research Paper

Association Between Cardiac Telomere Length and Lipid Peroxidation in Pulmonary Hypertensive Chickens

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ABSTRACT

Background: Pulmonary hypertension syndrome (PHS) in broiler chickens is exacerbated by cold stress, leading to physiological responses that can adversely affect cardiac health. This study investigates the relationship between heart telomere length and lipid peroxidation in chickens experiencing PHS due to cold stress.

Materials and Methods: A total of 31-day-old male Ross 308 broiler chicks were divided into control and cold-stress (PHS) groups, with the latter exposed to decreasing temperatures to induce PHS. At 21 and 42 days of age, we assessed the right ventricular to total ventricular (RV:TV) ratio, relative heart telomere length through real-time quantitative PCR, and serum malondialdehyde (MDA) levels as a marker of lipid peroxidation.

Results: The RV:TV ratio was significantly higher in the PHS group at both 21 days and 42 days compared to controls. Relative telomere length was significantly reduced in the PHS group at 42 days ($P<0.05$), while MDA levels were elevated at this age ($P<0.05$). A negative correlation between telomere length and MDA levels was observed at 42 days ($P<0.05$).

Conclusion: Cold stress-induced PHS in broiler chickens leads to increased oxidative stress, as evidenced by elevated MDA levels and reduced telomere length. The findings suggest that oxidative damage may accelerate telomere attrition, linking environmental stressors to cardiac dysfunction in poultry.

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Introduction

Pulmonary hypertension syndrome (PHS) and ascites are recognized as physiological responses to cold stress in broiler chickens. In response to chronic exposure to low temperatures, these birds typically exhibit an increase in metabolic activity and oxygen requirements [1]. This heightened demand for oxygen leads to hypoxia, which activates neuronal and hormonal mechanisms to compensate for the oxygen deficiency. Consequently, this increased stroke volume, cardiac output, and heart rate [2]. The enhanced cardiac output subsequently raises vascular pressure within the lungs and pulmonary arteries, culminating in pulmonary hypertension. This pressure overload imposes significant strain on the right ventricular muscle wall, leading to right ventricular hypertrophy [3, 4].

Over time, these physiological adaptations can result in a range of adverse outcomes, including right atrioventricular valve insufficiency, dilation of the right ventricle, right ventricular failure, edema, hydropericarditis, and abdominal ascites [5]. Numerous studies have documented alterations in various hormones implicated in this disorder, including endothelin [6-8], angiotensin [9, 10], nitric oxide [11, 12], thyroxine, triiodothyronine [13], and serotonin [14]. Furthermore, an imbalance between oxidant and antioxidant systems, which leads to increased production of reactive oxygen species (ROS), plays a critical role in the pathogenesis of PHS [15, 16].

Telomeres are specialized structures located at the ends of linear chromosomes, composed of repetitive DNA sequences and associated proteins that protect chromosome integrity. They play a crucial role in cellular aging and stability, as they shorten with each cell division, ultimately limiting the replicative capacity of cells [17]. This shortening is a natural part of the aging process; however, it can be accelerated by various stressors, particularly oxidative stress [18]. Oxidative stress occurs when there is an imbalance between the production of ROS and the body's ability to neutralize them with antioxidants [19]. High levels of ROS can cause damage to cellular components, including DNA, proteins, and lipids. Telomeres are particularly susceptible to oxidative damage due to their high guanine content, which can lead to mutagenic lesions, such as 8-hydroxy-deoxyguanosine (8-OHdG) [20, 21]. This oxidative damage can result in single-strand breaks in telomeric DNA, leading to telomere shortening and dysfunction [22]. Numerous studies have demonstrated a strong association between oxidative stress and accelerated telomere attrition [20, 22, 23]. This relationship is further complicated by the fact that oxidative stress not only accelerates telomere shortening but also decreases telomerase activity, the enzyme responsible for maintaining telomere length [23]. As telomeres shorten and telomerase activity declines, cells may enter a state of senescence or undergo apoptosis, contributing to age-related diseases and conditions such as cancer, cardiovascular disease, and metabolic syndrome [24].

In various forms of heart disease, including heart failure and pulmonary hypertension, which are closely linked to oxidative stress and inflammation, there is a progressive loss of cardiac cells [25]. This loss represents a fundamental pathogenic characteristic of heart disease and has motivated researchers to investigate the mechanisms that govern the survival and replication of cardiac cells [25]. Evidence indicates that during heart failure, cells with the shortest telomeres exhibit increased expression of senescence markers such as p16, along with pro-apoptotic factors including the transcription factor p53, an essential regulator of apoptosis, and cell-cycle checkpoint kinase 2. These findings underscore the association between telomere-induced senescence and apoptosis with the loss of cardiac cells in heart disease [25]. The interplay between oxidative stress, inflammation, and telomere dynamics is crucial for understanding the pathophysiology of heart disease [26].

Accordingly, the present study induces PHS in broiler chickens through exposure to cold stress. Following this induction, the research evaluated the relative heart telomere length and its relationship with lipid peroxidation during two phases of rearing.

Materials and Methods

Induction of PHS by cold stress

A total of 30 one-day-old male Ross 308 broiler chicks were randomly assigned to floor pens and raised for 42 days. The chicks were divided into a control group and a cold-stress treatment group (PHS group), with 15 chicks per group. The control group was housed under standard conditions, with unrestricted access to water and a diet formulated according to breeder guidelines for Ross 308. The PHS group was maintained in a separate room under identical standard conditions, except for temperature variations. To induce PHS, the cold-stress group's room temperature was systematically decreased following the protocol established by Teshfam et al. (2006) [27]. Accordingly, both the control and PHS groups were main-

tained at a temperature of 28 ˚C until day 8 of age. Then, the room temperature for the PHS group was gradually lowered to 14 ˚C, until the end of the experiment while the temperature was 21-22 ˚C for the control group. At the end of the experiment, the food conversion rate (FCR) in each group was calculated.

Sampling and calculation of pulmonary hypertensive index

At 21 and 42 days of age, 12 chickens from each group were randomly selected. The blood samples were collected from the brachial vein before euthanasia. The hearts were dissected from the body following euthanasia. The right ventricular to total ventricular weight ratio (RV:TV ratio) was subsequently calculated. An RV:TV ratio greater than 0.29 was indicative of PHS accompanied by ascites in broiler chickens [28]. The tissue samples were frozen in liquid nitrogen and stored at -70 ˚C until the extraction of DNA. The serum of each blood sample was separated and prepared for malondialdehyde (MDA) measurement.

DNA extraction and real-time quantitative polymerase chain reaction

DNA was extracted from right ventricular tissues using a DNA extraction Kit (DNPTM Kit, SinaClon BioScience, Karaj, Iran), according to its guidelines. The resulting DNA pellet was resuspended solvent buffer. To assess the telomere length, a relative quantitative real-time polymerase chain reaction (RT-qPCR) was conducted using an SYBR Green PCR kit (Takara Bio., Japan). Primers for telomere sequences and the reference gene tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (*YWHAZ*) were synthesized as described previously [29, 30]. The sequence of primers included the following items:

Telomere (tel1): CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT;

(tel2): GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT;

YWHAZ

Forward: AGGAGCCGAGCTGTCCAATG;

Reverse: TCCAAGATGACCTACGGGCTC.

The amplification reactions for both telomere and *YWHAZ* were conducted in a final volume of 10 µL, utilizing 15 ng of DNA per reaction. Amplifications were carried out in triplicate using a Rotor-Gene 6000 thermocycler (Qiagen, Australia). The PCR protocol for telomere amplification consisted of an initial denaturation step at 95 ˚C for 10 min, followed by 20 cycles of denaturation at 95 ˚C for 15 s and annealing/extension at 54 ˚C for 2 min. The amplification procedure for *YWHAZ* commenced with an initial denaturation phase at 95 ˚C for 2 min. This was followed by 40 cycles, each comprising a denaturation step at 95 ˚C for 15 s and an annealing/extension phase at 62 ˚C for 30 s. To ensure the absence of contamination, a no-template control reaction was included in the experimental design. The threshold cycle numbers (Ct) and mean efficiency values (E) for both targets were calculated using LinRegPCR software, version 2012.0 (Amsterdam, Netherlands). Relative telomere length was subsequently determined according to the methodologies established by Hassanpour et al. (2023) [30].

Thiobarbituric acid reactive substances (TBARS) assay

The reagents employed in the assay were sourced from Sigma-Aldrich (St. Louis, MO, USA). The MDA, a well-established biomarker for lipid peroxidation, was quantified in serum samples utilizing the TBARS assay. To facilitate protein precipitation, trichloroacetic acid was introduced to the serum samples, after which the resulting supernatants underwent centrifugation. Following centrifugation, the samples were incubated for 10 min in a boiling water bath after the addition of an equivalent volume of thiobarbituric acid (TBA). Absorbance was then measured at 532 nm using a spectrophotometer (Corning 480, USA) after allowing the samples to cool. The data generated from the TBARS assay were subsequently converted to micromolar units (µM) [31].

Statistical analysis

Data are presented as Means±SE. The Kolmogorov–Smirnov test was conducted to assess the normality of the data. For normally distributed data, parametric tests were employed for comparisons. Statistical comparisons between experimental groups were performed using the independent Student t-test. The relationship between groups was evaluated by logarithmic regression (Pearson). All statistical analyses were conducted using SPSS software, version 26 (SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered statistically significant.

Results

PHS index and total FCR

The ratio of RV:TV as an indicator of PHS was higher in the PHS group than in the control group at 21 days (control: 0.204±0.004, PHS: 0.274±0.020, P=0.002) and 42 days (control: 0.229±0.014, PHS: 0.342±0.013, P=0.001) of age. The total FCR (1-42 days) was significantly increased in the PHS group (1.98±0.03) compared to the control group (1.64±0.01; P=0.001).

Estimation of cardiac telomere length and serum MDA level and their association

The results of real-time PCR analysis for telomere length are presented in Figure 1. The *YWHAZ* gene, utilized as a reference, was successfully detected in all heart samples (right ventricle) during the DNA amplification process. At 42 days of age, broilers in the PHS group exhibited a significantly reduced relative telomere length in their right ventricular tissues compared to those in the control group (P<0.05). There was no significant difference in telomere length observed between the two groups at 21 days of age (P>0.05).

Figure 1 also presented the comparison of MDA levels between control and PHS groups at 21 and 42 days. The MDA levels were elevated in the PHS group relative to the control group at 42 days (P<0.05), while no significant change was noted at 21 days (P>0.05).

Figure 2 showed logarithmic regression graphs between heart telomere length and serum MDA in the chickens with PHS . This analysis revealed a negative correlation between relative telomere length and serum MDA levels in broilers at 42 days of age (P<0.05). This correlation was not significant at 21 days of age (P>0.05). These findings suggest an association between oxidative stress, as indicated by MDA levels, and telomere length in broilers, particularly at 42 days.

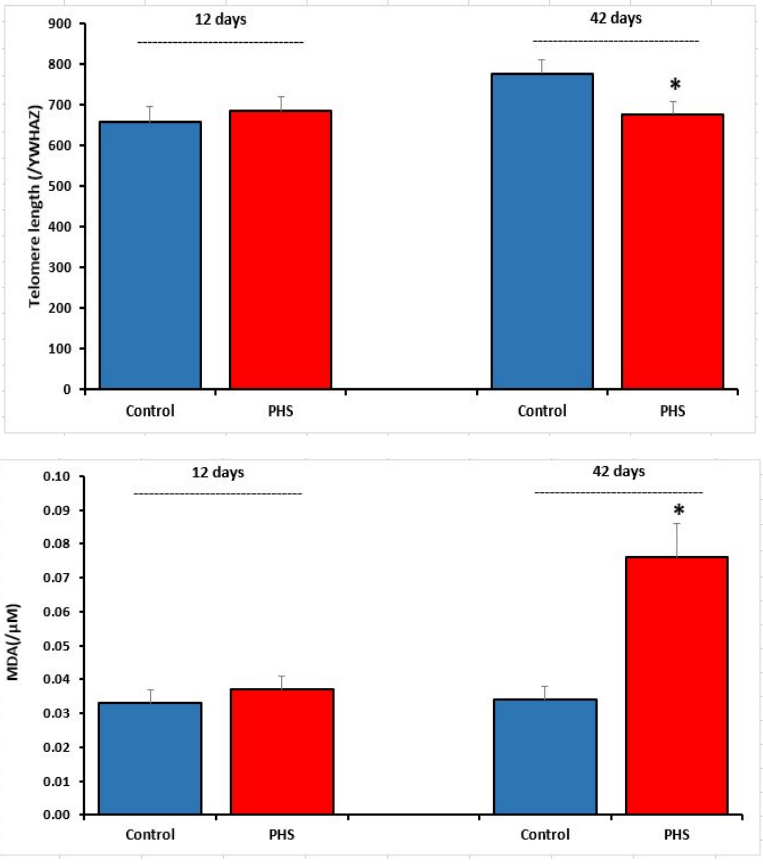

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Figure 1. Comparison of heart telomere length (up) and serum MDA (down) in chickens with PHS in 21 and 42 days

MDA: Malondialdehyde; PHS: Pulmonary hypertension syndrome.

*Significant difference between treatments (P<0.05).

Notes: Values are Means±SE.

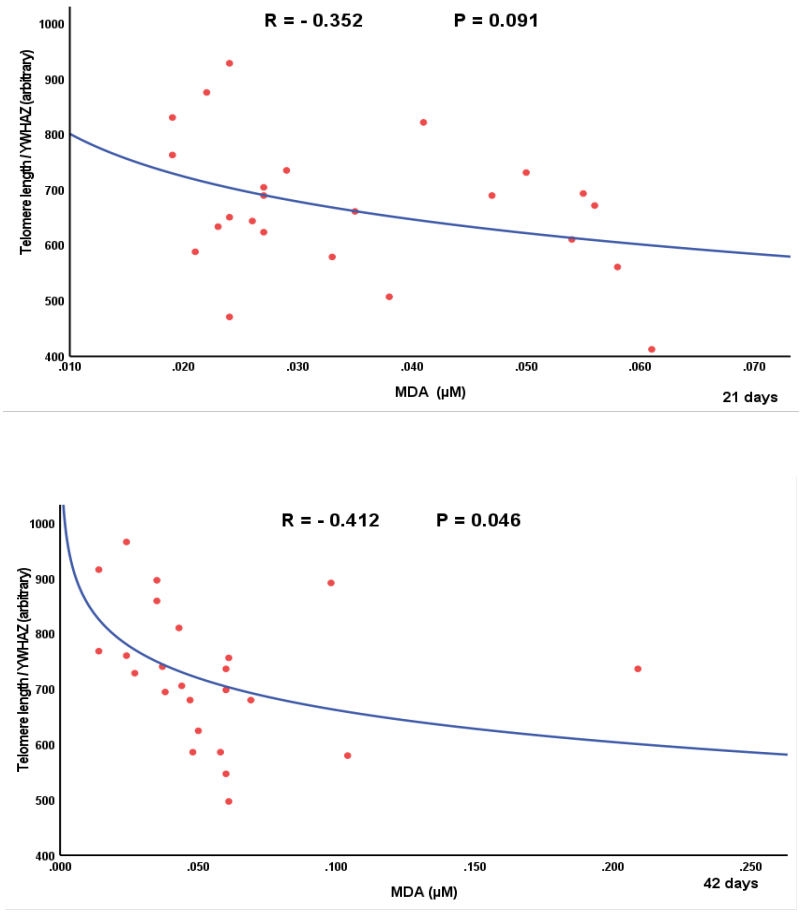

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Figure 2. Logarithmic regression graphs between heart telomere length and serum MDA in chickens with PHS at days 21 and 42
MDA: Malondialdehyde; PHS: Pulmonary hypertension syndrome.
Notes: P<0.05 is statistically significant. R is the correlation coefficient in regression analysis.

Discussion

Cold stress is a significant environmental factor that plays a crucial role in the onset of PHS in broiler chickens [32], as evidenced by many studies that highlight the effects of environmental stressors on telomere dynamics in birds. Various factors, such as overcrowding, high stocking density, and inadequate feeding regimens have been linked to telomere attrition [33]. During stress, the hypothalamus-pituitary-adrenal (HPA) axis becomes overactivated, influencing telomere dynamics through increased oxidative stress and impaired antioxidant defenses, ultimately leading to telomere shortening [34]. Cold stress also negatively impacts feed conversion efficiency in our study. This indicates that chickens affected by PHS required more feed to achieve comparable weight gain than their healthy counterparts, further emphasizing the economic implications of cold stress on poultry production [35]. In the present study, short-term exposure to cold stress did not yield significant changes in telomere length among the chickens, which aligns with findings by Angelier et al. (2018) [34]. They noted that while short-term stress can elevate glucocorticoid levels, it may also enhance antioxidant protection, thereby mitigating oxidative damage. Supporting this, Bahadoran et al. (2021) reported an elevated RV:TV ratio in chickens subjected to cold stress at 21 days of age, indicating early cardiac hypertrophy [13]. This hypertrophy may arise from damage or loss of cardiomyocytes, leading to compensatory hypertrophy of the remaining healthy cells [13]. It is well established that cardiomyocyte telomere length is positively correlated with cell size, and telomere length can vary across cells within a hypertrophied heart [25].

The present study investigated the relationship between heart telomere length and lipid peroxidation in broiler chickens experiencing PHS under cold stress conditions.

The results reveal a significant increase in the RV:TV ratio in chickens exposed to cold stress, confirming the successful induction of PHS particularly in 42 days (>0.29). This finding is consistent with previous research demonstrating the detrimental effects of cold stress on cardiac function in poultry [36]. While physiological adaptations to hypoxia, such as right ventricular hypertrophy, are well-documented, this study elucidated the molecular consequences of these adaptations, particularly regarding oxidative stress. A critical finding of our study was the significant reduction in relative heart telomere length observed in the PHS group at 42 days of age. This shortening is particularly concerning, as telomere attrition is associated with cellular senescence and apoptosis, processes exacerbated by oxidative stress [37]. The negative correlation between serum MDA levels, a biomarker for lipid peroxidation, and telomere length at this age underscores the role of oxidative damage in accelerating telomere shortening [37]. These results are consistent with existing literature linking oxidative stress to telomere dynamics in various heart disease models [38, 39].

The implications of these findings extend beyond poultry health, as the association between oxidative stress, telomere shortening, and cardiac dysfunction has broader relevance in both veterinary and human medicine. Understanding how oxidative stress influences telomere dynamics may provide insights into developing therapeutic strategies aimed at preserving cardiac health in conditions characterized by chronic stress and inflammation.

Lipid peroxidation is a key contributor to oxidative stress, which has a substantial effect on telomere shortening. Elevated levels of ROS can cause oxidative damage to cellular membranes, resulting in lipid peroxidation. This process generates reactive aldehydes, such as MDA, which further exacerbate cellular damage [40]. These byproducts can interact with various cellular macromolecules, including DNA, generating mutagenic lesions that are particularly harmful to telomeric DNA [40]. As mentioned, telomeres, composed of repetitive guanine-rich sequences, are inherently vulnerable to oxidative damage, as guanine bases are more prone to oxidation, resulting in the formation of 8-OHdG, and breaking the telomeric regions [20].

As telomeres sustain oxidative damage, their structural integrity is compromised, leading to progressive shortening. This shortening is exacerbated by the inhibition of telomerase activity, the enzyme responsible for adding telomeric repeats to chromosome ends. Under oxidative stress conditions, telomerase expression and activity can be significantly reduced, impairing the cell's ability to maintain telomere length. The combination of telomere shortening and decreased telomerase activity creates a feedback loop that accelerates telomere attrition, pushing cells toward a state of crisis [41].

The consequences of telomere shortening due to lipid peroxidation are very complicated. One major outcome is cellular senescence, where cells enter a permanent state of growth arrest. Senescent cells can accumulate within tissues, contributing to age-related degeneration and functional decline, particularly in high-turnover tissues like the cardiovascular system. The relationship between oxidative stress, telomere attrition, and disease risk is significant, resulting in various age-related diseases, including cardiovascular diseases [42].

Conclusion

This study highlights the significant impact of cold stress on the cardiac health of broiler chickens, specifically through its role in inducing PHS . The findings demonstrate a clear association between elevated serum MDA levels, indicative of increased oxidative stress, and reduced telomere length in cardiac tissues, particularly at 42 days of age. These results suggest that oxidative damage plays a crucial role in telomere attrition, which may contribute to cellular senescence and apoptosis in cardiac cells.

Ethical Considerations

Compliance with ethical guidelines

All the procedures in this study were approved by the Institutional Animal Care and Use Committee of Shahrekord University, Shahrekord, Iran (Code: IR.SKU.REC.1400.079), based on the Welfare Standard of the 1964 Declaration of Helsinki.

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Authors' contributions

Study design: Shahab Bahadoran, Hossein Hassanpour and Mohammad-Reza Akbari; Major experimental work, Data analysis, and writing the manuscript: Navid Farhadi, Shahab Bahadoran, and Hossein Hassanpour.

Conflicts of interest

The authors declared no conflict of interest.

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