Research Article

Oxidative Stress in Liver Tissues of Marek's Disease Affected Layer Chicken

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Abstract

Marek's disease is a lymphoproliferative and neuropathic disease of domestic chickens which was found to be caused by a highly contagious, cell-associated, oncogenic herpes virus. Increased generation of ROS is a feature of many viral infections. Hence an experiment was conducted to study the activities of antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT), glutathione-S-transferase, Glutathione peroxidase and reduced glutathione in liver tissues of Marek's disease (MD) affected chicken in layers of 35 to 40 weeks age. A total of ten control and ten MD affected layer chicken were collected from poultry farms in Chittoor district. The histopathological examination revealed severe infiltration of pleomorphic lymphoid cells and PCR analysis showed positivity of MDV-1 serotype at 314 bp region in MD affected liver. The SOD, CAT, GST, GP_x activities and GSH levels significantly reduced in MD affected chicken liver when compared to control. Lipid peroxidation (malondialdehyde) levels were measured to know the intensity of oxidative stress. Malondialdehyde levels were significantly elevated in MD affected chicken liver compared to control. These results indicate that MD virus causes oxidative stress in layers which may be due to reduced activity of antioxidant enzymes in liver tissue.

Keywords: Marek's disease, SOD, Catalase, GST, GP_x, GSH, Malondialdehyde

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Introduction

Marek's Disease is one of the economically important poultry diseases all over the world. Due to potentially high mortality rate and reduced performance MD infection is recognized as a significant health risk to poultry farming [1]. In chicken, MD occurs at 3–4 weeks of age or older and is most common between 12 and 40 weeks of age in layers. Increased generation of ROS is a feature of many viral infections and can be caused by both direct effect of virus on cells and inflammatory responses of the host [2].

In many viral infections, oxidative stress is primarily due to increased generation of reactive oxygen species (ROS) which modulate the permissiveness of cells to viral replication, regulate host inflammatory and immune responses, and cause oxidative damage to both host tissue and progeny virus [3]. To protect themselves, body maintains antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT) [4].

Superoxide dismutase catalyzes the breakdown of the superoxide anion into oxygen and hydrogen peroxide [5] and this enzyme forms the first line of defense against superoxide radicals. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen [6]. Glutathione is important particularly in the regulation of redox state and prevention of the cell damage induced by oxidative stress. Glutathione is the most important freely available antioxidant, which acts directly as an antioxidant and also participates in catalytic cycles of several antioxidant enzymes such as GP_x, GR and GST [7]. Glutathione Peroxidase plays an important role in the detoxification of hydrogen peroxides with the conversion of reduced glutathione (GSH) to Glutathione Disulfide (GSSG). Glutathione-S-transferase is believed to detoxify endogenous substances that are formed as a consequence of oxidative stress.

Most of the electrophilic molecules, such as endogenous oxidative stress products, carcinogens, drugs, pesticides and herbicides, pass through the liver before they reach and accumulate in target tissues. Hence, it is essential to study the antioxidant enzyme activities and oxidative stress in liver tissues of birds. Therefore, the present experiment was carried out to study the activities of SOD, Catalase, GPx, GST, levels of GSH and malondialdehyde in liver tissues of Marek's disease (MD) affected layer chicken liver as compared to control.

Material and Methods

Source of poultry birds

All the birds were layers with 35 to 40 weeks of age and were procured from various poultry farms in Chittoor district. The birds showing clinical signs of MD and control birds of the same age group were obtained from the farms.

Collection of tissues

Ten layer birds each for control and MD affected group of 35 to 40 weeks age were collected and sacrificed for collection of liver samples. Liver tissues collected for histopathological studies were fixed in 10% neutral buffered formalin (pH 7.2). The samples were processed and sections of 4-7 µm were cut and stained with Haematoxylin and Eosin stain (H&E). The specimens were examined under light microscope [8]. DNA isolation and purification was carried out using kit method (Himedia).

Polymerase chain reaction (PCR)

Marek's disease virus positive DNA sample obtained from Department of Biotechnology, TANUVAS, Chennai was used as a positive control for all PCR reactions. A known positive and negative control comprising of nuclease free water were included in the test and amplification was performed in Thermo cycler [9]. The specificity of PCR products was confirmed by the appearance of the fragments of predicted size on the agarose gel.

Assay of lipid peroxidation

In liver tissues, thiobarbituric acid reactive substances (TBARS) were estimated by the method of [10].

Determination of antioxidant parameters

Tissues were blotted dry, thawed and homogenized at 4ºC in 3 volumes of 0.25M sucrose containing 0.07M phosphate buffer (pH 7.2), 10mM EDTA and 0.1% Triton X-100. Post mitochondrial supernatant was prepared by centrifuging at 12,000 x g for 15 min at 4ºc. These tissue preparations were used immediately for measuring enzyme activities.

SOD activity was measured according to [11]. Catalase activity was measured by the method of [12]. Glutathione content was determined according to the method of [13]. Glutathione peroxidase(GPx) activity was assayed by the method of [14]. Glutathione-S-transferase (GST) activity was determined by the method of [15].

Statistical analysis

The results obtained were analyzed for paired't' test using SPSS version 20.0. Differences were considered as significant at $p<0.05$.

Results and Discussion

Liver enlargement with multifocal grayish white nodules of 2-5 mm in diameter was observed in all cases (**Figures 1** and **2**).

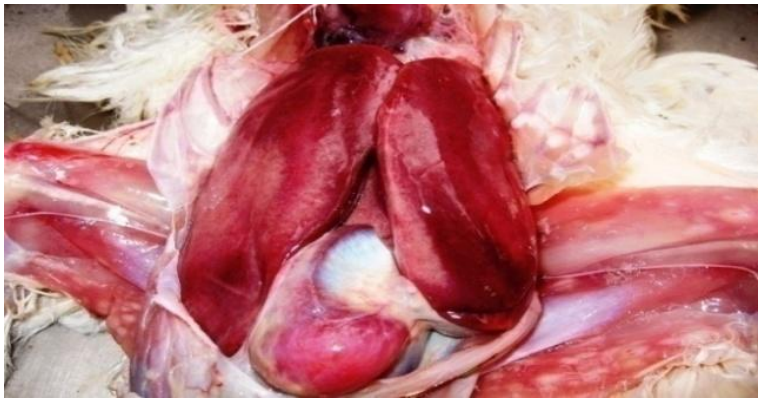


Figure 1 MD- liver: diffuse enlargement of liver occupying the entire abdominal cavity

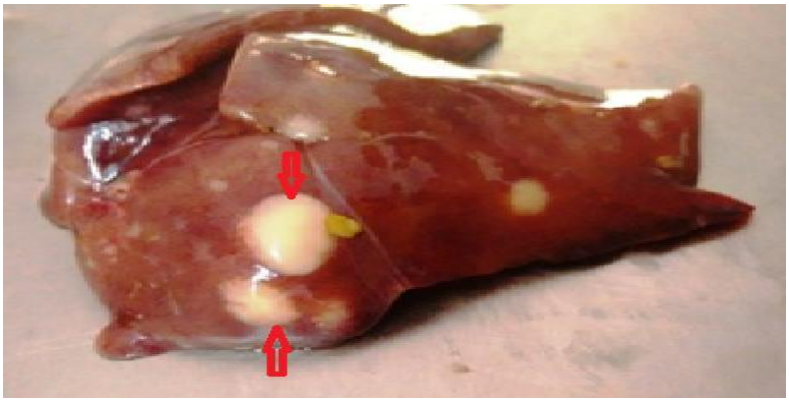


Figure 2 MD liver: Enlarged liver with multiple nodular growth and pale appearance

Histopathology

In MD affected chicken histopathological examination of liver tissues was done using H &E stain and observed severe infiltration of pleomorphic lymphoid cells which was the characteristic feature of liver cells affected with MD (**Figures 3** and **4**) [16].

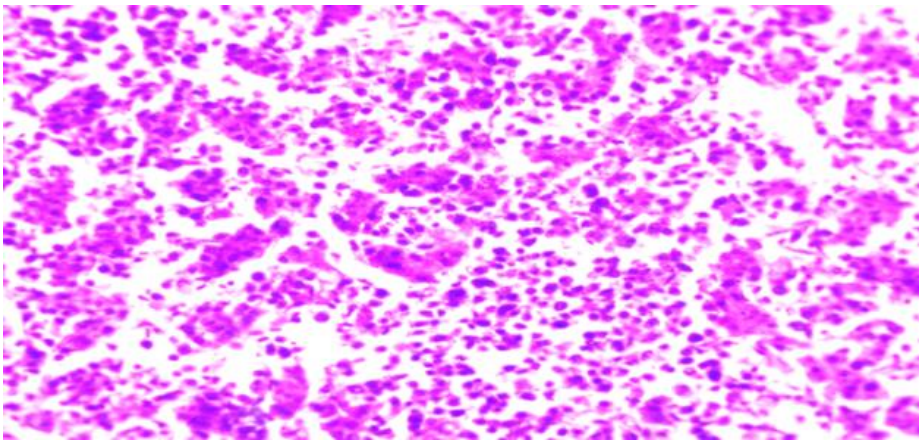


Figure 3 MD Liver: Severe infiltration of pleomorphic lymphoid cells. H&E x100

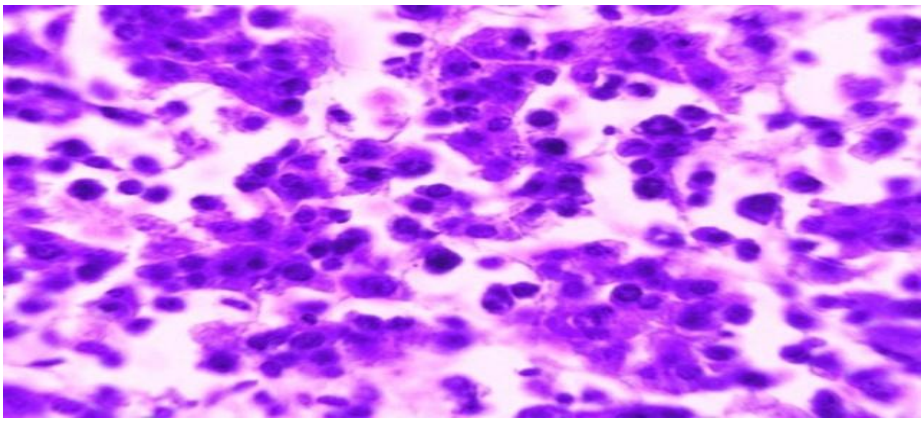
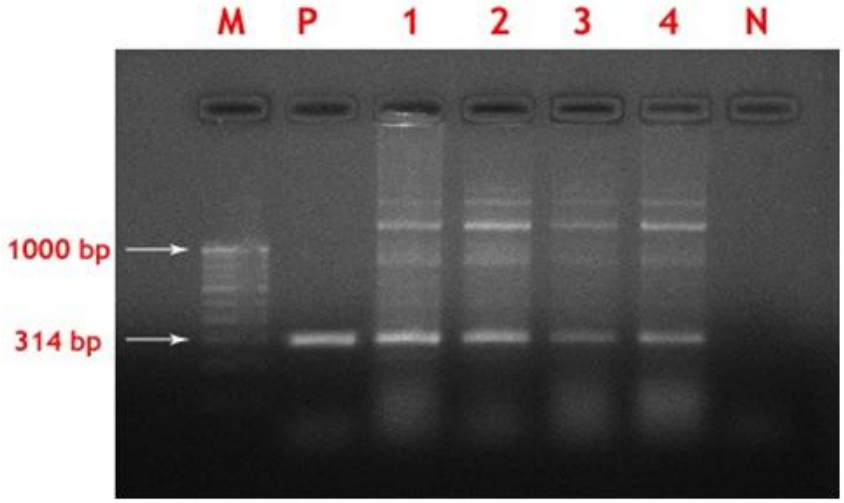


Figure 4 MD Liver: Severe infiltration of pleomorphic lymphoid cells. H&E x400

PCR analysis

The purity of DNA isolated from normal and MD affected birds was found to be optimum for PCR analysis. Primers were used to amplify the 132 bp repeat region in MD suspected cases using PCR. All the samples were positive for serotype-1 specific MDV yielding a 314 bp product in PCR amplification and showed 100 % positivity. This showed that the 314 bp product consisted of two copies of 132 bp tandem repeats (264 bases) along with 50 bases of primers. All the samples of MD showed two copies of 132 bp tandem repeats and results were shown in **Figure 5**.



PCR amplified samples showing positivity at 314 bp repeat region in Agar Gel electrophoresis

M → Marker 100bp **1, 2, 3, 4** → MD liver samples showing positivity at 314 bp

N → Negative control **P** → Positive control showing positivity at 314 bp

Figure 5 Agar Gel Electrophoresis of PCR product

Oxidative stress and anti-oxidant enzymes

Oxidative stress is a state characterized by imbalance between pro-oxidant molecules, comprising reactive oxidants and anti-oxidant defenses has been found to play an important role in poultry reproduction. Viral infections via oxidative stress may also contribute to hepato-carcinogenesis. Previously, it has been reported that virus induced pathogenecity mediates oxidative stress, thereby affecting target organs. Further, it has also been claimed that ROS play an important role in many viral infections and disease progression [3]. Many reports claim that quantification of malondialdehyde (MDA) levels has been used as an indicator of oxidative stress. Hence in the present study, the levels of TBARS (MDA) were estimated to determine the role of oxidative stress in MD affected chicken. Significant increase in the TBARS (179.82%) was observed in of MD affected chicken liver compared to control may be due to oxidative stress (**Table 1**). Similar findings were observed by [16] in plasma of MD affected chicken where the levels of TBARS and DNA damage was higher in MD affected chicken compared to control due to oxidative stress.

Table 1 Effect of MD virus on levels of TBARS and activities of antioxidant enzymes

Groups	**Group-I (Control)**	**Group-II (MD)**
TBARS (nmol of MDA/g of tissue)	135.78 ± 4.73^{a}	379.95 ± 5.08^{b}
SOD (U/mg/min)	0.022 ± 0.0004^{a}	0.015 ± 0.0006^{b}
CATALASE (U/mg/min)	9.93 ± 0.27^{a}	5.72 ± 0.20^{b}
Glutathione (GSH) (mg/g)	0.8055 ± 0.022^{a}	0.3905 ± 0.021^{b}
Glutathione Peroxidase (GP_x) (µ moles of GSH/min/mg)	0.076 ± 0.0017^{a}	0.034 ± 0.0010^{b}
Glutathione-S-transferase (GST) (U/mg)	6.22 ± 0.08^{a}	3.06 ± 0.10^{b}

Values are mean ± SE (n = 10)
Means with different superscripts differ significantly within the row ($p < 0.05$).

Superoxide dismutase is considered as the main element in the first line of antioxidant defense since, superoxide radical is the main free radical produced in physiological condition in the cell [17]. Significant decrease in SOD activity (32%) was noticed in MD affected chicken compared to control (Table 1) and our results are in accordance with [18] who reported reduced activity of SOD in brain tissues of NDV infected chicken compared to control.

Catalase catalyzes the conversion of hydrogen peroxide to water there by providing protection against ROS [19]. The hydrogen peroxide formed by SOD activity is detoxified by catalase. The reduced catalase activity (43%) in liver

of MD affected chicken compared to control (Table 1) may be due to excess utilization of the enzyme. Similar findings were observed by [20] in liver of NDV infected chicken in which decreased catalase activity was observed compared to control. Decreased catalase activity is due to the inflammation which leads to increased production of H_2O_2 intracellularly causing DNA damage thereby promoting cancer in MD affected chicken. Hepatic catalase activity decreased in patients with malignant diseases. The reduction in SOD and catalase activities may be due to their increased utilization in scavenging lipid peroxides as well as their sequestration by tumor cells [21].

Glutathione is important particularly in the regulation of redox state and prevention of the cell damage induced by oxidative stress. Glutathione is the most important freely available antioxidant, which acts directly as an antioxidant and also participates in catalytic cycles of several antioxidant enzymes such as GP_x, GR and GST [7]. It was found to be an important modulator of the life cycle of influenza virus and its levels were decreased during viral replication [22]. Hence it is essential to investigate GSH levels which were found to be significantly decreased (52%) in liver tissue of MD affected chicken compared to control (Table 1). Reduction in GSH content may be due to decreased GSH synthesis or an increased rate of loss due to increased consumption, degradation or transport. Our results are in accordance with [16], who also reported reduced levels of glutathione in plasma of MD affected chicken compared to control. [23] reported that GSH consumption may increase in viral infection as a result of increased oxidative stress.

Glutathione Peroxidase plays an important role in the detoxification of hydrogen peroxides with the conversion of reduced glutathione (GSH) to Glutathione Disulfide (GSSG). In the present study, the decreased activity of GP_x (55%) in MD affected chicken compared to control (Table 1) indicates improper detoxification of hydrogen peroxide radicals due to reduced activity of CAT (43%). A significant decrease in GSH content in MD affected chicken compared to their respective controls might be due to reduced activity of GP_x. It has been documented that GP_x is an allosteric enzyme and thus the presence or lack of Co-substrate GSH has significant effect on its activity [22]. Our results are in agreement with [5] where decreased SOD activity was accompanied by decreased GP_x activity and increased lipid peroxidation.

Glutathione-S-transferase is involved in the detoxification of endogenous substances that are formed as a consequence of oxidative stress. It plays a key role in the detoxification and reduction of reactive oxygen species (ROS). They are involved in the development of resistance towards chemotherapy agents, insecticides, herbicides and microbial antibiotics [24]. Our results showed significant reduction in GST activity (51%) in liver of MD affected chicken compared to control (Table 1). Similar findings were observed by [20] in liver of NDV infected chicken where the GST activity was reduced compared to control.

Conclusion

The results obtained in the present study showed significant increase in TBARS, reduced SOD, CAT, GPx and GST activities and GSH levels MD affected chicken liver compared to control. The results confirm that oxidative stress is responsible for reduced antioxidant enzyme activity in liver of MD affected chicken. A better understanding of the role of oxidative damage in viral infections may lead to improved therapeutic strategies that will reduce the extent of tissue damage during viral infections.

References

[1] Nair, V. (2005). Evolution of Marek's disease—a paradigm for incessant race between the pathogen and the host. Vet. J, 170, 175–183.
[2] Schwarz, K. B. (1996). Oxidative stress during viral infection: a review. Free Radic Biol Med, 21(5):641-649.
[3] Valyi-Nagy, T. and Dermody, T. S. (2005). Role of oxidative damage in the pathogenesis of viral infections of the nervous system. Histol Histopathol, 20: 957-967.
[4] Champe, C. P., Harvey, R. A. and Ferrier, D. R. (2007): Transamination: the funneling of amino groups to glutamate. In: Lippincott's Illustrated Reviews: Biochemistry. 4 groups to glutamate. In: Lippincott's Illustrated Reviews: Biochemistry. 4th ed., Williams & ed., Williams &Wilkins. Philadelphia, USA. pp. 250-252.
[5] Surai, P. F. (2016). Antioxidant Systems in Poultry Biology: Superoxide Dismutase. Animal Nutrition, 1:(1:8).
[6] Khan, N. and Farzana, N. N. (2014). Antioxidant enzymes and protein profiles in wheat seedlings under abiotic stress. American Journal of Research Communication, 2(12):155-167.
[7] Biswas, S. K., and Rahman, I. (2009). Environmental toxicity, redox signaling and lung inflammation: The role of glutathione. Molecular Aspects of Medicine, 30(1): 60-76.
[8] Singh, U.B. and Sulochana, S. (1997). A practical manual of histopathological and histochemical techniques, Kothari Publications, Bombay, pp. 154.

[9] Sambrook, J., and Russell, D. (2001). Molecular Cloning: a Laboratory Manual, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
[10] Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351-358.
[11] Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry, 247(10): 3170-3175.
[12] Beers, R. F. and Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry, 195(1): 133-140.
[13] Ellman, G. L. (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 82: 70-77.
[14] Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science, 179: 588-590.
[15] Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. Journal Biological Chemistry, 249: 7130-7139.
[16] Hikmet Keles, A., Fatih Fidan, I., Hakki Cigerci, Ismail Kucukkurt, Erkan Karadas, Yilmaz Dundar (2010). Increased DNA damage and oxidative stress in chickens with Natural Marek's Disease. Veterinary Immunology and Immunopathology, 133: 51–58.
[17] Halliwell, B. (2012). Free radicals and antioxidants: updating a personal view. Nutr Rev, 70(5): 257-65.
[18] Subbaiah, K. C., Rajendra, W., and Lokanatha, V. (2013).Newcastle disease virus (NDV) modulates pro/antioxidant status in different brain regions of chicken. Free Radicals and Antioxidants, 3(2): 81–86.
[19] Ahmed, R., Kima, Y. H., Kim, M. D., Kwon, S. Y., Cho, K., Lee, H. S. and Kwak, S. S. (2010). Simultaneous expression of choline oxidase, superoxide dismutase and ascorbate peroxidise in potato plant chloroplasts provides synergistically enhanced protection against various abiotic stresses. Physiologia Plantarum, 138: 520-533.
[20] Subbaiah, K. C., Raniprameela, D., Visweswari, G., Rajendra, W., and Lokanatha, V. (2011). Perturbations in the antioxidant metabolism during Newcastle disease virus (NDV) infection in Chicken: protective role of vitamin E. Naturwissenschaften, 98(12):1019-26.
[21] Manimaran, A., and Rajneesh, C. P. (2009). Activities of antioxidant enzyme and lipid peroxidation in ovarian cancer patients. Academic Journal of Cancer Research, 2(2): 68-72.
[22] Fraternale, A., Paoletti, M. F., Casabianca, A., Nencioni, L., Garaci, E., Palamara, A. T., and Magnani, M. (2009). GSH and analogs in antiviral therapy. Mol Aspects Med, 30(1-2):99-110.
[23] Gil, L., Martinez, G., Gonzalez, I., Tarinas, A., Alvarez, A., Giuliani, A., Molina, R., Tapanes, R., Perez, J., León, O.S. (2003). Contribution to characterization of oxidative stress in HIV/AIDS patients. Pharmacol Res, 47(3):217-24.
[24] Tew, K. D. (1994). Glutathione-associated enzymes in anticancer drug resistance. Cancer Res, 54(16):4313-20.

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