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Biochemical and Histopathological Changes in Immune and Non Immune Broilers after Inoculation of Field Infectious Bursal Disease Virus

Beenish Zahid[1,*], Asim Aslam[2], Zafar Iqbal Chaudhry[2] and Raheela Akhtar[2]

[1]*Department of Zoology, University of the Punjab, Lahore, Pakistan*

[2]*Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan*

ABSTRACT

The present study was conducted to compare the biochemical and histopathological changes in response to pathogenic field infectious bursal disease virus (IBDV). The Bursa of fabricius was collected from poultry flocks of Punjab Pakistan for isolation of IBD virus by inoculating in embryonated chicken eggs through chorioallantoic membrane. The two hundred birds were divided into 4 groups A, B, C and D). The birds in group A were commercial broiler chicks having maternal derived antibodies and were challenged with IBDV field isolate at the rate of 0.1ml of EID50 (virus titer $10^{5.50}$/100µl) on 2nd week of age through eye drops. Group B specific antibody negative (SAN) chicks were challenged with same dose at 2nd week of age. While the group C and D was unchallenged control containing commercial and SAN chicks. The Blood samples were collected from group A, B, C and D on 3rd, 5th and 7th day of post infection for biochemical analysis. The Albumin and total protein values were significantly low ($P<0.05$) in infected groups A and B as compared to control groups C and D on 5th and 7th day. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly ($P<0.05$) high in infected groups A and B. On 3rd, 5th and 9th day five birds from each group were slaughtered and histopathological lesions observed in bursa, spleen and thymus and compared with control. Mild to severe hemorrhages and lymphocytic depletion, follicle necrosis, leukocytic infiltration, cyst formation and fibrous tissue proliferation at medullar region of thymus and bursa were observed. These results indicated that pathogenic field IBDV caused the serum biochemical changes by damaging the liver and kidney tissues with histopathological effect on lymphoid organs of chicken.

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Authors' Contribution
AA and BZ conceived and designed the study and executed serum biochemical tests. ZIC and BZ performed histopathological studies. RA analyzed the data. BZ wrote the article.

Key words
IBDV, Alanine aminotransferase, Aspartate aminotransferase, Albumin, Total protein.

INTRODUCTION

Infectious Bursal Disease Virus (IBDV) also known as Gumboro disease, is highly contagious and immunosuppressive disease (Okwor *et al*., 2012). IBD is an acute, highly spreading disease may lead to high mortality rates. Due to the mortality and immunosuppressive effects, the disease has marked economic significance to the poultry sector throughout the world (Rauf, 2011). The IBD etiological agent is double stranded RNA virus having two segments which belongs to the *Birnaviridae* family and consisted of two serotypes, designated as serotypes 1 and serotype 2 (Carballeda *et al*., 2011).

Only type 1 serotype is pathogenic for the domesticated chicken. Numerous serotype 1 strains, differing in antigenicity have been identified and classified as classic and variant. Classical virulent strains in chicken, causes severe inflammation of bursa resulted in lymphocytic depletion and bursal atrophy in IBD challenged birds, ultimately causing immunosuppression (Atif *et al*., 2014). The very virulent strain of IBD virus had 80 % sequence homology with classical IBDV strains (Jackwood *et al*., 2009).

The damaging effect of virus can be evaluated at organ level, and at the level of cellular alterations. The clinical infection of IBDV based on few factors like type of viral strain, the dose of virus, route of virus entry, presence or absence of maternal antibodies, type of birds and age factors (Muller *et al*., 2003). The bursa of Fabricius becomes enlarged and shows pale yellow discoloration. Intra-follicular hemorrhages may be found and pin point hemorrhages on the skeletal muscles are usually prominent (Ingrao *et al*., 2013).

The acute phase of the disease lasts for 6-10 days and is characterized by atrophy of bursa along with depletion of B-cells (Uddin *et al*., 2012) in bursal follicles, the other lymphoid organs such as spleen and cecal tonsils are also affected. Immunosuppression occurs in clinical and subclinical form where both humoral and cellular immune responses are compromised and thus making birds more vulnerable to other secondary infections and reduced

* Corresponding author: bz-leo@hotmail.com
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response to vaccination (Musa *et al*., 2010).

Besides, it exhibits several pathological changes as part of the pathogenesis of the disease which could essentially be explained in biochemical changes in relation to the effect of the virus in several organ among others, liver and kidney (Abidin *et al*., 2014). The elevation in serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) at day 3 pi and/or days 5 and 7 pi in the IBD group suggested pathological involvement of liver and kidney which are common sequels in IBDV infection especially following secondary viremia (Tesfaheywet *et al*., 2012). The objective of present study is to evaluate the pathogenesis of virulent strain of field IBDV by estimating biochemical and histopathological changes produced in commercial broilers (immuned birds) and specific antibody negative chicks (Non immuned birds).

MATERIALS AND METHODS

Experimental design

Two hundred day old broiler chicks were procured and reared under standard housing conditions in University of Veterinary and Animal Sciences, the birds were fed with commercially prepared feed. The humoral antibody titer against IBD of reared birds were checked through enzyme linked immunosorbent assay in order to check the status of maternally derived antibodies both in commercial broilers and specific antibody negative (SAN) chicken at 0day. The SAN chicks were serologically negative for IBD. The birds were divided into 4 groups (A, B, C and D), fifty birds in each group. The birds in group A were commercial broiler chicks having maternal derived antibodies and were challenged with IBDV field isolate at the rate of 0.1ml of EID50 (virus titer 105.50/100µl) on 2nd week of age through eye drops (Abdel-Alim and Saif, 2001). Group B SAN chicks were challenged with same dose at 2nd week of age. While the group C and D were unchallenged control containing commercial and specific antibody negative chicks.

Virus isolates

The collected bursa of fabricus field tissue samples were inoculated in 10 day-old embryonated chicken eggs through chorio-allantoic membrane (CAM) route for IBDV at 0.2 ml (0.1 ml virus suspension + 0.1 ml antibiotic mixture) of inoculums. For IBDV samples of CAM were collected with PBS (phosphate buffered saline) to prepare 50% suspension and stored at -80°C for further use (Majed *et al*., 2013). Embryo infectious dose 50 (EID 50) of isolated field infectious bursal disease virus was estimated (Camilotti *et al*., 2016).

Histopathological examination

On day 3rd, 5th and 9th of post infection, from each group five birds were slaughtered, gross and histopathological lesions on lymphoid organs bursa, thymus and spleen in different groups were observed and tissue samples were preserved in 10% formalin for histopathological study (Subtain *et al*., 2011).

Collection of sera for biochemical analysis

From each group five birds were selected for blood collection. Blood samples were collected from group A, B, C and D on 3rd, 5th and 7th day of post infection, three mL of blood was collected from birds of each group via jugular vein or intra-cardiac route using a 23 G needle and 3.0 ml syringe. At room temperature collected blood was allowed to clot and serum was separated. The collected serum samples were stored at -20C^{o} till further use (Emadi *et al*., 2011).

Liver function tests (LFT)

Among LFTs hepatic serum enzyme levels of ALP, AST, ALT, total protein and albumin were analyzed by chemical analyzer (URIT-800) using "Human kit" to determine the changes in their levels as a result of IBD virus (Tesfaheywet *et al*., 2012).

Statistical analysis

The serum enzyme level data was analyzed statistically through analysis of variance and enzyme values in different treatment groups were compared through duncan's multiple range test by using SAS (Statistical Analysis Software).

RESULTS

In the present study bursal histological changes observed in group A were minimal, in group B mild changes observed in lymphoid organs whereas in group C and D no pathological changes observed. Mild lymphocytic depletion and epithelial cells hyperplasia observed in thymus (Fig. 1A). Congestion and hemorrhages observed in spleen. On 5th day of post infection Moderate histological changes observed in different organs in group A, severe changes observed in group B. Typical histological changes associated with IBDV infection, including lymphoid depletion, necrosis and hemorrhages in bursa (Fig. 1B), infiltration of leukocytic cells and cell debris of necrosed tissues observed in infected organs of IBDV inoculated birds of group B (SAN) chicks.

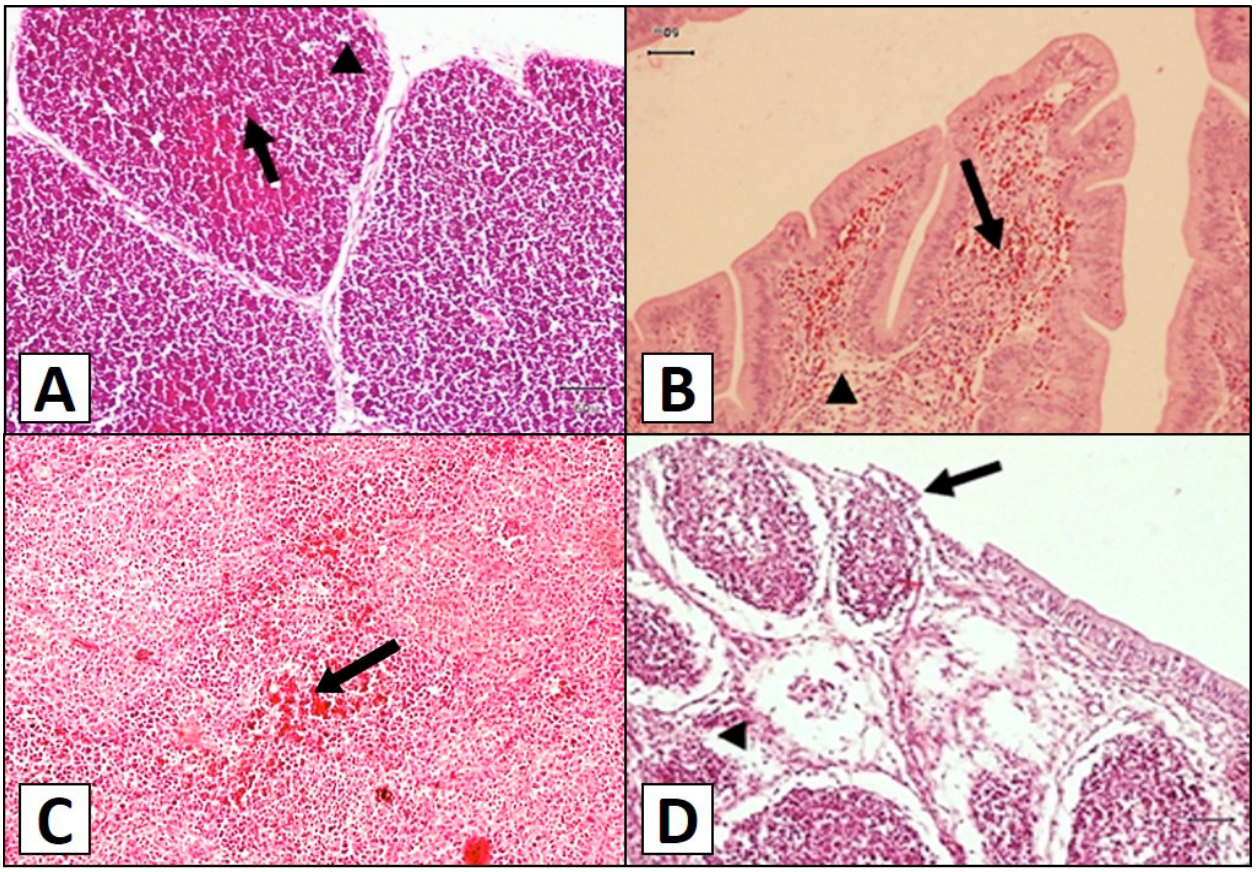

Fig. 1. Histological structure of chicken thymus, bursa and spleen. **A**, thymus of group A at 3rd day post-infection (PI) showing hemorrhages (arrow) and mild lymphocytic depletion (arrowhead) in the follicles; **B,** Bursa of group A at 5th day PI showing severe hemorrhages (arrow) and lymphocytic depletion (arrowhead) in bursal follicles. **C,** spleen of group B at 5th day of PI showing severe congestion (arrow) and leukocytic infiltration (arrowhead); **D,** bursa from group A at 9th day of PI showing severe lymphoid follicle necrosis (arrowhead) with degraded epithelium (arrow).

The spleen of group A showed mononuclear cell infiltration and sever congestion on 5th day of postinfection (Fig. 1C). In bursa of group B severe damage included lymphoid follicles atrophy, epithelial cell degradation (Fig. 1D) and leukocytes infiltration on 9th day of postinfection. Severe hemorrhages and lymphoid cell necrosis observed at medullar region of thymus. The bursa of Fabricius of group A on 9th day showed follicular atrophy, cyst formation and fibrous tissue proliferation.

Serum biochemical parameters of ALP, AST, ALT, and total protein and albumin values were evaluated (Table I). On third day of post infection albumin and total protein serum level were significantly ($P<0.05$) low in group B. Total protein and albumin values were significantly low ($P<0.05$) in infected groups A and B on 5th and 7th day of post infection as compared to control groups C and D. ALT, AST and ALP values were significantly high in infected groups A and B.

DISCUSSION

IBD causes the considerable economic losses through high mortality and immunosuppression in poultry. The present experiment was conducted to evaluate the serum biochemical parameters in field Infectious bursal disease virus in broiler chicken including changes in activities of ALT, AST, ALP and concentrations of albumin and total protein, and histopathological changes in immune organ were investigated.

Histopathological lesions were observed in lymphoid organs of infected birds, changes were compared with the control groups. Pathological lesions were scored mild in start, followed by moderate and severe. On third day of post infection mild lymphocytic depletion and hyperplasia of epithelial cells observed in bursa, mild hemorrhages and congestion was observed in thymus correspond to the result reported by (Atif *et al*., 2014).

Table I.- Serum concentration of albumin, total protein, ALT, AST and ALP in different treatment groups on day 3, 5 and 7 post infection.

Days	Groups	Day 3	Day 5	Day 7
Albumin (g/dl)	A	1.59±0.01^{b}	1.32±0.03^{b}	1.09±0.02^{b}
	B	1.51±0.01^{c}	1.41±0.06^{b}	1.06±0.01^{b}
	C	1.71±0.02^{a}	1.73±0.05^{a}	1.75±0.02^{a}
	D	1.68±0.01^{a}	1.74±0.04^{a}	1.78±0.04^{a}
Total protein (g/dl)	A	4.10±0.02^{d}	3.98±0.04^{c}	3.11±0.02^{d}
	B	4.43±0.02^{c}	3.57±0.04^{d}	3.28±0.03^{c}
	C	4.98±0.03^{b}	5.44±0.05^{a}	5.65±0.03^{a}
	D	5.13±0.02^{a}	5.27±0.04^{b}	5.42±0.02^{b}
ALT (u/L)	A	4.50±0.01^{b}	7.12±0.02^{a}	10.30±0.01^{b}
	B	5.16±0.01^{a}	6.75±0.02^{b}	12.59±0.02^{a}
	C	3.52±0.03^{c}	3.65±0.01^{c}	3.79±0.05^{c}
	D	3.48±0.02^{c}	3.57±0.03^{d}	3.82±0.04^{c}
ALP (u/L)	A	204±17.2^{a}	210±5.22^{b}	226±8.63^{ab}
	B	215±4.32^{a}	238±4.58^{a}	254±6.39^{a}
	C	186±3.60^{a}	198±5.87^{b}	206±8.63^{b}
	D	195±4.82^{a}	200±7.22^{b}	218±8.47^{b}
AST (u/L)	A	210±6.46^{b}	307±4.33^{b}	368±4.76^{b}
	B	300±10.3^{a}	353±6.64^{a}	396±6.88^{a}
	C	174±6.05^{c}	179±10.1^{c}	188±10.2^{c}
	D	180±6.68^{c}	180±6.33^{c}	185±7.60^{c}

ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase. Values with different superscripts within a column differ significantly (P<0.05). Group A, commercial IBDV infected chicks; Group B, specific antibody negative IBDV infected chicks; Group C, commercial chicks control; Group D, SAN control.

IBDV has greater affinity to the lymphocytes of the thymus than the spleen (Zubeedy *et al*., 2013; Al-Jubori, 2009). The virus destroyed the T lymphocytes thus causes severe immune suppression by destroying both B lymphocytes of bursa of Fabricius and T lymphocytes of thymus and finally failure to optimal response of vaccine (Uddin *et al*., 2012). On 5th day of postinfection moderate histopathological lesions observed depleted and necrosed B lymphocytes observed at medullar area of bursal lymphoid follicle, depleted lymphocytes replaced by heterophils and reticuloendothelial cells. The immature B-cells is believed to be first site of replication for IBD virus where virus causes damage to B cells in lymphoid follicles of bursa (Chen *et al*., 2009). The typical histological bursal lesions were similarly reported by (Murmu *et al*., 2014; Sing *et al*., 2015) that included moderate to severe lymphoid depletion in bursal follicle, cyst formation, follicular necrosis and hemorrhages formation in follicles.

Serum biochemical parameters like ALT, albumin, total protein, AST and ALP were measured on 3rd, 5th and 7th day of post-infection in infected groups. Albumin and total protein values were significantly (P<0.05) reduced in IBDV infected groups as compared to control groups. Total protein level decreased due to impaired liver function and kidney damage also led to protein loss (Zeryehun *et al*., 2012). Other causes of hypoproteinemia were anorexia, dehydration and diarrhea in IBDV infected birds. This statement is supported by Kudair and Al-Hussary (2010) who demonstrated that abnormal amount of certain enzyme at intercellular level in blood are result of damage to an organ or tissue. The ALT, AST and ALP values were significantly increased (P<0.05) in IBDV challenged birds. IBDV causing hepatocytes necrosis and rupturing of hepatocytes that elevated ALT values (Abidin *et al*., 2013). In IBDV infection liver and kidney were adversely affected that leads to increase values of serum enzymes. Similar findings were reported by Abidin *et al*. (2014) and Roosevien (2006).

CONCLUSION

The finding of present investigation indicated that serum biochemical changes in field infectious bursal disease virus were consistent with the pathogenicity of IBDV infection. These biochemical changes in IBDV infection are mostly related to damage to liver and kidney. Therefore, these parameters (biochemical changes and histological changes) are essential in clinicopathological assessment and also contribute to understand the pathogenesis of IBD field infection.

Statement of conflict of interest

Authors have declared no conflict of interest.

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