Short Communication

Cytological Changes in Chicken Infectious Anemia Virus Infected Bone Marrow in Broilers

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ABSTRACT

This study was designed to compare the cytological changes in chicken infectious anemia virus (CIAV) infected bone marrow in broiler chicken. Bone marrow samples were collected from proximal tibiotarsus and femur of each of the five healthy and CAV-infected flocks under natural condition. The bone marrow smears were fixed, stained with Romanowsky Giemsa Stain (RGS) and examined under microscope. Results showed a significant difference between the cellularity of normal and CAV-infected birds. While comparing healthy and infected chicken bone marrow, we observed a decreased mean erythroid cell percentage (39.95 versus 17.95%), myeloid cell percentage (57.29 versus 21.98%) and the mean M/E ratio (1.43 versus 1.22), respectively. Bone marrow smears were normocellular among healthy birds while, in diseased chicken, severe aplasia was noted. The bone marrow findings suggested an easy, quick and cost-effective approach for supportive diagnosis of chicken anemia virus infection and therefore could be replicated for efficient disease diagnostics in resource-limited setting.

Poultry industry is one of the most vibrant segment of livestock sector in Pakistan with a current investment of more than Rs. 700 billion (Pakistan Economy Survey 2018 – 2019). It provides a high proportion of cheapest protein derived from the animal source. However, the industry is threatened by a number of bacterial, viral, fungal and parasitic infections. Among these, many of the pathogen causes immunosuppression in birds and, subsequent to primary or secondary infection, results in failures in outcome of vaccination ultimately leading to decrease production, economic overburden, enhanced mortality and morbidity. Indeed, as a result of acquired immunosuppression, the birds become more prone to secondary viral, bacterial, parasitic and fungal infections (Schat, 2003).

Among the viral immunosuppressive diseases, Chicken infectious anemia virus is one of the devastating viral disease. The virus belongs to the genus *Gyrovirus* and family Circoviridae. It is a DNA virus that has a closed circular, negative sense, single-stranded genome. Post-infection mortality and morbidity may reach up to 55% and 80%, respectively (Lai *et al*., 2013). The disease is characterized by aplasia of bone marrow and generalized lymphoid atrophy with associated immunosuppression. Although conventional serological and molecular diagnostics is being used for identification of the virus, they are laborious, time-consuming cost-ineffective and much has been elucidated in the subject matter. In contrast, there is an absolute paucity of research and relevant data for bone-marrow studies and based diagnosis of CIAV. In fact, being actively involved in providing immunity to birds (Rubin and Strayer, 2008), the study of bone marrow may provide useful information not only about the immune status of a chicken but also prediction about presence or absence of immunosuppressive agent such as CIAV. This is true because bone marrow study facilitates diagnosis of different types of anemias, leukopenia, thrombocytopenia, pancytopenia, leukemia and other unexpected change in peripheral blood cells as has previously been documented (Thrall *et al*., 2004; Campbell, 1995; Chand *et al*., 2015). With this brief background, the aim of this study was to determine the quantitative cellular changes, morphological alterations and ratio of myeloid to erythroid series cell in normal and CAV infected birds in bone marrow smears. Based on the cytology of the bone marrow and the circulating blood cells, this study will help clinicians to have a presumptive diagnosis of CIAV and devise

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subsequent treatment and control interventions.

Materials and methods

This study involved farms in Lahore district and its surroundings. We included this particular area because poultry is too dense here and occurrence of multiple infection across the area is not very uncommon. During a period of one year (2017–2018), we screened a total of fifteen broiler flocks having the capacity of 5000–30000 birds each. Each of the farm was suspected of having CIAV on the basis of clinical symptoms that included poor growth, anemia, anorexia and depression. Bone marrow samples were obtained from each of the flock and transferred to University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore. The negativity and positivity of each of the flock was confirmed through PCR as described previously (Herman *et al.*, 2012). The samples were processed as pool of samples from each of the individual flock. Finally, after PCR confirmation, a total of five CIAV positive along with an equal number of negative broiler flocks were selected for bone marrow examination. From each flock, bone marrow (n=5) were collected from tibiotarsus and femur of chickens during necropsy and smears were made. The smear slides were fixed with methanol and stained by Giemsa stain (Al-Sadi and Hussein, 2010). The slides were then examined by microscope to evaluate cellularity of the bone marrow, to identify different stages of erythroid, myeloid and thrombocyte precursors maturation. Myeloid Erythroid Ratio (M:E) was also noted in each slide. Five hundred cells parameter was used for differential counting of all marrow cells precursors. Developmental stages of erythroid maturation including pro-erythroblast, basophilic normoblast, polychromatic normoblast and orthochromatic erythroblast were identified and calculated. Likewise, the developmental stages of myeloid progenitors (promyelocytes, myelocytes, meta myelocytes, stab form and mature lobulated granulocytes) were also calculated. The M/E ratio was calculated by dividing total number of nucleated cells of granulocytic group with total number of nucleated erythroid cells.

Complete concerned data was analyzed by using SPSS (23.0) and final results were declared as mean ± SEM.

Results and discussion

In CIAV perspective, the bone marrow-based immunosuppression studies are limited and a much of detailed analysis has previously been documented for other avian pathogens such as infectious bursal disease (IBD) and Newcastle disease (ND). This was the reason that results interpretation along with evidences are documented

or referenced while comparing other immunosuppressive agents available in the literature so far.

Table I.- Comparison of cytological changes in CAV infected and healthy bone marrow cells in broiler chicken.

	CIAV infected bone marrow (%)	Health bone marrow (%)
Erythroid maturation stages		
Pro- erythroblast	3.20 ± 0.25	7.20 ± 0.50
Basophilic erythroblast	2.70 ± 0.40	5.00 ± 0.60
Polychromatic erythroblast	8.70 ± 0.38	20.50 ± 0.58
Orthochromatic erythroblast	3.35 ± 0.30	7.25 ± 0.55
Total erythroid series cells	17.95 ± 0.33	39.95 ± 0.55
M:E ratio	1.22 ± 0.15	1.43 ± 0.28
Myeloid maturation stages		
Myeloblast	0.26 ± 0.15	0.60 ± 0.20
Pro-myelocyte	1.40 ± 0.20	3.50 ± 0.30
Myelocyte	13.34 ± 0.95	35.50 ± 1.12
Meta myelocyte	2.9 ± 0.35	7.0 ± 0.52
Band cell	2.4 ± 0.18	5.55 ± 0.30
Heterophil	0.40 ± 0.12	1.2 ± 0.25
Eosinophil	0.18 ± 0.80	0.49 ± 0.25
Basophil	0.05 ± 0.04	0.14 ± 0.10
Monocyte	0.30 ± 0.10	1.0 ± 0.15
Megakaryocyte	0.50 ± 0.20	1.5 ± 0.4
Total	21.98 ± 0.30	56.48±0.35
Lymphocyte	0.25 ± 0.12	0.80 ± 0.20

Immune system in chicken is constituted by lymphoid organs which provide a strong protective mechanism against all bacterial, viral and fungal infections. These lymphoid organs are labelled as primary or secondary lymphoid organs. Thymus, bone marrow and bursa of *Fabricius* are grouped under the heading of primary lymphoid organs whereas spleen, collections of mucosae related lymphoid tissues as secondary lymphoid organs (Riddel, 2001). In chicken development of erythrocytes and production of platelets takes place within the dilated vascular channels whereas production of granulocytic cells occurs outside the vascular sinuses (Bounous *et al.*, 2000). Our study highlighted the depression of bone marrow activity in CIAV infected birds as compared to healthy birds. A depletion in mean percentage of erythroid precursors in bone marrow of CIAV infected bird (17.9±0.30) compared to healthy ones (39.95±0.55) was observed (Table I). Similarly, depletion in mean percentage of myeloid series precursors in infected bone

marrow was observed (21.98±0.30) than in healthy ones (57.28±0.35). Decreased in M/E ratio of 1.22±0.15 was observed in infected chicken as compared to normal chicken (Table I). Our study results agree with the findings of a research conducted on bone marrow cytological changes in IBD, ND and Colibacillosis infected birds in comparison with healthy birds. In his study, deletion in erythroid and myeloid progenitor series were reported in infected birds bone marrow cytology (Wani *et al*., 2016); however, the depletion in the percentage of both progenitor series noted was non-significant when compared to control group results. In our study the difference in infected and control group cytology is significant. This might be due to the differences in the viruses used in their study, managemental practices, bird maternal immunity, use of intoxicated feed *etc*. In our study, a decrease in lymphoid progenitors were evidenced in infected bone marrow than in the control group. This finding is in line with the results of study conducted previously on IBD, ND and colibacillosis (Wani *et al*., 2016). In this study, it was noticed that among the cells of erythroid series, highest percentage of polychromatic erythroblast was found in both infected and healthy birds however the percentage of subject cells were less (8.70 ± 0.38) in infected than in control group (20.50 ± 0.58) (Table I). The morphology of cells was also examined in infected and control group. It was interesting to note that no differences in the morphology of cells were seen in both study groups. These polychromatic erythroblasts were circular in shape with a basophilic cytoplasm. In polychromatic erythroblast nucleus was small as compared to cytoplasm and was having dense clumped chromatin whereas orthochromatic erythroblasts were oval in shape with irregularly condensed chromatin material. Similar findings were noted in ducks and quails by Campbell and Coles (1986) and Tadjalli *et al*. (2002).

Among the granulocytic cells, myelocytes with eccentric nucleus and secondary granules were predominant cells which could be labelled as heterophils, basophils and eosinophils considering the characteristics of secondary granules. Metamyelocytes possess bean shaped nucleus along with secondary granules. Stab cells or band cells showed band shaped nucleus without lobulation. Monoblast and monocytes were having irregular shaped nucleus with transparent basophilic cytoplasm containing only few granules. Megakaryocytes were the largest cells in the bone marrow smear having large multi lobulated nucleus with granular cytoplasm. Lymphoblast and prolymphocytes were relatively small in size having high nuclear cytoplasmic ratio and scanty cytoplasm without granules. These mentioned morphological findings were found in concordance with the findings of earlier researchers (Tadjalli *et al*., 1997).

Conclusion

The bone marrow examination showed a significant difference in the bone marrow cellularity of normal and CAV infected bird. There was marked depression in erythroid, myeloid and megakaryocyte precursors along with developing cells, which ultimately leads to anemia, leucopenia and thrombocytopenia. Hence, bone marrow examination is easy, rapid and cost-effective analysis which may aid in the presumptive diagnosis of Chicken Infectious Anemia Virus in field contidions.

Statement of conflict of interest

Authors declared no conflict of interest.

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