Indian J. Vet. Pathol., 35(1) : 36-40, 2011

Sequential haematological and biochemical changes in experimentally induced hydropericardium syndrome in immunocompromised broiler chickens

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Received: 27.12.2010; Accepted: 25.4.2011

ABSTRACT

Kumar, Sharad and Gupta, R.P. (2011). Sequential haematological and biochemical changes in experimentally induced hydropericardium syndrome in immunocompromised broiler chickens. Indian J. Vet Pathol., 35 (1): 36-40.

The effects of cyclosporin A (T-cell suppressor) was studied in chickens experimentally infected with hydropericardium syndrome (HPS) virus. Forty five broiler chicks at the age of 18 days were injected with cyclosporin A @100mg/kg body weight by intramuscular route and subsequently at 3 days intervals upto the age of 30 days while forty five birds were injected with placebo of ethanol and castor oil only. At the age of 21 days, each group was further divided into two subgroups and the birds of one of the subgroups from each group were inoculated with HPS virus infected liver suspension (ID_{50}) subcutaneously. Haematological studies revealed the significant decrease in Hb concentrations in both the infected groups on days 4 and 6 PI and total leucocyte count from day 4 PI but in CsA plus HPS group the decrease in TLC was significant from day 2 PI and the values were significantly lower as compared to HPS group at different intervals. This decrease in TLC was due to lymphocytopenia. Biochemical studies revealed the significant decrease in serum total protein and albumin concentrations in HPS group as compared to control from days 4 to 9 PI and significant increase in the activities of serum aspartate transaminase, alanine transaminase, lactate dehydrogenase and creatine phosphokinase though the increased activities of these enzymes were of higher magnitude in CsA plus HPS group. In the pericardial fluid too, the activities of these enzymes were significantly higher in CsA plus HPS group as compared to HPS group. It may be concluded that the severity of HPS infection was of higher magnitude in the immunocompromised chickens.

Keywords: Broiler chickens, cyclosporin A, haematological changes, hydropericardium, immunosuppression

INTRODUCTION

Hydropericardium syndrome (HPS) is a disease of three to six-week-old broiler chicks, causing significant economic losses to the poultry farmers. The etiological agent of HPS has been identified as fowl adenovirus serotype 4 (FAV)[1]. However, some workers suggested that FAV alone might not be able to cause the disease and proposed that prior immune suppression or synergism with other viruses such as chicken anemia virus, infectious bursal disease virus or other viruses is necessary to induce HPS in chickens[2,3]. Little experimental information is available in the literature on the effects of T cell suppressive agents on haematological and biochemical parameters in HPS affected chickens. The objective of the present study was to investigate the effect of cyclosporin-A (CSA), a known T-cell suppressor on haematological and biochemical parameters in experimentally HPS infected broiler chickens.

MATERIALS AND METHODS

Experimental chicks and feed

Day old commercial broiler chicks of Cobb strain were procured from a local hatchery having no earlier history of occurrence of hydropericardium syndrome (HPS) in the parent flock and reared under strict hygienic conditions. All the birds were given *ad libitum* alfatoxin/ mycotoxin free and autoclaved standard chick feed and provided clean drinking water through out the experiment. The approval for conducting the experiment was taken from the Institutional Animal Ethics Committee, CCS HAU, Hisar. Cyclosporin–A was dissolved in a solution containing 5.5 parts absolute ethanol and 13 parts sterilized castor oil to get the dose rate of 100mg/kg body weight.

Experimental design

Ninety broiler chicks at the age of 18 days were divided randomly into two groups viz. group A and group B of 45 chicks each. Each bird of the group A was injected with cyclosporin A @100mg/kg body weight by intramuscular route on 18th day of age and subsequently at 3 days intervals upto the age of 30 days whereas the birds of group B were injected with placebo of ethanol and castor oil. At the age of 21 days, three birds from each group were sacrificed and then each group was further divided into two subgroups i.e. group A into groups A1 and A2 of 27 and 15 chicks, respectively and group B into group B1 and B2 of 27 and 15 chicks,

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respectively. Each bird of groups A1 and B1 was injected 0.5 ml of 10^{-3} diluted HPS virus inoculum as infective dose (ID50) calculated using the method of Reed and Muench[4], subcutaneously whereas the birds of groups A2 and B2 were injected with liver inoculum prepared from healthy birds.

Blood samples were collected from three birds from each group on 0,2,4,6,9 and 13th day post infection separately in sterile vials containing 2 mg/ml of ethylene diamine tetra acetate (EDTA) for haematological studies and in sterile vials for serum separation. For differential leucocyte count (DLC) the smear was prepared and fixed with methanol. After blood collection, these birds were sacrificed and the pericardial fluid, if any was collected aseptically in sterile, graduated centrifuge tubes for measuring volume and biochemical studies.

Haematological and biochemical changes

Total leucocyte count (TLC) was determined in bright line improved Neubauer haemocytometer as per method described by Natt and Herrick[5]. The blood smear, prepared from fresh blood were stained with Wright staining method. Haemoglobin (Hb) concentration was estimated by cyanmet haemoglobin method[6]. Serum and pericadial fluid samples were analyzed for concentration of total protein and albumin and activities of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) using Chemistry Auto Analyzer (RA-50, Bayer diagnostics). Statistical significance was assessed by using analysis of variance[7].

RESULTS

Haematological values

Mean haemoglobin (Hb) concentrations of different experimental group were as given in Table 1. A significant decrease in Hb concentrations was observed in both the infected groups on days 4 and 6 PI as compared to control. The difference between the Hb values of both the infected groups was non significant. CsA administration did not cause any significant effect on Hb concentration. Mean total leucocyte count for different experimental groups was given in Table 2. A

Table 1. Haemoglobin concentrations (g/dl) in chickens of different experimental groups (mean±S.E.)

Groups	Post infection period (days)					
	0	2	4	6	9	13
Control	5.50±0.10[a]	5.47±0.53[a]	6.00±0.30[a]	6.20±0.40[a]	5.63±0.26[a]	6.20±0.40[a]
CsA	5.86±0.15[a]	6.17±0.12[a]	6.03±0.03[a]	6.33±0.38[a]	5.97±0.03[a]	6.23±0.14[a]
HPS	5.50±0.10[a]	5.67±0.35[a]	5.07±0.07[b]	4.80±0.29[b]	5.47±0.40[a]	6.30±0.17[a]
CsA+HPS	5.86±0.15[a]	5.17±0.31[a]	5.57±0.06[b]	4.63±0.23[b]	6.23±0.09[a]	6.33±0.33[a]

Table 2. Total leucocyte counts (10^3/µl) in chickens of different experimental groups (mean±S.E.)

Groups	Post infection period (days)					
	0	2	4	6	9	13
Control	23.50±0.45[a]	22.33±1.92 [a]	22.33±1.83 [a]	24.17±2.13 [a]	22.60±0.33 [a]	25.33±1.30 [a]
CsA	22.67±1.01 [a]	19.00±1.04 [a]	15.33±0.60 [b]	17.00±0.76 [b]	16.50±0.58 [b]	19.00±0.76 [b]
HPS	23.50±0.45 [a]	19.00±0.29 [a]	18.17±1.31 [b]	19.17±0.44 [b]	17.83±1.05 [b]	20.33±1.65 [b]
CsA+HPS	22.67±1.01 [a]	14.00±0.32 [b]	15.67±0.93 [b]	17.67±1.61 [b]	17.17±1.17 [b]	17.00±0.70 [b]

a,b,c : Means with unlike superscript in a column were significantly different, P<0.05

Table 3. Percent heterophils (H) and lymphocytes (L) in chickens of different experimental groups (mean±S.E.)

Groups		Post infection period (days)					
		0	2	4	6	9	13
Control	H	23.67±0.88[b]	26.67±0.33[b]	26.00±1.15[b]	27.67±0.33[a]	26.33±0.33[a]	25.33±0.33[a]
	L	62.67±0.33[a]	61.33±0.88[a]	64.33±2.60[a]	61.00±0.58[b]	67.67±1.20[a]	66.00±0.58[a]
CSA	H	27.00±2.31[b]	37.00±2.08[a]	34.00±0.00[a]	30.00±0.58[c]	32.00±0.58[b]	28.33±0.33[ac]
	L	58.00±0.58[a]	47.00±0.58[b]	52.67±0.83[b]	49.00±0.58[a]	55.00±0.58[b]	59.00±0.58[b]
HPS	H	23.67±0.88[b]	25.33±7.03[ab]	30.00±4.62[b]	26.00±1.15[a]	25.33±2.45[a]	21.33±1.33[ab]
	L	62.67±0.33[a]	60.00±11.85[a]	56.00±13.86[b]	70.00±1.15[b]	66.67±12.34[ab]	68.67±1.76[a]
CSA+HPS	H	27.00±2.31[b]	25.33±5.81[b]	34.33±0.33[a]	36.37±0.88[b]	36.00±0.58[b]	30.67±1.76[c]
	L	58.00±0.58[a]	55.33±1.76[b]	51.00±0.58[b]	51.33±0.33[a]	55.67±0.33[b]	56.00±1.00[b]

a,b,c : Means with unlike superscript in a column were significantly different, P<0.05

Table 4. Absolute heterophils (H) and lymphocytes (L) (10^3/µl) in chickens of different experimental groups (mean±S.E.)

Groups		Post infection period (days)					
		0	2	4	6	9	13
Control	H	4.96±0.42^a	5.95±0.37^a	5.80±0.56^a	6.68±0.73^a	5.95±0.31^a	6.41±0.36^a
	L	14.09±0.11^b	13.69±0.35^a	14.36±1.65^a	14.74±0.45^a	15.29±0.26^a	16.71±1.33^a
CSA	H	5.57±0.23^a	7.03±0.08^a	5.21±0.20^a	5.09±0.14^a	5.27±0.08^a	5.39±0.26^a
	L	12.95±0.24^b	8.93±0.57^b	8.07±0.34^{bc}	8.34±0.46^b	9.08±0.37^c	11.22±0.53^b
HPS	H	4.96±0.42^a	4.79±1.38^a	5.45±0.43^a	5.48±0.51^a	4.61±0.45^a	4.78±0.31^a
	L	14.09±0.11^b	10.09±1.35^{ab}	10.51±0.27^b	12.95±0.20^a	11.65±0.48^b	13.57±0.32^b
CSA+HPS	H	5.57±0.23^a	4.94±0.43^a	5.42±0.35^a	6.71±0.21^a	5.93±0.19^a	5.21±0.45^a
	L	12.95±0.24^b	7.76±0.40^b	7.17±0.58^c	8.14±0.32^b	9.19±0.48^c	9.52±0.25^c

a,b,c : Means with unlike superscript in a column are significantly different, P<0.05

Table 5. Values of volume (ml) and AST, ALT, LDH and CPK activities (IU/L) in pericardial fluid of HPS infected groups (mean±S.E.)

Groups	Parameter	Post infection period (days)		
		2	4	6
HPS	volume	1.00±0.58	9.33±0.67	0.67±0.33
	AST	414.60±2.19	264.10±2.25	260.15±2.76
	ALT	6.10±1.03	6.53±0.20	5.95±0.15
	LDH	351.33±7.76	248.13±4.17	359.75±0.55
	CPK	278.30±1.21	112.60±1.09	112.95±0.45
CsA+HPS	volume	1.67±0.88	14.33±1.20*	1.00±0.58
	AST	559.00±5.19**	572.73±1.45**	404.20±0.40**
	ALT	25.23±1.54**	12.63±1.88*	7.65±0.05*
	LDH	407.83±1.21*	358.30±2.25*	406.25±0.25*
	CPK	635.60±5.66**	316.70±1.85**	201.25±0.35**

** Significantly different from HPS group P<0.01; * Significantly different from HPS group P<0.05.

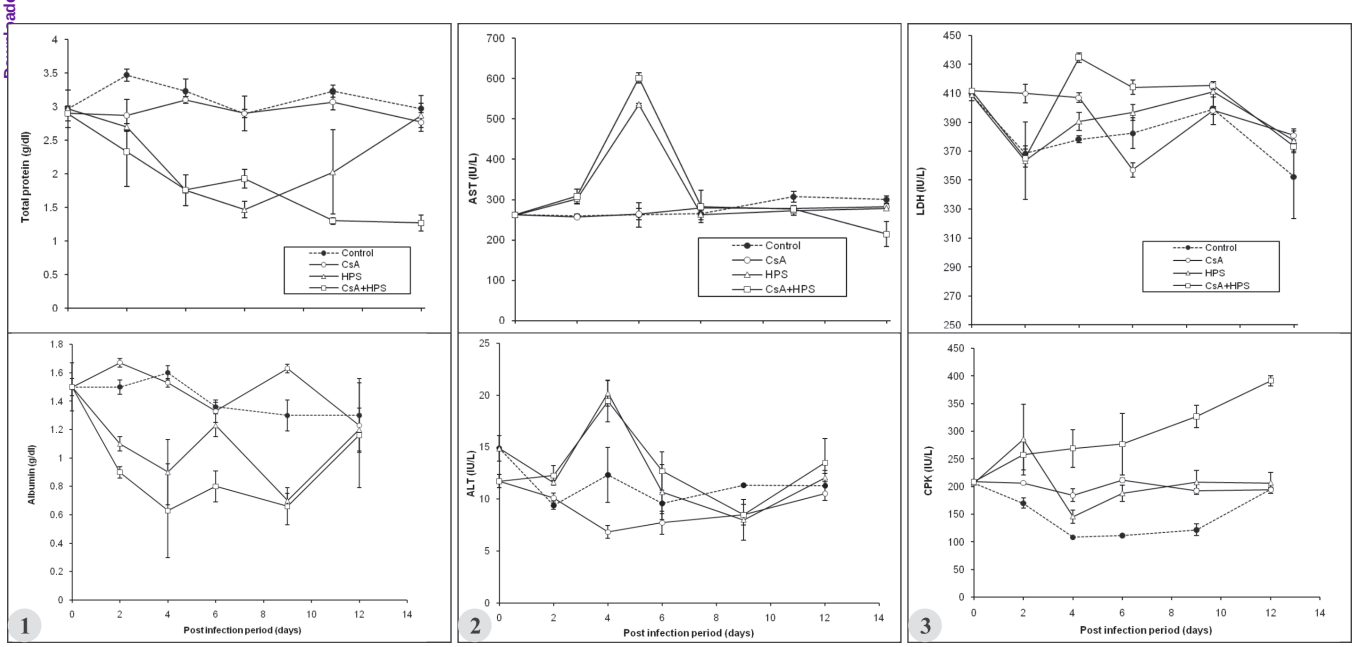

Fig. 1. Serum total protein and albumin concentrations in chickens of different experimental groups (mean±S.E.); **Fig. 2.** Serum aspartate transaminase and alanine transaminase activities in chickens of different experimental groups (mean±S.E.); **Fig. 3.** Serum lactate dehydrogenase and creatine phosphokinase activities in chickens of different experimental groups (mean±S.E.)

significant decrease in TLC was observed in HPS group as compared to control from day 4 PI. In CsA plus HPS group, the decrease in TLC was significant from day 2 PI as compared to control. Furthermore, the values of TLC in CsA plus HPS group were lower as compared to HPS group at different intervals. CsA alone also caused significant decrease in TLC from day 4 PI as compared to control. Mean per cent values of heterophils and lymphocytes for each experimental group were as given in Table 3 and their absolute values in Table 4. There was no significant difference in mean values of per cent heterophils in HPS infected birds from that of control at the different intervals. However, significant increase in per cent heterophils was observed in CsA group from day 2 PI and in CsA plus HPS group from day 4 PI as compared to control. A significant decrease in per cent lymphocytes was noticed in HPS group on day 4 PI, and CsA plus HPS groups from day 2 PI as compared to control. There was no significant difference in absolute count of heterophils among the different experimental groups. A decrease in absolute count of lymphocytes was observed in both the infected groups as compared to control from day 2 PI but this decrease was more significant in CsA plus HPS group. CsA alone also caused significant reduction in absolute lymphocyte count from day 2 PI as compared to control.

Serum biochemical values

Mean serum total protein and albumin concentrations of both groups were as illustrated in Fig 1. A significant decrease in serum total protein and albumin concentrations was observed in both HPS infected groups as compared to control from days 4 to 9 PI. Mean serum aspartate transaminase (AST) and alanine transaminase (ALT) values of different experimental groups were illustrated in Fig 2. A significant increase in serum AST and ALT activities was observed in both the infected groups on days 2 and 4 PI as compared to control and this increase was more significant in CsA plus HPS group. Mean serum lactate dehydrogenase (LDH), creatine phosphokinase (CPK) activities were illustrated in Fig 3. There was increase in serum LDH and CPK activities in both the infected groups as compared to control from day 4 to 9 PI onwards but this increase was significant only in CsA plus HPS group.

Pericardial fluid studies

Mean values of volume and activities of AST, ALT, LDH and CPK in pericardial fluid in both the infected groups were as given in Table 5. Mean values of pericardial fluid volume in CsA plus HPS group were higher as compared to HPS group from days 2 to 6 PI but this increase was statistically significant on day 4 PI. The pericardial fluid in the birds of control and CsA groups at different intervals was almost negligible and could not be quantified. A significant increase in AST, ALT, LDH and CPK activities in pericardial fluid was observed in CsA plus HPS group as compared to HPS groups from days 2 to 6 PI.

DISCUSSION

A significant decrease in Hb concentrations in both the HPS infected groups on days 4 and 6 PI was almost of the same magnitude indicating no effect of CsA administration on Hb concentration. The reduction in Hb concentrations in HPS affected chicks has also been reported by other workers[8]. Internal haemorrhages noticed in cardiac muscles and some other organs of both the HPS infected groups might have caused reduction in haemoglobin concentration. Significant decrease in total leucocyte count due to lymphocytopenia was noticed earlier in CsA plus HPS group i.e. from day 2 PI and even the values at different intervals were significantly lower as compared to HPS group. Lymphocytopenia has also been reported in natural cases of HPS in chickens[8]. The mechanism by which HPS caused lymphocytopenia is not clear. However, the findings of lymphocytopenia were supported by the facts that HPS caused depletion of lymphocytes in spleen, thymus and bursa of Fabricius. A significant decrease in serum total protein observed in HPS infected birds was due to hypoalbuminemia might be attributed to acceleration in protein catabolism because of the stress of the infection and fever. Secondly, it might be secondary to the increase in globulin concentration (not estimated in the present study) since colloid osmotic pressure has to be maintained within normal limits by a regulatory mechanism[10]. Hepatic necrosis/hepatitis which is a consistent finding in HPS might have also contributed to the hypoalbuminemia since liver diseases have been reported to inhibit albumin synthesis[11]. On day 13 PI, serum albumin values in both the infected groups reached almost to the control value indicating recovery in the values.

Serum AST and ALT activities were increased significantly in both the infected groups during early acute phase of the infection though the increase was more significant in CsA plus HPS group. More or less similar results have been reported in natural and experimental cases of HPS in the literature[12,13]. The increased activity of AST was probably due to myocarditis as reported in the HPS infected birds, since this enzyme is present in large quantities in cardiac muscles[9,10]. The increased activity of serum ALT observed in the infected groups might be due to excessive release of this enzyme from the liver as a result of hepatic necrosis/hepatitis since an increase in the activity of serum ALT is considered to be a sensitive indicator of hepatic cell damage and alteration in the permeability of the hepatic cell membrane[14]. Increased activity of serum LDH in both the infected

groups was observed during acute phase of the infection particularly when severity of the lesions was at peak though this increase was significant only in CsA plus HPS group. Difference in increased activity of LDH between both the infected groups could be correlated with the severity of lesions in cardiac muscles, hepatocytes and lungs since this enzyme is present in the cells of these organs[10]. The increase in serum CPK activity in CsA plus HPS group was more as compared to HPS group at the different intervals. The elevated CPK activity in HPS infected chicks might be attributed to cardiac lesions which has been reported in HPS affected chickens by many workers[9,15,16]. Furthermore, the quantitative differences in the results of serum CPK activity due to the infection in both the infected groups might be because of differences in the severity of cardiac lesions. Nevertheless, the increase in serum CPK activity in human beings has been reported to be a reliable indicator of myocardial infarction[15].

The amount of pericardial fluid in CsA plus HPS group was significantly higher as compared to HPS group. This difference in the amount of pericardial fluid might be due to difference in the severity of the infection between both the infected groups as CsA administration caused immunosuppression which might be responsible for the severe lesions of HPS in CsA plus HPS group. It appears that no biochemical study has been carried out in pericardial fluid of HPS affected birds. However, in the present study, the biochemical studies in pericardial fluid of HPS infected birds revealed that there was significant increase in the activities of AST, ALT, LDH and CPK in the pericardial fluid of CsA plus HPS group as compared to HPS group. It seems that these enzymes might have leaked in the pericardial fluid from serum since the values of these enzymes in serum were significantly higher in both the infected groups. On the basis of these results, it was concluded that the severity of HPS was enhanced due to T-cell immunosuppression.

ACKNOWLEDGEMENTS

The financial support to first author in the form of Junior Research Fellowship by Indian Council of Agricultural Research, New Delhi is duly acknowledged.

REFERENCES

1. Toro H, Prusas C, Raue R, Cerda L, Geisse C, Gonzalez C and Hess M. 1999. Characterization of fowl adenoviruses from outbreaks of inclusion body hepatitis/hydropericardium syndrome in Chile. *Avian Dis.*, **43:** 262-270.
2. Survashe BD, Pandit SV, Ghalsasi GR and Mallikarjun S. 1996. Inclusion body heptitis/hydropericardium syndrome (Leechi heart disease) in India - An overview. Proc. 20th World's Poult. Cong. New Delhi. 2-5 Sept. pp. 375-380.
3. Toro H, Gonzalez C, Cerda L, Hess M, Ryes E and Geisse C. 2000. Chicken anemia virus and fowl adenovirus: association to induce the inclusion body hepatitis/hydroperi cardium syndrome. *Avian Dis.,* **44:** 51-58.
4. Reed LV and Muench H. 1938. A simple method of estimating fifty per cent and points. *Am. J. Hyg.*, **27:** 493-497.
5. Natt MP and Herrick CA. 1955. The effect of caecal coccidiosis on the blood cells of the domestic fowl. *Poultry Sci.*, **34 :** 1100-1106.
6. Schalm OW, Jain NC and Carroll EJ. 1975. *Veterinary Haematology*, Lea and Febiger, Philadelphia.
7. Snedecor WG and Cochran GW. 1967. *Statistical Methods*. The Iowa State University Press, Ames, Iowa, USA.
8. Asrani RK, Gupta VK, Sharma SK, Singh SP and Katoch RC. 1997. Hydropericardium hepatopathy syndrome in asian poultry. *Vet. Rec.,* **141 :** 271-273.
9. Kumar S and Gupta RP. 2003. Biochemical and pathological changes in experimentally induced hydropericardium syndrome in broiler chickens. *Haryana Vet.*, **42**: 56-60.
10. Benjamin MM. 1978. *Outline of Veterinary Clinical Pathology*. 3rd edn., The Iowa State University Press Ames. Iowa, USA.
11. Kirsch RE. 1983. Protein synthesis. In: *Clinical Hepatology*, G.Csmos and H. Thaler (eds), Springer-Verlag, Berlin, pp. 78-86.
12. Iqbal MJ, Bhatti BM and Khan KM. 1994. Studies on serum enzymes in hydropericardium syndrome (*Angara disease*) of broilers. *Pakistan Vet. J.*, **14** : 83-85.
13. Kumar S and Khrole MU. 1999. Role of immuno-suppression in pathogenesis of hydropericardium syndrome : *Indian J. Poult. Sci.,* **34** : 220-223.
14. Christen P and Metzler DE. 1985. Transaminases. In: *Aminotransferases in Human Pathology and Clinical Chemistry,* E. Schmidt and F.W. Schmidt (eds.), Wiley, New York, pp. 587-624
15. Cotran RS, Kumar V and Robbins SL. 1994. *Pathological Basis of Diseases*. W.B. Saunders Co., Philadelphia, 5th edn. pp. 528-538.
16. Akhtar S. 1994. Hydropericardium syndrome in broiler chickens in Pakistan. *World's Poult. Sci. J.*, **50** : 177-182.