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Clinico-pathological and Immunological Changes in Chickens Infected with Chicken Anemia Virus

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Abstract

Chicken infectious anemia virus (CIAV) is an immunosuppressive viral disease causing high economic losses in poultry industry. In this study, 20 farms were represented for a prevalence study of the disease in Ismailia governorate, Egypt.ON532690.1 and ON532691.1 Isolates from bone marrow, thymus loops, liver, and spleen of broiler farms exhibiting some clinical and postmortem signs were used for reverse transcription polymerase chain reaction assay. RT-PCR was used to amplify a 418bp product of the CIAV VP1 gene. Three farms out of 20 (15%) were positive. Phylogenetic tree of partial vp1 amino acids were classified into three groups according to change in H/22/N-Q amino acid indicated that there are three CIAV different strains circulating in Egypt. Hematological investigation revealed significant decrease in RBCs count, hemoglobin concentration, and packed cell volume declared normocytic normochromic anemia. The immunological studies revealed a significant decrease in serum lysozyme, nitric oxide (NO), antioxidants (CAT and GSH), total protein, and in the majority of serum protein fractions in infected chickens (G2) compared to apparently healthy (G1) while there were marked increase in G2 than G1 in A: Gratio. This result guides to review the vaccination programs against CIAV in Egypt for improving the immune response against the infection.

KEYWORDS
Broiler, CAV, PCR, Immunity, Hematology.

INTRODUCTION

Chicken infectious anemia virus (CIAV) is an immunosuppressive viral disease with severe economic impacts on broilers. It was first identified as a circovirus in Japan in 1978 (Yuasa *et al*., 1979). The virus belongs to genus Gyrovirus of the family Circoviridae which is a small, single strand, negative sense, circular DNA genome with three overlapping open reading frames (ORFs). The ORFs named VP1, VP2, and VP3 with 1350, 651, and 366 nucleotides respectively. The CAV's Amino Acid (A.A.) composition is extremely restrictive, with major differences in some regions in the VP1 gene called Hyper Variable Region (HVR) at 139-151 A.A. (Zhang *et al*., 2013). It encodes for three viral proteins, VP1 is the major structural protein, VP2 is a scaffolding protein, and VP3 is non-structural apoptin protein that induced apoptosis to thymocytes and hemopoietic cells in infected chickens (Castano *et al*., 2019).

CIAV infections are manifested by either clinical or subclinical symptoms (Davidson *et al*., 2004). The infection may manifest differently and with varying degrees of severity in young chicks. These symptoms include stunting, increased mortality, anemia, bone marrow cell depletion, subcutaneous hemorrhage, and atrophy of secondary lymphatic organs (Dhama *et al*., 2008; Schat, 2009).

CIAV infections can spread both vertically and horizontally in chickens. Young chicks are affected clinically by vertical transmission at 10–14 days old, which impairs their immune systems. Older birds can also be infected by horizontal transmission causing mainly the sub-clinical disease (McNulty, 1991). Importantly, CIAV is increasing in prevalence and infection increases susceptibility to a wide variety of other avian viral and bacterial pathogens, presumably through immunosuppression of the CIAV-infected bird (Todd, 2004).

CIAV infection compromises immune response through lymphoid depletion and causes a global immune deregulation with focus on T-cells inhibition (Giotis *et al*., 2015). Additionally, CIAV infection can inhibit the IFN-γ/ macrophages/NO axis, as one of CAV's many immunosuppressive effects and thus attenuate nitric oxide (NO) production in HD11 chicken macrophages (Ester and Ragland, 2020). Whereas, the suppression of macrophages functions severely compromise the host innate and adaptive immunity (Kaspers *et al*., 2008) and subsequently lead to failure of vaccination in chicken farms.

The aim of this work was to detect chicken anemia virus (CAV) circulating in Egypt and studying its molecular characterization. In addition to assess changes in immunological and hematological markers and the effect of CAV on the antioxidant enzymes along with the background information and clinical symptoms that aid in the diagnosis of CAV disease.

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MATERIALS AND METHODS

Ethical approval

There was no experimental work done on chicken during this study, it was only employed for samples collection. Handling of chickens was done in accordance with the European Communities Council Directive 1986 guidelines and approved by the National Animal Health and Research Institute, Giza, Egypt.

Field samples

A total of 185 samples of broilers were collected, aged 15-35 days. Samples were randomly collected from apparently healthy and clinically diseased chickens from 20 broiler flock in Ismailia, Egypt, during the period from September 2021 to August 2022. Samples collected from each chicken were tissue samples, whole blood, and serum. These birds suffered from anemia, uneven growth and vaccine failure with several complications and suspected to be infected with CIAV. The tissue samples (thymus loops, bone marrow, liver, spleen) were homogenized in PBS containing antibiotics (penicillin 1000 IU/ml and streptomycin 1 mg/ml) using electrical homogenizer. The mixture was frozen and thawed three times then centrifuged at 3000 rpm for 20 minutes and the supernatant was collected and preserved at -80°C for PCR testing.

Nucleic acid extraction and PCR

The DNA was extracted from the tissue samples using the QIAampminielute virus spin kit (Qiagen, Germany, GmbH). The oligonucleotide primers 5′-CTAAGATCTGCAACTGCGGA-3′and reverse: 5´-CCTTGGAAGCGGATAGTCAT-3´) were supplied by Metabion, Germany and used to amplify 418 base pairs (bp) of VP1 gene of CIAV (Hussein *et al*., 2002). Polymerase chain reaction (PCR) was performed using theEmeraldAmp® GT PCR kit (TaKaRa Bio, Inc., Shiga, Japan) following the manufacturer's instructions (Hussein *et al*., 2002).

Sequencing and phylogenetic analysis

It was done according to Tamura *et al*. (2018), two representative samples were sent for sequencing in Animal Health Research Institute, El-Dokki, Egypt.

Hematological studies

Whole blood samples of broiler chicken were evaluated for hematological parameter, red blood corpuscles (RBCs 10^6/µl), hemoglobin (Hb g/dl), packed cell volume (PCV %), and blood indices; mean corpuscular volume (MCV fl), mean corpuscular hemoglobin (MCH pg.), mean corpuscular hemoglobin concentration (MCHC %), total leukocytic count (WBCs x10^3/ µl), and differential leukocytic count (Lewis *et al*., 2006).

Lysozyme activity assay

The activity of serum lysozyme was measured according to Schultz (1987).

Measurement of serum nitric oxide (NO)

It was estimated as previously described by Rajaraman *et al*. (1998).

Serum protein electrophoretic pattern

Total protein and electrophoretic pattern analysis were carried out in all serum samples using the polyacrylamide gel technique described by Sonnenwirth and Jarett (1980).

Measurement of serum antioxidants (CAT and GSH)

The levels of CAT enzyme were estimated in all serum samples by using colorimetric method according to Aebi (1984). The serum GSH levels were measured following Biodiagnostic manufacturer's instructions according to Beutler *et al*. (1963).

Statistical analysis

The obtained results were statistically analyzed using IBM SPSS software version 23.0 (IBM SPSS Statistics for Mac OS, Armonk, NY, USA). Results were expressed as mean values±standard error (SE) and compared by one-way ANOVA (P≤0.05).

RESULTS

Clinical observations

The most common signs were paleness in addition to ruffled feathers and uneven growth with weakness or healthy failure. Many other complications such as diarrhea, respiratory manifestation and locomotor disorders were observed in broiler chicken flocks.

Postmortem finding

The most prominent postmortem lesions were observed in lymphoid organs. Thymus was varied from congested to atrophied. Liver was clearly pale in color. Spleen was in variable size and color from small pale colors to enlarged with ecchymosis hemorrhages. Bone marrow was pale. Hemorrhagic lesion appeared in muscles specially the breast muscles.

CIAV detection using RT-PCR

Out of 20 broiler chicken farms CIAV samples tested with Rt-PCR, 3 farms (15%) were positive. All Rt-PCR positive samples showed specific bands at 418 bp on agarose gel (Fig. 1).

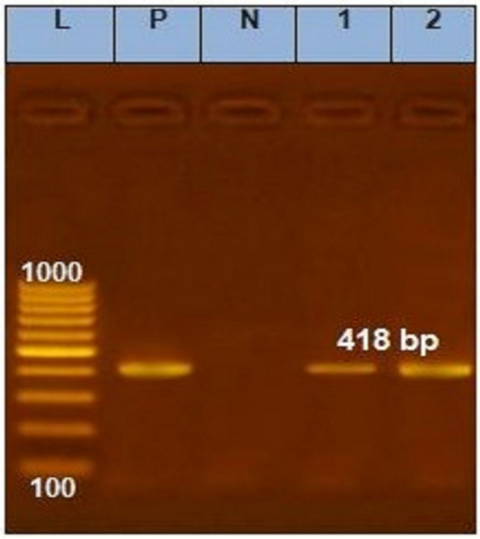

Fig. 1. Gel electrophoresis of Rt-PCR showing 418 bp band with positive control (P) and positive samples (lanes 1,2) and no band was observed in negative control (N). M= 100 bp ladder.

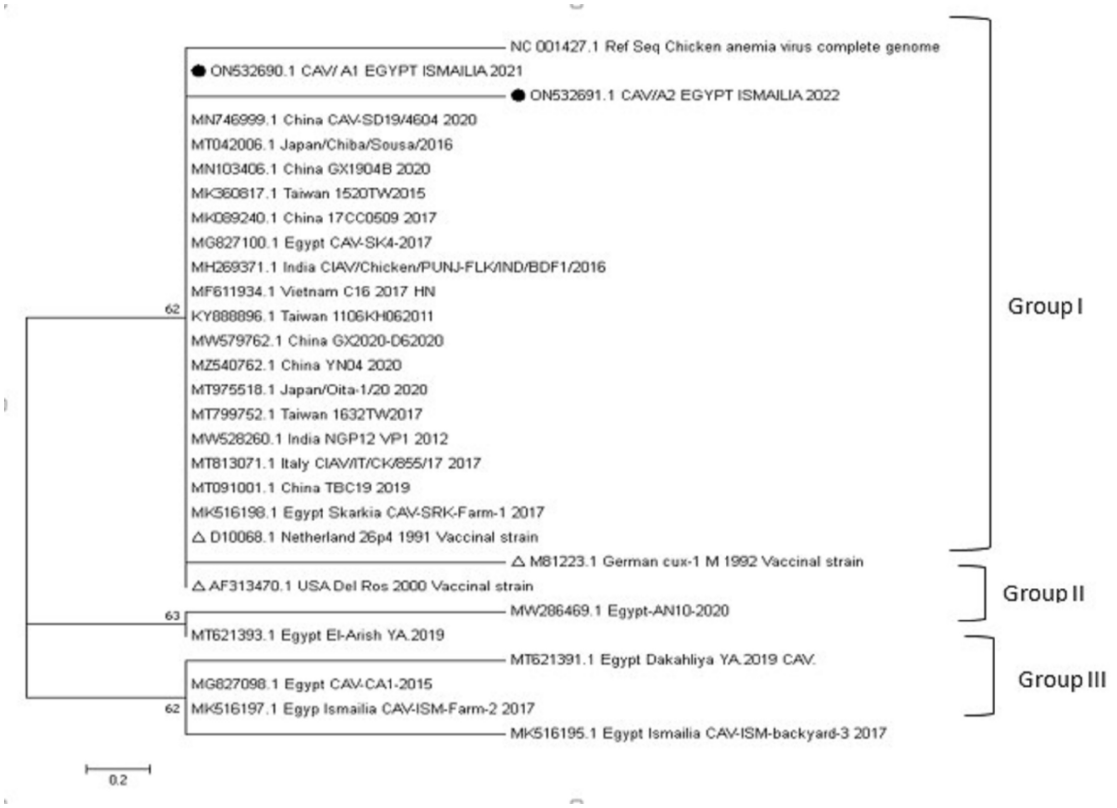

Fig. 2. Phylogenetic tree of two Egyptian CIAV isolates from GenBank. Phylogenetic relationship of 1000 bootstrap was conducted using MEGA version 7.0.26.

Sequence analysis of partial VP1 sequences of two CAV isolates

There are changes in nucleotides at positions A/832/G, C/837/T, T/892/G in both isolated strains in compere with reference strain.

Studies of partial vp1 amino acids sequence in two isolated strain showed change in amino acid at position S/14/A, while change at position N/63/S in the A2 isolate strain (Table 1); The phylogenetic study of partial vp1 amino acids resulted into classification the phylogenetic tree into three groups (Fig. 2), it was depending on the change in amino at position H/22/N-Q, the isolated strains were located in phylogenetic group I with the vicinal strains, and China , Japan, India, Vietnam, Italy, Taiwan, Egypt (Sharqia) while group II and III include others Egyptian strains indicated that there are three strains circulating in Egypt depend on the change in amino at position H/22/N-Q.

Hematological findings

RBCs count (0.78±0.64 x10^6/µl), hemoglobin concentration (6.48±0.11 g/dl) and packed cell volume (18.96±0.19%) all showed a significant decrease ($p<0.05$), while mean corpuscular volume (107.72±3.73 fl), mean corpuscular hemoglobin (36.82±1.3 pg) and mean corpuscular hemoglobin concentration (34.18±0.7 %) showed non-significant change compared to the apparently healthy one. The total leukocytes count (TLC ×10^3/µl) declared a significant decrease (32.7±1.16) ($p< 0.05$) as a result of significant decrease in the lymphocyte (16.20±0.45), monocyte (0.76±0.07) and eosinophil (0.69±0.08). However, heterophils, showed a significant increase (15.10±0.56) ($p< 0.05$) in the diseased chicken compared to apparently healthy one. While basophils declared non-significant change (Table 2).

Serum lysozyme activity and nitric oxide assays

The results of the serum lysozyme and NO assays (Fig. 3) revealed significant decrease ($P\leq0.05$) for both parameters in the CIAV infected chickens (G2) compared to the apparently healthy chickens (G1). Where the lysozyme levels were 24.47±1.62 and 17.29±0.37 µg/ml, and the NO levels were 8.09±1.18 and 4.05±0.94 µmol/ml in G1 and G2, respectively.

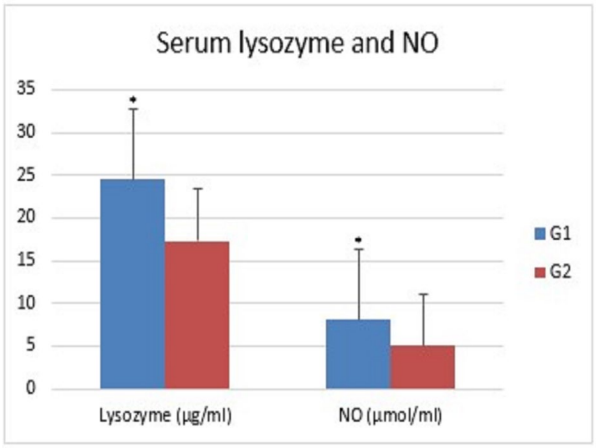

Fig. 3. The effect of CIAV infection on the serum levels of lysozyme and NO. G1 is apparently healthy chickens and G2 is CIAV diseased chickens. Data are presented as means with error bars. Columns with an asterisk indicates significantly different from the other columns within the same parameter ($p\leq0.05$).

Serum antioxidants (GSH and CAT)

The findings of the antioxidant enzymes evaluation indicated that CIAV infection in G2 induced significantly decreased levels ($P\leq0.05$) of GSH and CAT enzymes (Fig. 4).

Total proteins and electrophoretic pattern analysis of chicken's serum

Most serum protein fractions (g/dl) revealed significant increase ($P\leq0.05$) in the apparently healthy chicken (G1) com

Table 1. Nucleotide identity (%) of the VP1 gene of the examined CIAV isolates with Egyptian, and vicinal sequences retrieved from Gen Bank.

Seq->	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
NC_001427.1 Ref Seq, USA, 1991		98%	97%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	95%	97%	95%	97%	97%	95%	98%	98%	97%	98%
ON532690.1 CAV/ A1 EGYPT ISMAILIA 20	98%		98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
ON532691.1 CAV/A2 EGYPT ISMAILIA 202	97%	98%		98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	95%	97%	95%	97%	97%	95%	98%	98%	97%	98%
MN746999.1 China CAV-SD19/4604 2020	98%	100%	98%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MT042006.1 Japan/Chiba/Souta2016	98%	100%	98%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MN103406.1 China GX19045, 2020	98%	100%	98%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MK360817.1 Taiwan 1520TW_2015	98%	100%	98%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MK080240.1 China ITCC0509, 2017	98%	100%	98%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MG827100.1 Egypt CAV-SK4-2017	98%	100%	98%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MH089371.1 India CIAV/Chicken/PUNJ-FLK	98%	100%	98%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MF611934.1 Vietnam C16_2017_HN	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
KY888886.1 Taiwan 1106H06_2011	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MW536762.1 China GX2020-D6_2020	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MZ540762.1 China YN04, 2020	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MT977518.1 Japan/Oita-I/20 2020	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MT799752.1 Taiwan 1632TW_2017	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MW536260.1 India NGP12 VP1 2012	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MT813071.1 Italy CIAV/IT/CK/855/17 2017	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MT091001.1 China TBC19, 2019	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		97%	98%	97%	98%	98%	97%	100%	100%	98%	100%
MW286469.1 Egypt-AN10-2020	95%	97%	95%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%		98%	95%	97%	97%	95%	97%	97%	95%	97%
MT621393.1 Egypt, El-Adsh YA 2019	97%	98%	97%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%		97%	98%	98%	97%	98%	98%	97%	98%
MT621391.1 Egypt, Dakahliya YA 2019 CAV	95%	97%	95%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	95%	97%		98%	98%	97%	97%	97%	95%	97%
MG827098.1 Egypt, CAV-CA1-2015,	97%	98%	97%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	97%	98%	98%		100%	98%	98%	98%	97%	98%
MK516197.1 Egyp, Ismailia CAV-ISM-Farm	97%	98%	97%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	97%	98%	98%	100%		98%	98%	98%	97%	98%
MK516195.1 Egypt, Ismailia CAV-ISM-backy	95%	97%	95%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	97%	95%	97%	97%	98%	98%		97%	97%	95%	97%
MK516198.1 Egypt, Sharkia, CAV-SRK-Farr	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%		100%	98%	100%
D10068.1 Netherland 26pf 1991 Vaccinal s	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%		98%	100%
M81223.1 German cux-1 M 1992 Vaccinal	97%	98%	97%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	95%	97%	95%	97%	97%	95%	98%	98%		98%
AF313470.1 USA Del Rot 2000 Vaccinal s	98%	100%	98%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	97%	98%	97%	98%	98%	97%	100%	100%	98%	

Table 2. Hematological parameters in chicken anemia diseased broiler chickens compared to apparently healthy one.

Parameters \ Groups	Apparently healthy chicken	Clinically diseased chicken
RBCs (x10^6/μl)	2.6±1.76^a	0.78±0.64^b
Hb (g/dL)	9.41±0.08^a	6.48±0.11^b
HCT (%)	26.55±0.12^a	18.96±0.19^b
MCV (fL)	102.12±6.65^a	107.72±3.73^a
MCH (pg)	36.19±1.16^a	36.82±1.3^a
MCHC (g/dL)	35.44±0.6^a	34.18±0.7^a
WBCs (x10^3/μl)	41.6±0.7^a	32.7±1.16^b
Heterophils (x10^3/μl)	13.09±0.47^b	15.10±0.56^a
Lymphocytes (x10^3/μl)	25.70±0.52^a	16.20±0.45^b
Monocyte (x10^3/μl)	1.52±0.10^a	0.76±0.07^b
Eosinophils (x10^3/μl)	1.29±0.09^a	0.69±0.08^b

Data are presented as Mean±SE. Mean values with different superscript a, b within the same column are significantly different at P<0.05.

pared to those of CIAV infected chickens G2). There was significant increase in G1 than G2 in α-1 globulin (0.47±0.03 and 0.39±0.01, respectively), β-1 globulin (0.65±0.04 and 0.44±0.01), γ-1 globulin (0.68±0.1 and 0.52±0.06), γ-2 globulin (0.48±0.08 and 0.26±0.01), total γ globulins (1.17±0.18 and 0.78±0.03), total globulins (3.08±0.19 and 2.482±0.09), and total proteins (4.26±0.11 and 3.587±0.12) (Fig. 5).

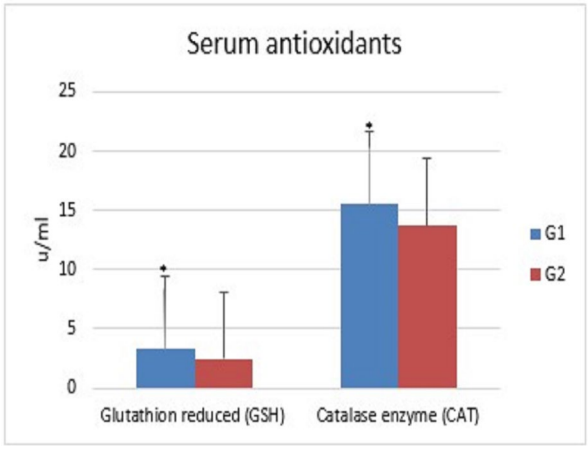

Fig. 4. The impact of CIAV infection on the serum enzyme levels (CAT and GSH). G1 is apparently healthy chickens and G2 is CIAV infected chickens. Data are presented as means with error bars. Columns with an asterisk indicates significantly different than the other columns within the same antioxidant (p≤ 0.05).

DISCUSSION

Chicken anemia virus is an immunosuppressive disease that circulates in several countries (Natesan *et al.*, 2006; Zhang *et al.*, 2013). In Egypt, the CAV was spread in different governorates causing high mortality and economic losses (Erfan *et al.*, 2018). In the current study, two positive samples from 3 farms out from 20 broiler farms (15%). The VP1 gene sequence is extremely to de termine the virulence of the virus. Phylogenetically, the VP1 gene was divided into many groups in the world that can easily identify the field and vaccines strains (Eltahir *et al.*, 2011).

In the present study, strain showed change in amino acid at position S/14/A, while change at position N/63/S in the A2 isolate strain. the change in amino acid at position H/22/N-Q, the isolated strains were located in phylogenetic group I with the vicinal strains, and China, Japan, India, Vietnam, Italy, Taiwan, Egypt (Sharkia), while group II and III included others Egyptian strains indicated that there are three strains circulating in Egypt depend on the change in amino acid at position H/22/N-Q. This result, agree with van Santen *et al.* (2001), where the amino acids changes in VP1 were S-14/A, H-21/R and H-22/Q. The nucleotide changes at position C-918/G affect VP1 amino acid at position H-22/Q. Abo-Elkhair *et al.* (2014) observed change at position H-22/Q amino acid of VP1 and detected VP1 amino acid change H-22/N amino acid in Egyptian isolates. While the obtained results agreed partially with Abdel-Mawgod *et al.* (2018) and Erfan *et al.* (2018) who detected the same amino acids mutation in some Egyptian isolates.

The chicken infectious anemia virus inhibits the differentiation

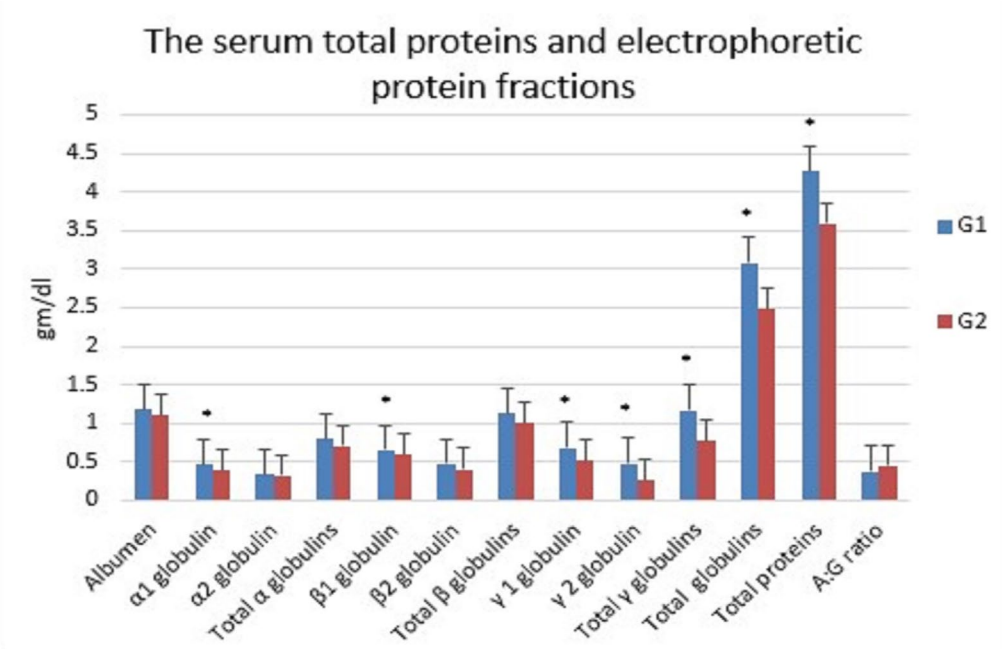

Fig. 5. Serum total protein and protein fraction concentrations (gm/dl). G1 is apparently healthy chickens and G2 is CIAV infected chickens. Data is shown as means with error bars. Columns with an asterisk indicates significantly different than the other column in the same fraction ($p \leq 0.05$).

and proliferation of hematopoietic precursor cells, resulting in transient destruction of the erythroblastoid and granuloblastoid cell lineages in the bone marrow, which is characterized by a drastic reduction in the production of mature red blood cells and myelopoiesis leading to hypoplasia, anemia and panleukopenia (Dhama *et al*., 2008). Haematopoietic precursor and thymic precursor cells in the bone marrow and thymus cortex, respectively, are the major cells targeted during the pathogenesis of CIAV infection (Adair, 2000).

Hemograms determined during disease conditions coupled with history and clinical signs, often assist to suspect the disease. Their use as a diagnostic aid in avian species, especially pet birds, is becoming increasingly popular. Quantitative changes in a particular leukocyte type indirectly reflect the nature of the disease process and the body's response. All such changes were clinically observed in CIAV infected chicken. Results from this study declared decreased values of RBCs count, Hb concentration and PCV indicating anemia (normocytic normochromic) which have previously been observed by Sommer and Cardona (2003); Wani *et al*. (2014) and Abdelwahab and Mansour (2019).

The innate immune system responds quickly to viral invasion and plays a major role in stimulating the adaptive immune system response, which generally leads to virus elimination (Koyama *et al*., 2008). The immunological components nitric oxide and lysozyme can be crucial in the innate immune response to viral infections (Xu *et al*., 2021). However, CAIV, as immunosuppressive virus, interferes with at least part of the host antiviral immune responses in chickens (Giotis *et al*., 2015), this was obvious in the current study where the NO and lysozyme, as innate immune parameters, were strongly affected by CAIV infection as their levels were significantly decreased in CAIV infected birds (G2) compared to those of the apparently healthy ones (G1). The lower NO levels were confirmed by Ester and Ragland (2020), who found that CAIV reduces NO generation in chicken macrophages because, the virus could interfere with signaling pathways connected to the Inducible Nitric Oxide Synthase (iNOS) expression and attenuate NO production where the NO molecule is produced under the effect of the iNOS. Whereas, viruses that interfere with expression of type 1 interferon, as in case of CAIV infection (Ragland *et al*., 2002), consequently impair expression of iNOS, since these signaling pathways overlap. On the other hand, the decreasing levels of lysozyme concentration in the current study could be related to the inhibitory effect of CAIV infection on the granulocyte macrophage-colony stimulating factor (GM-CSF) in young chicks (Basaraddi *et al*., 2013), as well as, on phagocytic and bactericidal activity of macrophages (Schat, 2009). These decreased levels of innate immune parameters can lead to down-regulation of the anti-pathogenic defense processes in CAIV infected chickens that may cause serious problem in chicken farms and enhance the secondary bacterial infections and trigger the morbidity and mortality particularly when co-infected with other viruses.

Viral invasion commonly could modify the scavenging antioxidant systems such as CAT and GSH and induce an impairment of the host redox homeostasis (Reshi *et al*., 2014). This was clear in the current study where both tested antioxidants (CAT and GSH) were significantly decreased in CAIV diseased group (G2) than the normal group (G1). This downregulation of the CAT, GSH and the immune parameters (NO and lysozyme) in G2 could be attributed to the apoptin-induced immunosuppression and apoptosis of the haematopoietic and lymphopoietic tissue (Adair, 2000), which in turn may influence synthesis of antioxidant enzymes including CAT enzyme and GSH. Furthermore, the apoptin was found to inhibit the transcription factors NF-κB and its antiapoptitic function (Chen *et al*., 2011), thus may diminish the glutathione S-transferase pi and glutathione peroxidase-1, production which are produced from the cells under the effect of the NF-κB (Schreiber *et al*., 2006). In the same regard, the apoptin-induced inhibition of NF-κB may be the cause of decreased NO level in CAIV group (G2), since the iNOS is heavily upregulated by the NF-κB (Morris *et al*., 2003).

Blood proteins are considered as important factors in the evaluation of health and immune status of chickens, as well as of production features (Filipovic *et al*., 2007). The obtained data of the serum protein electrophoresis show that CAIV infection in G2 induced obvious downregulation in total serum proteins associated with marked lower values of the albumen and total globulins (α, β and γ-globulins) fractions in addition to increase in A/G ratio. These results were come in consistence with Mostafa *et al*. (2021), who reported that, CAIV induce hypo-proteinemia, hypo-albumenemia, hypo-globulinemia in chickens. This obtained results may be attributed to the decreasing of synthesis of the serum protein fractions from the damaging haematological and lymphopoietic tissues, as well as, to the occurrence of anorexia, decreased feed intake, kidney, and liver dysfunction (Dhama *et al*., 2008) which effect on proteins synthesis and thus the level of albumen, total globulins and total proteins in CAIV infection.

On the other hand, the drop in total globulins is indicative of a decrease in immunoglobulins which are the main constituent of the γ and β- globulin fractions, that have been linked to immunosuppression and decreased disease resistance in chicken infected

with CAIV. Furthermore, the decreased total protein fractions in CAIV could lead to severe problem as blood proteins have many important physiological roles in the body including the maintenance of homeostasis (Piotrowska *et al*., 2011).

CONCLUSION

There are three strains in Egypt according to change in amino acid H/22/N1Q based on the phylogenetic analysis. The CIAV infection in chicken induced changes in the hematological markers, downregulation of the innate immune parameters, impairment of the scavenging antioxidant system and decrease concentration of different serum protein fractions, which support the evidence of the oxidation stress and immunosuppressive nature of the CIAV infection. So, it is recommended that more scientific research be done on the chicken anemia virus to follow up the changes that occur in the amino acids, and thus the work of appropriate vicinal programs.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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