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Research Article

Applications of Sialyltransferase as a Diagnostic Biomarker for Newcastle Disease Virus Infection in Chickens

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Abstract

Newcastle disease virus (NDV) poses a significant economic threat to chicken production. Early and precise diagnosis of NDV infection is crucial for the prevention of outbreaks. Due to paucity of reports on sialyltransferases (STs) in NDV-infected chickens, this study explored ST expression levels as a diagnostic biomarker for NDV infection in chickens and its role in endogenous control or resistance to infection. The study focused on β-galactoside α-2,3-sialyltransferase (ST3GAL6) and β-galactoside α-2,6-sialyltransferase (ST6GAL1) in kidney and liver tissues. Fifty, four-week-old chickens, placed at random into infected and control groups of 25 each were used. Samples were collected daily pre- and post-infection (pi) and analysed for packed cell volume (PCV). ST gene expressions were analysed using the Step One Real-Time PCR system. The study reported a decrease in mean PCV from day 2 pi until day 5 pi, reaching its lowest of 16.00 ± 1.20%, followed by a gradual increase from day 6 pi onwards. The infected group exhibited significantly ($p < 0.05$) lower mean PCV values compared to the control group on days 5, 6, 7, and 8 pi. Significant outcome revealed mRNA levels of ST3GAL6 and ST6GAL1 from the livers and kidneys of infected chickens were significantly ($p < 0.05$) higher than those of controls. ST3GAL6 was much more elevated in the kidney (44.79 ± 2.95 fold) while ST6GAL1 was higher at 31.72 ± 1.66 elevation in the liver of the infected chickens respectively on days 9 pi. These findings suggest that upregulation of ST3GAL6 and ST6GAL1 expression in NDV-infected chickens play a vital role in resialylating desialylated erythrocytes, aiding recovery and erythrocyte mass stabilization.

Keywords: Newcastle Disease Virus; Sialyltransferase; Biomarker; Chicken; Resistance; Gene Expression; ST3GAL6; ST6GAL1

Introduction

Newcastle disease a highly contagious viral disease that afflicted a wide range of avian species, including chickens [1]. Infection by Newcastle disease virus (NDV) caused severe symptoms in the respiratory and nervous systems that resulted in high mortality rates in infected birds [2,3]. Rapid diagnosis of NDV infection is crucial for controlling the spread of the virus and preventing outbreaks in other neighbouring poultry establishments. However, diagnostic methods for NDV infection, such as virus isolation and serological tests, encountered limitations in terms of sensitivity, specificity and turnaround time, in addition to the need for specialized laboratory facilities with biosafety containment, which made traditional diagnostic methods less practicable during epidemic situations where rapid diagnosis was crucial [4-7]. Therefore, there is a need for the development of new biomarkers for NDV infection that can provide more accurate and timely diagnosis.

Sialyltransferases, a family of enzymes that catalysed the transfer of sialic acid to glycoproteins and glycolipids, a post-translational modification sialylation played important roles in various biological processes, including cell signalling, immune system regulation and pathogen recognition [8,9]. Sialylation is a process that involves the transfer of sialic acid residues to glycan chains by sialyltransferases, which are a group of enzymes that play a critical role in regulating the biological functions of glycoproteins. In some instances, sialylation process is accompanied desialylation of blood cells for the prolongation of the life span of the resialylated blood cells [10-12]. Previous studies showed that sialylation patterns could be altered during viral infections, including NDV infection [13-15].

Indeed, much earlier report showed that the interaction of NDV with cells deficient in N-glycoprotein expression was less efficient and required N-linked glycoproteins for efficient attachment and entry into host cells [16].

In relation to viral sialidases in the pathogenesis of Newcastle disease, a study with 50 each of pigeons, guinea fowls, ducks and chickens revealed the least erythrocyte surface sialic acid concentrations of 7.88 ± 2.51 in pigeons with much higher concentrations of 14.6 ± 2.51, 17.6 ± 2.51 and 14.2 ± 2.51 mg/mg in guinea fowls, ducks and chickens respectively, [17], as exploitable variations.

The much higher concentrations of erythrocyte surface sialic acids [18], the existence of additional type of erythrocyte surface sialic acid [19] and the possession of more resistant side chains on the structure of the erythrocyte surface sialic acids by the trypanotolerant N'dama breed of cattle against the trypanosusceptible Zebu breed [20] were strongly linked to the trypanotolerance of the N'dama. However, the role of sialylation in NDV infection is not well understood, in particular, since the production and characterization of sialidase from NDV Kudu 113 strain [21,22].

The use of sialyltransferase expression levels as a biomarker for NDV infection has several potential applications. First, sialyltransferase might be used as a diagnostic tool for NDV infection in chickens [23]. The upregulation of sialyltransferase expression levels in NDV-infected chickens may be used as an early biomarker of infection, allowing for timely and accurate diagnosis. Second, sialyltransferase may be used as a prognostic tool for NDV infection in chickens. The level of sialyltransferase expression may potentially be used to predict disease severity and mortality rates in infected birds, allowing for more targeted treatment and management strategies. Finally, the use of sialyltransferase as a biomarker for NDV infection may facilitate the development of new diagnostic strategies for this disease. The endogenous defence and/or resistance from resialylation can be exploited. This study was designed to investigate the dynamics of sialyltransferase gene expression in serum and tissue samples of layers experimentally infected with NDV Kudu 113 strain.

Materials and Methods

Experimental design and animal care

A total of 50 4-week-old Isa Brown layer pullets were obtained from a commercial hatchery and separated at random into two groups: the experimental (n = 25) and the control (n = 25) groups. The experimental group was inoculated with NDV Kudu 113 strain via intramuscular route at a dose of 10^4 EID50 per bird, while the control group also received intramuscularly an equivalent volume of phosphate-buffered saline (PBS). The groups of the pullets were housed at different locations in enclosed buildings.

Ethical clearance

Ethical approval for the use of birds for the study was obtained from the Animal Use and Care Committee of Ahmadu Bello University, Zaria, with the approval number ABUCAUC/2021/016.

Sample collection and processing

At 9 days post-infection (pi), three birds from each group were humanely euthanized by cervical dislocation, and tissue samples (kidney and liver) were collected for sialyltransferase gene expression. The tissue samples were immediately placed in RNA stabilizing reagent (RNAlater®, Thermo Fisher Scientific) and stored at -80 °C until when required for the analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from tissue samples using the Total RNA Purification Kit (Jena Bioscience GmbH Germany) according to the manufacturer's instructions. A DNA-free kit (Life Technologies) was used to remove residual genomic DNA that might be contained in RNA samples. RNA concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the SOLIScript RT cDNA synthesis kit (Solis BioDyne Tartu Estonia) following the manufacturer's instructions.

Quantitative real-time PCR (RT-qPCR)

Quantitative real-time PCR (RT-qPCR) was performed to measure the expression levels of sialyltransferase genes in tissue samples using a StepOne Real-Time PCR system (Applied Biosystem, Singapore). The primer sequences for the sialyltransferase genes (Table 1) were designed based on previously published sequences [24-26]. The housekeeping gene β-actin was used as a reference gene for normalization. A 20 µL PCR reaction containing PCR components listed in table 2 was used. PCR cycling parameters were an initial denaturation at 95 °C for 12 minutes and 40 cycles of denaturation at 95 °C for 15 seconds, annealing for 1 minute at 60 °C and extension at 72 °C for 20 seconds. Melting curves were generated after each run to confirm a single PCR product (from 60 °C to 95 °C, increasing 1 °C/3 sec).

Genes	Primers	Sequences (5' to 3')
ST3GAL6	Forward Reverse	GGAGAGAAGGAACGCCCTAA ACTGGCACACAGGAACGG
ST6GAL1	Forward Reverse	TGGGTCGCTGTGCTGTT TGGGAGTTGACAAGACGAATC
β-actin (endogenous housekeeping)	Forward Reverse	GAGGCTACAGCTTCACCACCACA CCACAGGACTCCATACCCAAGAA

Table 1: Primer sequences used for RT-PCR.

Key: ST3GAL6 = β-galactoside α-2,3-sialyltransferase; ST6GAL1 = β-galactoside α-2,6-sialyltransferase; G = Guanine; C = Cytosine; T = Thymine; A = Adenine

Components	Volume (µL)	Concentration
Template cDNA	4	
EvaGreen Master Mix	4	5x
Forward Primer	0.3	10mM
Reverse Primer	0.3	10mM
H_2O	11.4	
Total volume	20	

Table 2: qPCR reaction mix components using cDNA and EvaGreen master mix.

Results

Decreases in the mean PCV commenced by day 2 pi and continued until day 5 pi with the lowest mean PCV of 16.00 ± 1.20%. Thereafter, the PCV increased gradually until the termination of the experiment at day 9 pi. The mean PCV of the control group were relatively constant throughout the experiment. The mean PCV were significantly ($p < 0.05$) lower in the infected group than those of the control group on days 5, 6, 7 and 8 pi (Table 3).

	DAYS												
	-3	-2	-1	0	1	2	3	4	5	6	7	8	9
Control	26.00 ± 2.90	27.67 ± 2.70	26.67 ± 2.00	27.67 ± 1.80	27.67 ± 1.50	27.67 ± 0.88	28.67 ± 1.90	28.33 ± 2.20	28.33 ± 3.20[a]	28.67 ± 3.00[b]	28.33 ± 2.70[c]	29.67 ± 2.00[d]	30.00 ± 3.10
Infected	25.00 ± 1.20	25.00 ± 1.20	25.00 ± 1.20	24.33 ± 0.67	24.67 ± 0.88	22.67 ± 1.20	21.67 ± 0.88	20.67 ± 1.30	16.00 ± 1.20[a]	16.67 ± 1.20[b]	17.67 ± 1.70[c]	18.33 ± 2.20[d]	24.00 ± 0.65

Table 3: Packed cell volume (Mean ± SEM) of Newcastle Disease Virus Kudu 113 strain infected and control chickens.

values with the same superscript alphabets along the same column are significantly different with $p < 0.05$.

The expression levels of mRNA of β-galactoside α-2,3-sialyltransferase (ST3GAL6) and β-galactoside α-2,6-sialyltransferase (ST6GAL1) as quantified in the liver and kidney of chickens infected with NDV Kudu 113 strain and non-infected chickens are presented in Table 4. The relative gene expressions of the infected and control chickens were significantly ($p < 0.05$) different. The ST3GAL6 activity in the infected chickens was higher in the kidney while the ST6GAL1 in the infected chickens was higher in the liver with values of 44.79 ± 2.95 fold and 31.72 ± 1.66 fold respectively. On the other hand, the relative expression of both the ST3GAL6 and ST6GAL1 genes in the liver and kidney of the control chickens were similarly less than 2.0-fold.

Sialyltransferases	Organs	Control	Infected	P values
ST3GAL6	Liver	1.01 ± 0.08	29.13 ± 1.73	<0.0001
	Kidney	1.07 ± 0.24	44.79 ± 2.95	0.0001
ST6GAL1	Liver	1.01 ± 0.08	31.72 ± 1.66	<0.0001
	Kidney	1.18 ± 0.48	12.64 ± 0.97	0.0005

Table 4: Relative gene expression (±SEM) of kidney and liver ST3GAL6 and ST6GAL1 in chickens infected with Newcastle Disease Virus Kudu 113 strain and non-infected chicken.

Key: ST3GAL6 = β-galactoside α-2,3-sialyltransferase; ST6GAL1 = β-galactoside α-2,6-sialyltransferase

Discussion

The expression levels of sialyltransferase genes, that is, ST3GAL6 and ST6GAL1 in the current study, were upregulated in liver and kidney tissue samples of infected chickens. This is being suggested to impact on immune cell modulation and inflammation during NDV infection. The roles of these sialyltransferases in the immune response to NDV infection involved modulating sialylation processes that impacted immune cell infiltration and inflammation. Specifically, ST6Gal1 had been associated with anti-inflammatory functions in the liver, affecting the infiltration of immune cells like NK cells, pDCs, and CD8+ T cells. Downregulation of ST6Gal1 had been linked to liver inflammation, suggesting a potential role in regulating immune responses during NDV infection [27]. Additionally, the upregulation of sialyltransferases like ST3Gal6 and ST6Gal1 can lead to hypersialylation, influencing disease progression and potentially serving as a target for controlling infections [28].

It is being suggested that upregulation may be an endogenous host defence response to the virus infection, to modify the glycosylation patterns of glycoproteins for an enhancement of their antiviral activities. Indeed, previous studies showed that changes in the sialylation patterns of IgG glycoproteins affected their binding affinity to Fc receptors and complement components, thus modulating their effector functions in the immune response against viruses [29-32].

Earlier report [11] revealed an upregulation of ST3Gal1 and ST6Gal1 in the liver and kidney of *Trypanosoma brucei brucei*-infected pigs, which supported a resialylation process onto desialylated erythrocytes and the expression levels of ST3Gal1 and ST6Gal1 suggested an involvement in immune cell infiltration, indicating a potential role in regulating immune responses during the infection. These observations highlight the multifaceted roles that ST3Gal6 and ST6Gal1 may play in the context of NDV infection in this current study ranging from inflammation modulation to immune response regulation.

It is scientifically noteworthy that the higher upregulation of ST6Gal1 in the liver of NDV-infected chickens in the current study, similarly occurred as higher upregulation of the same ST6Gal1 of hepatic and renal tissues of *Trypanosoma brucei brucei*-infected pigs [11]. While ST3Gal6 is additionally upregulated in the current study with NDV-infected chickens, ST3Gal1 was additionally upregulated in the *T. brucei brucei*-infected pigs [11]. It is being speculated that a particular type of sialic acid that responds to the resialylation process by ST6Gal1 is present on the cells, particularly, erythrocytes of chickens in response to NDV infections, in the present study and pigs in response to *T. brucei brucei* infection [11] and chickens as reported earlier [16]. This speculation derives support from the report that efficient interaction of NDV with target cells required α-2,3- and α-2,6-N-linked sialic acids [16]. In addition, studies on the phenomena that endowed the N'dama and

Zebu breeds of cattle with resistant and non-resistant erythrocyte sialic acids respectively [18-20] may reveal identical sialic acids that may respond to the resialylation activity of β-galactoside α-2,6-sialyltransferase (ST6GAL1), same STGal1 that may be exploited for the design of a non-empirical control measures, common to these animals.

The upregulation of sialyltransferases, particularly ST3Gal6 and ST6Gal1, was notably observed on day 9 post-infection in chickens in the current study when PCV was highest. This temporal pattern of upregulation may serve as a valuable biomarker for the detection of NDV infection, indicating a specific molecular response that can be utilized for early identification and monitoring of the disease progression. However, conducting longitudinal studies with multiple time points to track the expression levels of sialyltransferases over time post-infection may provide a more comprehensive understanding of their dynamics as biomarkers [33].

Similarly, the upregulation of sialyltransferases, particularly ST6Gal1, observed which contributed to the recovery from anaemia in NDV-infected chickens in the study, also occurred in *T. brucei brucei*-infected pigs [11]. This suggests that there is a common sialic acid on the erythrocytes of chickens and pigs responding to ST6Gal1 sialylation.

In conclusion, the findings from this study underscore the significance of sialyltransferases, specifically ST3Gal6 and ST6Gal1, as potential diagnostic markers for NDV infection in chickens. The upregulation of these enzymes with concurrent increase in the PCV in the NDV-infected chickens not only reflects a host response to viral infection but also suggests their involvement in modulating immune responses, inflammation, and glycosylation patterns that influence disease progression. The temporal pattern of upregulation post-infection highlights a potential use for these sialyltransferases as early biomarkers for detecting NDV infection.

Conclusion

However, it is recommended that further longitudinal and strain-specific studies are warranted to validate their utility across different NDV strains and tissues, ultimately enhancing our understanding and application of sialyltransferases as diagnostic tools in veterinary medicine. Also, the study only assessed sialyltransferase gene expression in the liver and kidney. Hence, to grasp tissue-specific glycosylation regulation fully, future studies should broaden analysis to include a wider range of tissues.

Bibliography

1. EW Aldous and DJ Alexander. "Newcastle disease in pheasants (*Phasianus colchicus*): A review". *The Veterinary Journal* 175 (2008): 181-185.

2. R Haddas. "Newcastle Disease Virus". BT - Encyclopedia of Sustainability Science and Technology, in: R.A. Meyers (Ed.), Springer New York, New York, NY (2020): 1-14.

3. PJ Miller., *et al*. "Newcastle disease: evolution of genotypes and the related diagnostic challenges". *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* 10 (2010): 26-35.

4. S Cardenas-Garcia., *et al*. "Development of an improved vaccine evaluation protocol to compare the efficacy of Newcastle disease vaccines". *Biologicals* 43 (2015): 136-145.

5. MB Bello., *et al*. "Diagnostic and Vaccination Approaches for Newcastle Disease Virus in Poultry: The Current and Emerging Perspectives". *BioMed Research International* 2018 (2018): 7278459.

6. Q Mao., *et al*. "Review detection of Newcastle disease virus". *Frontiers in Veterinary Science* 9 (2022).

7. M Munir., *et al*. "Biological characterization and phylogenetic analysis of a novel genetic group of Newcastle disease virus isolated from outbreaks in commercial poultry and from backyard poultry flocks in Pakistan". *Infection, Genetics and Evolution: Journal of Molecular Epidemiology And Evolutionary Genetics in Infectious Diseases* 12 (2012): 1010-1019.

8. F Li and J Ding. "Sialylation is involved in cell fate decision during development, reprogramming and cancer progression". *Protein Cell* 10 (2019): 550-565.

9. A Varki and R Schauer. "Essentials of Glycobiology, in: A. Varki, R.D. Cummings, J.D. Esko, H.H. Freeze, P. Stanley, C.R. Bertozzi, G.W. Hart, M.E. Etzler (Eds.), Sialic Acids, 2nd edition., Cold Spring Harbor Laboratory Press, New York (2009).

10. KAN Esievo., *et al*. "Variation in erythrocyte surface and free serum sialic acid concentrations during experimental Trypanosoma vivax infection in cattle". *Research in Veterinary Science* 32 (1982): 1-5.

11. JA Atata., *et al*. "Upregulation of sialyltransferases ST3Gal1 and ST6Gal1 promotes stabilization of erythrocyte mass and recovery of anemia in Trypanosoma brucei brucei-infected pigs". *Research in Veterinary Science* 145 (2022): 102-108.

12. YX Huang., *et al*. "Restoring the youth of aged red blood cells and extending their lifespan in circulation by remodelling membrane sialic acid". *Journal of Cellular and Molecular Medicine* 20 (2016): 294-301.

13. BR Wasik., *et al*. "Effects of Sialic Acid Modifications on Virus Binding and Infection". *Trends Microbiol* 24 (2016): 991-1001.

14. P Fournier and V Schirrmacher. "Oncolytic Newcastle Disease Virus as Cutting Edge between Tumor and Host". *Biology (Basel)* 2 (2013): 936-975.

15. SS Jang., *et al*. "α2,3-Linked Sialic Acids Are the Potential Attachment Receptor for Shaan Virus Infection in MARC-145 Cells". *Microbiology Spectrum* 10 (2022): e0125622.

16. L Sánchez-Felipe., *et al*. "α;2-3-And α;2-6-N-linked sialic acids allow efficient interaction of Newcastle disease virus with target cells". *Glycoconjugate Journal* 29 (2012): 539-549.

17. NM Useh., *et al*. "Comparative studies on erythrocyte sialic acid levels in apparently healthy indigenous Nigerian poultry species". *Cell Biochemistry and Function* 24 (2006): 143-146.

18. KA Esievo., *et al*. "Erythrocyte surface sialic acid in Ndama and Zebu cattle". *Journal of Comparative Pathology* 96 (1986): 95-99.

19. KA Esievo., *et al*. "Electrophoresis of bovine erythrocyte sialic acids: existence of additional band in trypanotolerant Ndama cattle". *Journal of Comparative Pathology* 102 (1990): 357-361.

20. A Shugaba., *et al*. "Biochemical differences (O-acetyl and glycolyl groups): in erythrocyte surface sialic acids of trypanotolerant N'dama and trypanosusceptible Zebu cattle". *Journal of Comparative Pathology* 110 (1994): 91-95.

21. SB Oladele., *et al*. "Effect of some inhibitors on neuraminidase of Newcastle disease virus Kudu 113 strain". *VETERINARSKI ARHIV* 72 (2002): 185-194.

22. SB Oladele., *et al*. "Preliminary report on neuraminidase, erythrocyte surface and free serum sialic acid concentrations in the serum of healthy and newcastle disease virus-infected chickens". *Revue D'élevage Et De Médecine Vétérinaire Des Pays Tropicaux* 55 (2002): 265-268.

23. M Rabiei., *et al*. "Indicators of the molecular pathogenesis of virulent Newcastle disease virus in chickens revealed by transcriptomic profiling of spleen". *Scientific Reports* 11 (2021): 1-14.

24. F Zhou., *et al*. "The expression pattern and histological distribution of sialyltransferases ST3Gal III in yellow chicken". *Veterinary Research Communications* 37 (2013): 285-291.

25. S Kidani., *et al*. "Analyses of chicken sialyltransferases related to O-glycosylation". *Journal of Bioscience and Bioengineering* 122 (2016): 379-384.

26. Y Kojima., *et al*. "Analyses of chicken sialyltransferases related to N-glycosylation". *Journal of Bioscience and Bioengineering* 119 (2015): 623-628.

27. R Liu., *et al*. "Downregulation of ST6GAL1 promotes liver inflammation and predicts adverse prognosis in hepatocellular arcinoma". *Journal of Inflammation Research* 15 (2022): 5801-5814.

28. S Chandel., *et al*. "Role of ST6GAL1 and ST6GAL2 in subversion of cellular signaling during enteroaggregative Escherichia coli infection of human intestinal epithelial cell lines". *Applied Microbiology and Biotechnology* 107 (2023): 1405-1420.

29. S Trzos., *et al*. "The role of N-glycosylation in B-cell biology and IgG activity. The aspects of autoimmunity and anti-inflammatory therapy". *Frontiers in Immunology*14 (2023).

30. LL Lu., *et al*. "Beyond binding: antibody effector functions in infectious diseases, Nat. Rev. Immunol. 18 (2018): 46-61.

31. MHC Biermann., *et al*. "Sweet but dangerous - the role of immunoglobulin G glycosylation in autoimmunity and inflammation". *Lupus* 25 (2016): 934-942.

32. S Ochoa-Rios., *et al*. "Analysis of N-linked Glycan Alterations in Tissue and Serum Reveals Promising Biomarkers for Intrahepatic Cholangiocarcinoma". *Cancer Research Communication* 3 (2023): 383-394.

33. AA Alturfan and E Emekli-Alturfan. "nteraction of Sialyltransferases, Sialidases and Sialic Acids in Liver Diseases and Applications to Biomarker Discovery BT - Biomarkers in Liver Disease, in: V.R. Preedy (Ed.), Springer Netherlands". *Dordrecht* (2016): 1-18.