

# URINARY N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE INDEX EVALUATION IN CATS WITH NATURALLY ACQUIRED FELINE IMMUNODEFICIENCY VIRUS INFECTION

## EVALUAREA INDEXULUI N-ACETIL- $\beta$ -D-GLUCOZAMINIDAZEI URINARE LA PISICI INFECTATE NATURAL CU VIRUSUL IMUNODEFICIENȚEI FELINE

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### ABSTRACT | REZUMAT

N-acetyl- $\beta$ -D-glucosaminidase (NAG) is an enzyme found in lysosomes, primarily originating in the proximal convoluted tubules of the kidney. It serves as a valuable tool for the early detection of various renal diseases. It is particularly noteworthy as a specific marker for renal tubular damage across different species. Given NAG's superior specificity and sensitivity when compared to traditional kidney disease markers like urea and creatinine, this clinical case study aims to assess the prevalence of tubular nephropathy in cats infected with Feline Immunodeficiency Virus (FIV) by measuring the enzymatic activity of the NAG index. Initially, an assessment of the FIV infection status of the cats was performed. Subsequently, urine samples were collected for NAG index assessment and urinary specific gravity. Blood samples were also collected for serum urea and creatinine determination. The results demonstrated that renal tubular injury is present in all FIV-positive cats from this study, as evidenced by NAG index values exceeding the normal reference values.

**Keywords:** NAG index, FIV, renal tubular injury

N-acetil- $\beta$ -D-glucozaminidaza (NAG) este o enzimă care se găsește în lizozomi, provenind în principal din tubulii contorți proximali ai rinichiului. Aceasta servește ca instrument valoros pentru detectarea precoce a diferitelor boli renale. NAG este un marker specific pentru leziunile tubulare renale la diferite specii de mamifere. Având în vedere specificitatea și sensibilitatea superioară a NAG în comparație cu markerii tradiționali ai bolilor renale, cum ar fi ureea și creatinina, acest studiu are ca și scop evaluarea prevalenței nefropatiei tubulare la pisicile infectate cu virusul imunodeficienței feline (FIV) prin măsurarea activității enzimatice a indexului NAG. Inițial, a fost efectuată evaluarea statutului de infecție cu FIV al pisicilor. Ulterior, au fost colectate probe de urină pentru evaluarea indicelui NAG și a densității urinare. De asemenea, au fost recoltate probe de sânge pentru determinarea ureei și a creatininei serice. Rezultatele au demonstrat prezența leziunilor tubulare renale la toate pisicile FIV pozitive din acest studiu, după cum o demonstrează valorile indicelui NAG care depășesc valorile normale de referință.

**Cuvinte cheie:** Index NAG, FIV, leziune tubulară renală

Feline Immunodeficiency Virus (FIV) is a significant viral infection affecting cats. This virus, often referred to as "feline AIDS," is a member of the lentivirus family, which includes HIV in humans. FIV primarily targets a cat's immune system, weakening its ability to fight off infections (2, 5, 9, 11). FIV is typically transmitted through bite wounds, making it more common in male outdoor cats that engage in territorial fights. However, it can also be transmitted from an infected mother to her kittens during birth or through nursing (9,11). FIV-infected cats may not show symptoms for years, but as the disease progresses, they can experience a weakened immune system, recurrent infections, weight loss, oral health problems, and

a decline in overall health (12). FIV infection in cats is a chronic viral condition that affects their immune system. Early detection, preventive measures, and proper management are crucial for maintaining the health and well-being of infected cats and preventing the spread of the virus within cat populations (19, 20).

The urinary NAG index, also known as the N-acetyl- $\beta$ -D-glucosaminidase index, is a valuable diagnostic tool in the fields of nephrology and renal medicine. This index measures the enzymatic activity of NAG in urine, providing crucial insights into the health and function of the kidneys, specifically the renal tubules. As a sensitive marker for renal tubular damage and disease, the urinary NAG index plays a significant role in the early detection and monitoring of various kidney conditions (6, 7, 8, 17, 18).

NAG is an enzyme naturally found in lysosomes within renal tubular cells, which are an integral part of

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the kidney's nephrons responsible for concentrating urine (18). When there is injury or damage to the renal tubules, such as in cases of infection, inflammation, ischemia, or toxins, the cells within these tubules can suffer various degrees of injury. In response to this damage, the NAG enzyme is released from the renal tubular cells into the urine (3, 6, 7, 17).

Elevated levels of NAG in the urine are often associated with renal tubular damage or injury. It serves as a sensitive marker for kidney conditions, particularly those affecting the renal tubules. Monitoring the urinary NAG index can help diagnose and manage various kidney diseases, such as acute kidney injury (AKI), chronic kidney disease (CKD), and other renal disorders (10,17, 18).

In HIV-positive human subjects, various studies have reported that HIV-associated nephropathies displayed distinct histopathological characteristics, including "collapsed" glomeruli, glomerular hyalinosis, tubular microcystic dilatation, and tubular cell degenerative alterations. Additionally, features like focal glomerular electron-dense deposits, numerous tubuloreticular inclusions, granular degeneration of nuclear chromatin, and specific nuclear body changes were observed in these patients (4).

Renal abnormalities were reported in experimentally FIV-infected cats, such as mesangial widening and glomerulonephritis, along with lower proteinuria and fewer tubular and interstitial changes. In contrast, naturally infected cats showed more severe kidney lesions, including interstitial infiltrates and renal amyloidosis. These findings resemble renal alterations seen in HIV-infected humans, suggesting a potential link between FIV infection and renal disease (1, 13, 14, 15, 16, 21).

The aim of this study was to highlight the incidence of tubular injury in FIV-positive cats through the evaluation of the urinary NAG index. The NAG enzyme is known to be a specific marker for the detection of proximal tubular active lesions in many species and can be used for the early detection of acute kidney injury.

## MATERIALS AND METHODS

**Sample Population:** The study involved a number of 15 FIV-positive cats, both males and females, aged between 2 and 8 years old, with sterile urine samples on urinary culture tests. After a thorough clinical examination, all the cats were tested to determine the FIV infection status using the VetExpert rapid FIV Ab Kit, based on a chromatographic immunological method for the qualitative detection of antibodies against feline immunodeficiency virus (FIV) in whole blood. Blood samples were collected from the jugular vein in heparin tubes. Urine samples were obtained through ultrasound-guided cystocentesis to ensure the collec-

tion of an uncontaminated urine sample. After aseptic preparation of the skin, approximately 5 ml of urine were collected using a sterile syringe and divided into two plain sterile tubes for the measurement of urinary creatinine, NAG enzyme levels, urinary specific gravity, and urine culture.

The urine was centrifuged at 2000 rpm for 5 minutes at 4°C, and the resulting supernatant was used for subsequent assessments. The enzymatic activity of NAG was determined at 37°C using a commercially available kit (Diazyme Lab, CA, USA). This kit consists of three reagents: R1-MNP-GlcNAc and HCL, R2-Citric Acid and Potassium Phosphate, and R3-Carbonate (Buffer). The method is based on NAG's capability to hydrolyze 2-methoxy-4-(2'-nitrovinyl)-phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (MNP-GlcNAc) into (2'-nitrovinyl)-phenol, with the formation of this compound being assessed using a spectrophotometer (Screen Master Touch, Hospitex Diagnostics®, Italy) set at a wavelength of 505 nm. Urine NAG levels were expressed in U/L.

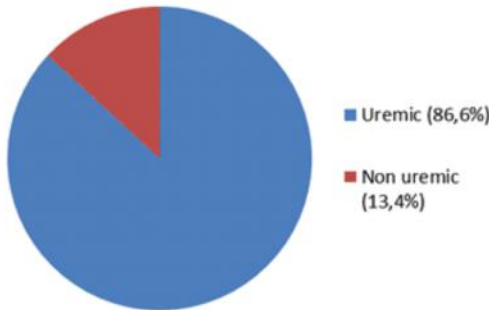
Urinary creatinine concentration was determined by a fixed-time colorimetric reaction using a commercial kit with two reagents: R1-Picric Acid and R2-Sodium Hydroxide and Disodium Phosphate. The method is based on the Jaffe reaction and consists of quantifying the rate of formation of the colour complex between creatinine and alkaline picrate at 37°C, the reading being performed after the calibration of the spectrophotometer at the 515nm wavelength (Chema Diagnostica®, Italy). The results were expressed in g/L.

The results of NAG and creatinine determinations were subsequently used to calculate the NAG Index based on the following formula: Index NAG (U/L) = NAG(U/L)/Creatinine(g/L). Urinary-specific gravity was evaluated using a urinary refractometer (Optika HR-160N®). Serum concentrations of creatinine and urea were measured using a spectrophotometric kinetic method for urea and the Jaffe reaction for creatinine. Urine cultures were performed on Columbia agar + 5% sheep blood and incubated for 24 hours at 37°C. Only cats with sterile urine samples were included in this study.

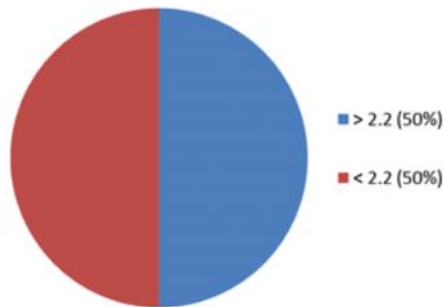
## RESULTS AND DISCUSSIONS

A total of 86.6% of the FIV-positive cats included in this study (n = 15) presented elevated serum urea nitrogen levels (> 34 mg/dL) (Fig. 1). Furthermore, 50% of the cats demonstrated elevated serum creatinine levels when compared to the upper reference range of 2.2 mg/dL (Fig. 2). The mean serum urea nitrogen concentration was 117.06±104.44 mg/dL, and the mean serum creatinine concentration was 3.19±3.14 mg/dL. Urea and creatinine are metabolic waste products produced by the body that the kidneys

normally filter from the blood and excrete into the urine. Elevated serum concentrations of these substances can be a sign that the kidneys are not effectively filtering and eliminating these waste products, suggesting potential kidney injury.



**Fig. 1.** Serum urea (mg/dL) in FIV-positive cats



**Fig. 2.** Serum creatinine (mg/dL) in FIV-positive cats

Urinary NAG index evaluation in all 15 FIV-positive cats demonstrated elevated values when compared to the literature reference range of  $1.38 \pm 0.45$  U/g, indicating that all individuals had renal tubular injury (Table 1). The mean NAG index value was  $39.92 \pm 39.54$  U/g. When the renal tubules, which are an essential part of the nephron, become damaged due to various factors such as infection, toxins, or other kidney-related conditions, the cells within these tubules can be harmed. Kidney tubular cells release NAG into the urine as a response to injury or damage. A high urinary NAG index is often considered a marker for renal tubular damage.

Urinary specific gravity: in 73.3% of cats hyposthenuria was demonstrated ( $SG < 1.020$ ), and 26.7% had a normal urinary specific gravity. The results indicate a deficiency in the urine concentration mechanism, which refers to the impaired ability of the kidneys to concentrate urine effectively. The regulation the concentration of solutes in urine takes place in the renal tubules. When renal tubules are damaged, they may not function properly, leading to an inability to concentrate urine effectively. As a result, the urine may be-

come excessively dilute, and hyposthenuria can occur.

**Table 1**  
**Urinary Nag index in FIV-positive cats**

Sample number	iNAG (U/g)
1.	21.78
2.	17.56
3.	19
4.	66.05
5.	12.95
6.	17.49
7.	19.3
8.	16.3
9.	157.2
10.	13.53
11.	76.83
12.	30.2
13.	20.60
14.	32.76
15.	77.26

**CONCLUSIONS**

All cats in this study had NAG (N-acetyl- $\beta$ -D-glucosaminidase) index values significantly above the standard reference range ( $1.38 \pm 0.45$ ). This renal marker exhibited a notably higher sensitivity compared to urea and creatinine. Cats that presented with serum urea (13.4%) and creatinine (50%) within the normal reference ranges had NAG index values exceeding the normal reference range. The NAG index specifically targets the site of kidney damage, indicating that cats infected with the FIV virus are experiencing renal tubular damage. Hyposthenuria was present in 73.3% of the FIV-positive cats that had high urinary NAG index values, indicating that urinary iNAG is more accurate in detecting tubular damage than urinary specific gravity.

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