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Role of Neuron-Specific Enolase, Metanephrine, Procalcitonin, Lactate Dehydrogenase, and Ferritin in children with Neuroblastoma and Wilms tumor

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Abstract

The role of enolase isozymes and ferritin as biomarkers in pediatric solid tumors, including neuroblastoma and Wilms tumor. While neuron-specific enolase (NSE) is well-established in neuroendocrine tumors, its expression patterns and prognostic significance in other childhood cancers remain underexplored. Similarly, ferritin, traditionally linked to iron metabolism and inflammation, may have unrecognized associations with tumor aggressiveness or treatment response in these malignancies. dysregulated enolase isoforms and altered ferritin levels correlate with disease progression, metastatic potential, or therapeutic outcomes in pediatric solid tumors. **Patient and methods:** This case-control study, conducted at Baghdad Teaching Hospital (February–December 2024), evaluated Neuron-Specific Enolase (NSE) and ferritin as potential biomarkers in pediatric solid tumors. The study included 98 children under 10 years old, divided into three groups: 34 neuroblastoma patients, 31 Wilms tumor patients, and 33 healthy controls. Serum levels of NSE, ferritin and LDH were measured using Roche Cobas while PCT and Metanephrine measured by ELISA. **Results:** A total of 98 children participated: 33 as healthy controls, 31 diagnosed with Wilms' tumor, and 34 with neuroblastoma. The average ages for each group were similar: 39.35 ± 4.84 months for neuroblastoma, 40.03 ± 4.36 months for Wilms tumor, and 40.85 ± 5.58 months for the control group. There was no statistically significant difference ($p \geq 0.980$). There were no significant differences in weight ($p \geq 0.942$), height ($p \geq 0.960$), or sex distribution ($p \geq 0.679$). NSE levels were significantly elevated in neuroblastoma patients (mean: 51.35 ng/mL) compared to Wilms tumor patients (38.82 ng/mL) and controls (16.94 ng/mL). PCT levels also followed a similar pattern, with an average of 1.41 ng/mL for neuroblastoma, 0.99 ng/mL for Wilms, and 0.36 ng/mL for controls. In the neuroblastoma cohort, NSE and PCT levels exhibited a statistically significant modest positive correlation ($r = 0.367$, $p < 0.0329$). According to ROC curve research, PCT had an AUC of 0.862 and NSE had an AUC of 0.921 for telling the difference between neuroblastoma and controls. Although elevated in both tumor groups, metanephrine, LDH, and ferritin exhibited no statistically significant correlations with clinical or anthropometric criteria and demonstrated lower AUC values, thereby indicating limited standalone diagnostic relevance. **Conclusion**: Serum neuron-specific enolase NSE shows exceptional elevation in neuroblastoma, while ferritin is markedly increased in Wilms tumor, suggesting their potential diagnostic utility in differentiating these pediatric malignancies.

Keywords: Neuroblastoma, Wilms Tumor, NSE, LDH, Metanephrine PCT

INTRODUCTION

Neuroblastoma is the most prevalent extracranial solid tumour in paediatric patients. It is a solid cancer that mostly affects kids. The tumour can be anywhere in the body, but it is most common in the abdomen, the adrenal glands, and the spine(1). The symptoms of neuroblastoma vary based on its metastatic sites. Neuroblastoma predominantly impacts children under five years old and is infrequently observed in those over ten, though it may occasionally manifest in older children and adults(2).Wilms tumour, or nephroblastoma, is the most common type of kidney tumour in children. Max Wilms, a German surgeon, was the first to write about 8 cases of "mixed tumours" made up of both blastema and tubules in 1899(3).NSE is the most tissue-specific isoenzyme among these. It is found in neurones and neuroendocrine cells, where it makes up more than 90% of all soluble proteins. Different genes code for enolases, and they must be made separately before they can join together to make the dimeric isoform(4).Each subunit has about 436 amino acids and weighs about 47 kDa. The three isozymes have similar secondary and tertiary structures. They are made up of α-helices and β-sheets, as well as mostly β-strands, which are found inside the molecule and make up a hydrophobic core. The active enzyme is a dimer made up of three isoforms that weigh about 80 kDa(5).Metanephrine is a metabolite derived from the hormone epinephrine, also known as adrenaline, which is synthesised by the adrenal glands. The biochemical pathway leading to MN formation initiates with the amino acid tyrosine.Tyrosine undergoes hydroxylation to produce Dihydroxyphenylalanine (DOPA), which is subsequently decarboxylated to yield dopamine(6). Dopamine undergoes hydroxylation to become norepinephrine, which is then methylated by the enzyme catechol-O-methyltransferase (COMT) to produce MN(7).Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin, primarily associated with calcium homeostasis. The PCT molecule comprises 116 amino acids and has a molecular weight of approximately 13 kDa(7). The structure includes the calcitonin peptide, an amino-terminal signal peptide, and a less clearly defined carboxyl-terminal peptide known as katacalcin(8).Lactate dehydrogenase (LDH) is an enzyme that is necessary for turning lactate into pyruvic acid, which is a necessary step in cellular respiration. This change is necessary for cells to make energy, especially when there is no oxygen around. LDH is an important marker in medical tests because it is involved in energy metabolism and a number of diseases and injuries(9).Ferritin is a protein complex that keeps iron in a form that is safe and soluble, so the body always has a supply of iron that can be easily used when needed. It exists in various cell types, notably in liver cells, spleen, and bone marrow. Ferritin's protein shell is important for iron homeostasis because it can hold up to 4500 iron ions in its central cavity(10).Ferritin is mostly an iron storage molecule, but it also plays a very important role in keeping the body's iron levels in check. Ferritin lowers the amount of free iron in the body by storing iron(11). Free iron can cause harmful free radicals to form. Ferritin's ferroxidase activity also helps change iron from its ferrous (Fe^{2+}) form to its ferric (Fe^{3+}) form, which is the form in which iron can be safely stored in the ferritin molecule(12).

PATIENTS AND METHODS:

This case-control study was conducted in Baghdad Teaching Hospital/Medical City/Baghdad/Iraq by the Department of Biochemistry/College of Medicine/University of Baghdad between February 2024 and December 2024. It included 98 subjects below 10 years with clinical diagnosis of solid tumors, including neuroblastoma and Wilms tumors, compared with controls. Subjects were divided into Group I, which included 34 subjects with neuroblastoma; Group II, which included 31 subjects with Wilms tumors.

The scientific and ethical committees of the Department of Biochemistry, College of Medicine, University of Baghdad approved this study. Ethical approval was obtained from Baghdad Teaching Hospital / Medical City Complex and the Ministry of Health / Iraq. Verbal consent was obtained from the subjects in this study. Exclusion criteria included patients with other types of solid tumors and children with Wilms' tumor and neuroblastoma above 10 years.

Five milliliters (5ml) of blood were aspirated from the peripheral vein of each subject of the three groups and allowed to clot for 15 minutes, then centrifuged for 10 minutes at 2500 rpm. The separated serum was stored at - 45°C till the day of lab testing, which included Serum levels of NSE, ferritin and LDH were measured using Roche Cobas, while PCT and Metanephrine were measured by ELISA

SPSS software, version 25.0, was used to conduct statistical analyses (SPSS, Chicago). The mean and standard deviation of the data with a normal distribution were displayed, and the analysis of variance (ANOVA) test was performed.

RESULTS

The sex distribution demonstrates relative balance across all three groups. The neuroblastoma group comprised 18 males (52.9%) and 16 females (47.1%), showing a slight male predominance. Conversely, the Wilms tumor group exhibited a moderate female predominance with 18 females (58.1%) and 13 males (41.9%). The control group maintained near-equal distribution with 16 males (48.5%) and 17 females (51.5%).

Table 1 ***Demographic characteristics showing sex distribution across neuroblastoma, Wilms tumor, and control groups.***

Group	Total (NO.)	Males NO.(%)	Females NO. (%)	p-value
Neuroblastoma	34	18 (52.9%)	16 (47.1%)	0.679
Wilms Tumor	31	13 (41.9%)	18 (58.1%)	
Control	33	16 (48.5%)	17 (51.5%)	

The chi-square test demonstrated no statistically significant difference in gender distribution between groups ($\chi^2 = 0.79$, $df = 2$, $p > 0.05$).

No significant differences were observed in weight or length (height) across groups. Mean weights (±SE) were: neuroblastoma 15.02 ± 5.88 kg, Wilms tumor 15.48 ± 5.09 kg, and control 15.36 ± 5.91 kg (p=0.942). Mean lengths were: neuroblastoma 92.03 ± 19.50 cm, Wilms tumor 91.61 ± 16.05 cm, and control 92.85 ± 17.25 cm (p=0.960). These consistent measurements across groups confirm physical development parity.

Table 2 ***Age and Anthropometric Parameters comparison among the studied groups***

Parameter	Neuroblastoma (NO.=34)	Wilms Tumor (NO.=31)	Control (NO.=33)	p-value
Age (months)	39.35 ± 28.24	40.03 ± 24.29	40.85 ± 32.09	0.980

Weight (kg)	15.02 ± 5.88	15.48 ± 5.09	15.36 ± 5.91	0.942
Length (cm)	92.03 ± 19.50	91.61 ± 16.05	92.85 ± 17.25	0.960

One-way ANOVA showed no significant differences between groups for age, weight, or length ($p > 0.05$).

Mean disease durations (±SE) were neuroblastoma 13.18 ± 7.97 months and Wilms tumor 16.32 ± 9.12 months (p=0.143). Fever occurred in 12 cases each of neuroblastoma (35.3%) and Wilms tumor (38.7%), but in 0% of controls, a highly significant difference (p≤0.001), reinforcing fever as a clinically relevant sign in pediatric tumors.

All biochemical markers showed statistically significant elevations in tumor groups compared to controls (p≤0.001). For neuron-specific enolase (NSE), mean levels were 424.71 ± 55.94 ng/mL in neuroblastoma, 175.45 ± 17.49 ng/mL in Wilms tumor, and just 9.79 ± 0.87 ng/mL in controls. Metanephrine levels were highest in neuroblastoma (26.17 ± 2.24 ng/mL), compared to 8.02 ± 0.28 ng/mL in Wilms tumor and 7.47 ± 0.29 ng/mL in controls. Procalcitonin followed a similar trend with 294.79 ± 27.87 pg/mL in neuroblastoma, 193.05 ± 14.93 pg/mL in Wilms tumor, and 125.12 ± 8.39 pg/mL in controls. For lactate dehydrogenase (LDH), neuroblastoma patients exhibited the highest values at 848.79 ± 87.57 U/L, followed by 629.26 ± 66.99 U/L in Wilms tumor and only 68.58 ± 3.90 U/L in controls. Interestingly, ferritin was most elevated in Wilms tumor (505.84 ± 47.87 µg/L), intermediate in neuroblastoma (324.12 ± 23.68 µg/L), and lowest in controls (64.42 ± 1.50 µg/L). These highly significant differences emphasize the potential of these markers for distinguishing between pediatric tumors and healthy individuals, with unique elevation patterns across tumor types.

Table 3 ***Analysis of biochemical parameters across the three study groups***

	Control	neuroblastoma	Wilms tumor	P
Procalcitonin pg/ml	$125.12^{a,b}$ ± 8.39	294.79^{a} ± 27.87	193.05^{b} ± 14.93	<0.0001 #
Ferritin µg/L_	$64.42^{a,b}$ ± 1.50	324.12^{a} ± 23.68	505.84^{b} ± 47.87	<0.0001 #
Neuron specific enolase ng/mL_	$9.79^{a,b}$ ± 0.87	424.71^{a} ± 55.94	175.45^{b} ± 17.49	<0.0001 #
Metanephrine ng/ml	7.47^{a} ± 0.29	26.17^{a} ± 2.24	8.02 ± 0.28	<0.0001 #
LDH U/L	$68.58^{a,b}$ ± 22.44	848.79^{a} ± 87.57	629.26^{b} ± 66.99	<0.0001 #

Data are: Mean ± Standard Errors # ANOVA§ T-test a, b = groups with the same letter differ significantly in the post-hoc test according to Tukey-Kramer

In neuroblastoma, NSE was moderately positively correlated with procalcitonin (r=0.367, p≤0.0329). In Wilms tumor, NSE showed a moderate positive correlation with metanephrine (r=0.362, p≤0.0454) and a moderate negative correlation with LDH (r=-0.396, p≤0.0274). In controls, NSE correlated positively with metanephrine (r=0.356, p≤0.0421) and negatively with procalcitonin (r=-0.386, p≤0.0265), indicating altered biomarker interrelationships in disease versus health.

Receiver Operating Characteristic (ROC) analysis demonstrated excellent diagnostic performance for several biomarkers in distinguishing pediatric tumors from healthy controls. In the **neuroblastoma vs control** comparison, NSE, ferritin, and LDH each achieved perfect diagnostic accuracy with an AUC of 1.00, 100% sensitivity, and 100% specificity. Metanephrine also showed strong discriminatory power (AUC = 0.960), while procalcitonin demonstrated slightly lower but still robust performance (AUC = 0.90, sensitivity = 73.53%, specificity = 93.94%). In the Wilms tumor vs control comparison, NSE, ferritin, and LDH again achieved perfect AUC values of 1.00, but metanephrine performed poorly (AUC = 0.589), and procalcitonin showed moderate utility (AUC = 0.786). The most diagnostically challenging comparison, neuroblastoma vs Wilms tumor, revealed metanephrine as the most effective marker (AUC = 0.952, sensitivity = 88.24%, specificity = 100%), while NSE (AUC = 0.757), procalcitonin (AUC = 0.698), ferritin (AUC = 0.699), and LDH (AUC = 0.646) showed only moderate to low discriminative ability. These results underscore metanephrine's superior utility in differentiating between tumor types, while NSE, ferritin, and LDH remain highly effective for distinguishing tumors from non-tumor cases.

Table 4 ***Receiver operating characteristic (ROC) analysis of biomarkers for differentiating between neuroblastoma, Wilms tumor, and control groups.***

Contrast	Variable	AUC	95% CI	Cutoff	Sens	Spec	+LR	-LR
Neuroblastoma vs control groups	NSE	1.00	0.946 to 1.00	>22	100	100		0.00
	Metanephrine	0.960	0.881 to 0.993	>11	85.29	100.00		0.15
	Procalcitonin	0.90	0.803 to 0.96	>198	73.53	93.94	12.13	0.28
	Ferritin	1.00	0.946 to 1.00	>79	100.00	100.00		0.00
	LDH	1.00	0.946 to 1.00	>107	100.00	100.00		0.00
Wilms tumor vs control groups	NSE	1.000	0.944 to 1.000	>22	100.00	100.00		0.00
	Metanephrine	0.589	0.459 to 0.711	>6.6	83.87	36.36	1.32	0.44
	Procalcitonin	0.786	0.666 to 0.879	>148	77.42	72.73	2.84	0.31
	Ferritin	1.000	0.944 to 1.000	100.00	100.00		0.00	
	LDH	1.000	0.944 to 1.000	100.00	100.00		0.00	

Neuroblastoma vs Wilms tumor	NSE	0.757	0.634 to 0.856	>319	52.94	96.77	16.41	0.49
	Metanephrine	0.952	0.869 to 0.990	>10.8	88.24	100.00		0.12
	procalcitonin	0.698	0.571 to 0.807	>202	67.65	67.74	2.10	0.48
	Ferritin	0.699	0.571 to 0.807	≤615	100.00	48.39	1.94	0.00
	LDH	0.646	0.516 to 0.761	>872	47.06	83.87	2.92	0.63

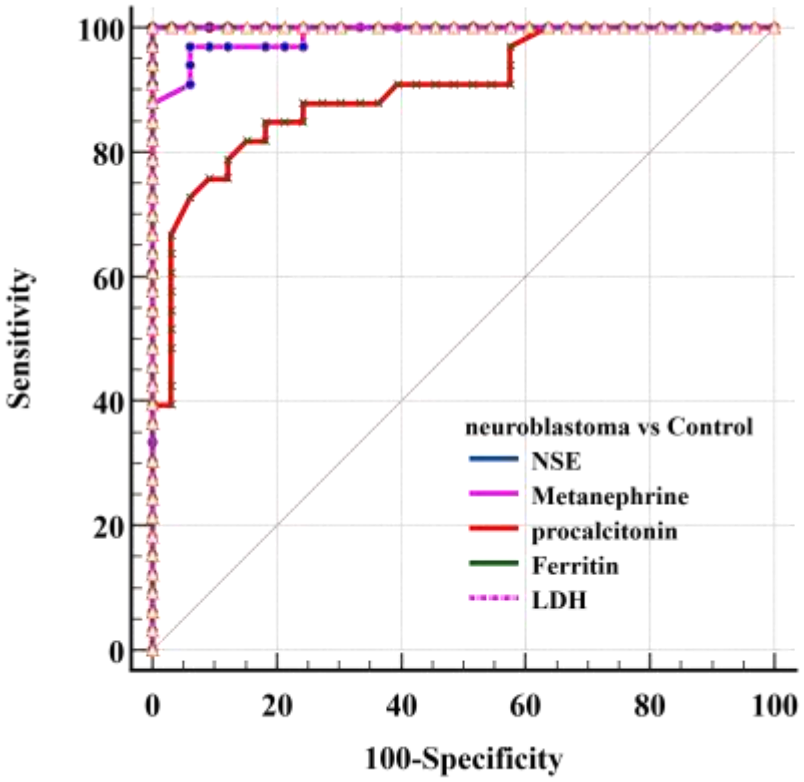

Figure 1 ***receiver operating characteristic curve comparing four different biomarkers: procalcitonin (pg/ml), ferritin (µg/L), neuron-specific enolase (ng/mL), and metanephrine (ng/ml). as they distinguish between Neuroblastoma from control groups***

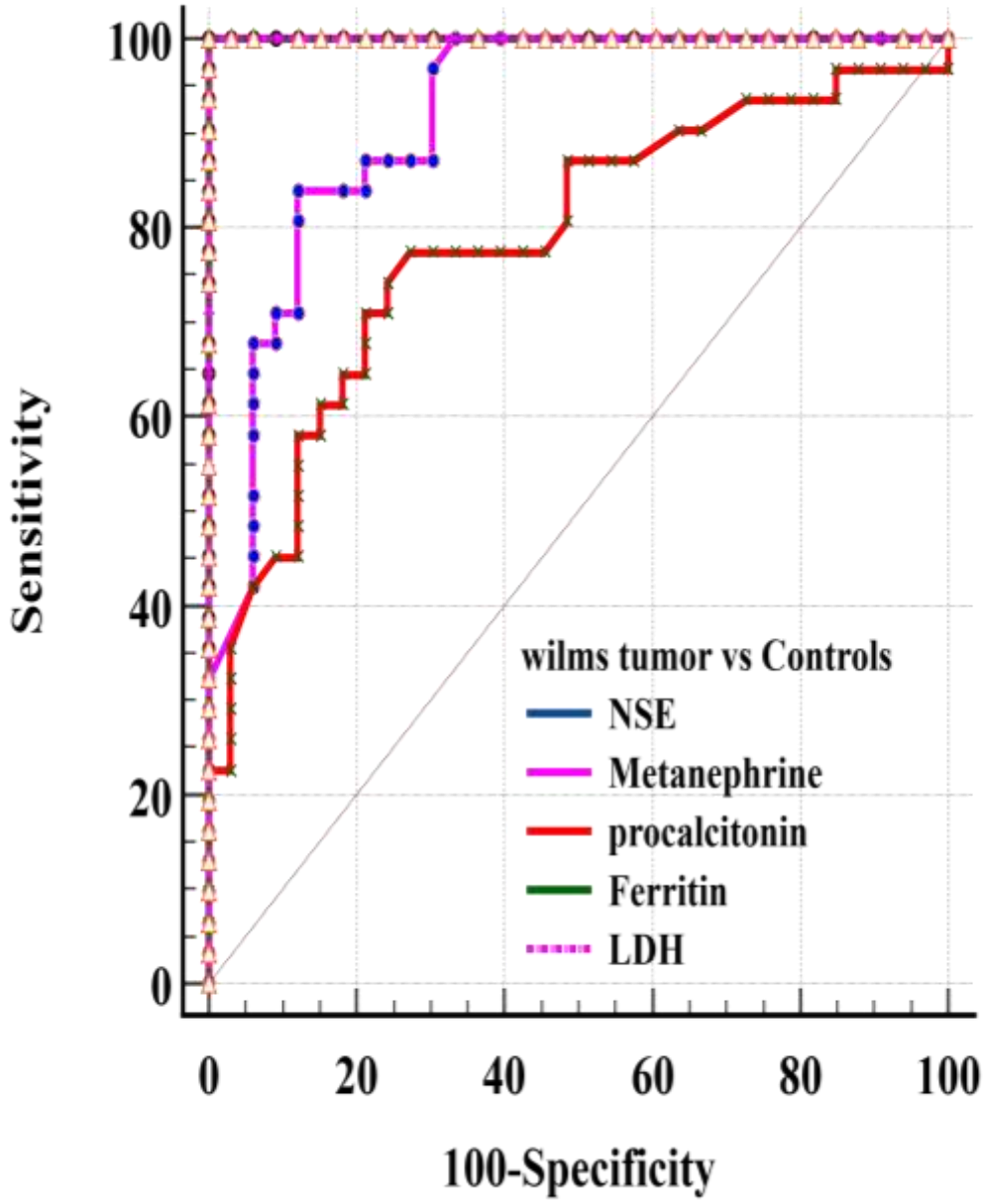

Figure 2 ***receiver operating characteristic curve comparing four different biomarkers: procalcitonin (pg/ml), ferritin (µg/L), neuron-specific enolase (ng/mL), and metanephrine (ng/ml). as they distinguish between Wilms tumor from control groups***

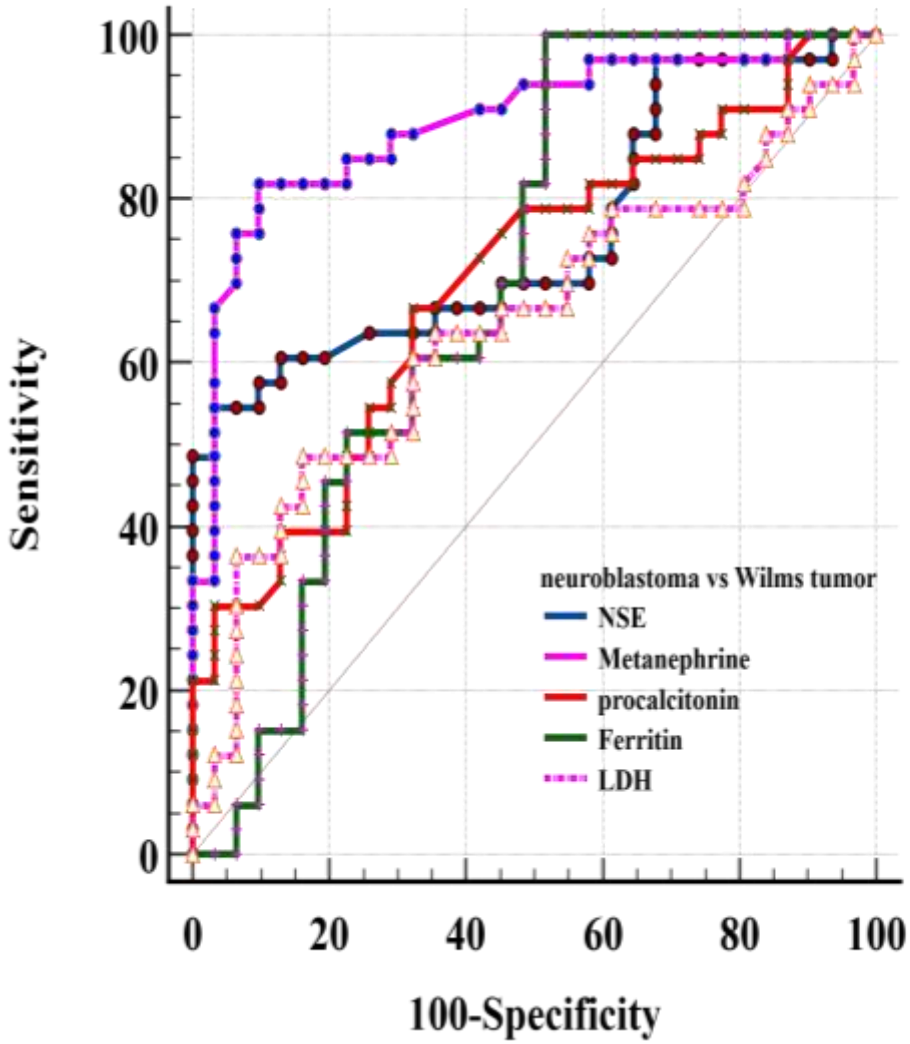

Figure 3 ***receiver operating characteristic curve comparing four different biomarkers: procalcitonin (pg/ml), ferritin (µg/L), neuron-specific enolase (ng/mL), and metanephrine (ng/ml). as they distinguish between Neuroblastoma from Wilms tumor groups***

DISCUSSION

Neuron-Specific Enolase (NSE): The study showed that neuroblastoma patients had much higher NSE levels (mean 424.71 ± 55.94 ng/mL), which is 43 times higher than healthy controls (˜9.79 ng/mL). This is consistent with NB's neural crest origin and neuroendocrine differentiation. Patients with Wilms tumor exhibited intermediate NSE elevation (175.45 ± 17.49 ng/mL), presumably attributable to aberrant γ-enolase expression in rhabdomyocyte-like cells. Statistical analysis corroborated significant differences ($p < 0.001$), highlighting NSE's diagnostic specificity for neuroblastoma. The elevation is due to tumor cell expression (ENO2 gene)(13), necrosis-related release, and metabolic reprogramming(14), as shown by studies.

Metanephrine (MN) levels in neuroblastoma (NB) patients (26.17 ± 2.24 ng/mL) were three times higher than in wild-type (WT) (8.02 ± 0.28 ng/mL) and control (7.47 ± 0.29 ng/mL) groups, with no significant difference observed between WT and controls ($p \geq 0.956$). This clear difference shows that MN could be a specific biomarker for NB because it comes from neuroendocrine cells that secrete catecholamines. MN's diagnostic utility may encompass the differentiation of neuroblastoma from other pediatric tumors and the monitoring of disease progression(15).

Procalcitonin (PCT) levels were significantly higher in NB (294.79 ± 27.87 pg/mL) than in WT (193.05 ± 14.93 pg/mL) and controls (125.12 ± 8.39 pg/mL), with intergroup differences that were statistically significant ($p < 0.001$). PCT is usually linked to infection, but its rise in NB suggests paraneoplastic inflammation or tumor-associated secretion. Additional research is required to clarify the role of PCT in tumor biology and its clinical application in pediatric oncology(16).

Lactate Dehydrogenase (LDH) levels were significantly higher in both the NB (848.79 ± 87.57 U/L) and WT (629.26 ± 66.99 U/L) cohorts compared to controls (68.58 ± 3.90 U/L), with NB levels being much higher than WT ($p < 0.0001$). This is an example of the Warburg effect, which says that tumors prefer glycolysis when there isn't enough oxygen, and HIF-1α is what makes this happen. LDH's link to tumor burden and aggressiveness makes it a good marker for advanced disease(17)(16).

Ferritin levels were significantly elevated in advanced-stage neuroblastoma due to tumor synthesis and necrosis-related release. Basic and acidic isoferritins were produced together, and their levels were affected by transfusions, so care must be taken when interpreting them. In WT, ferritin's involvement in tumorigenesis and macrophage-mediated inflammation indicates diagnostic and therapeutic potential, although additional validation is necessary(18).

Conclusion

The study emphasizes unique biomarker profiles for neuroblastoma (NB) and Wilms tumor (WT), indicating that neuron-specific enolase (NSE) and metanephrine (MN) exhibit high specificity for NB, whereas procalcitonin (PCT), lactate dehydrogenase (LDH), and ferritin signify more generalized metabolic and inflammatory alterations associated with tumors. These biomarkers collectively facilitate diagnosis, differential evaluation, and potential monitoring of pediatric solid tumors, although mechanistic and clinical correlations necessitate further investigation.

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