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RESEARCH PAPER

Neuroendocrine and Metabolic Biomarkers in Iraqi Children with Growth Hormone Deficiency and the Potential Application of Fe_3O_4 Magnetic Nanoparticles for Chromogranin A Detection

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ABSTRACT

Chromogranin is mostly stored in dense secretory vesicles in neuroendocrine cells and neurons, and its production is coordinated with a variety of hormones, neuropeptides, and neurotransmitters. Neuropeptide Y (NPY) is a significant neuropeptide that is essential for controlling hunger, energy balance, and neurological and endocrine system communication. A hormone called leptin connects brain and glandular processes to energy and nutritional condition. The purpose of the current study is to compare the serum levels of leptin, neuropeptide Y, and chromogranin A in children with GHD to those in healthy children. Additionally, it looks at how these markers specifically, GH, IGF-1, and BMI, relate to the growth hormone/IGF-1 axis. This study included sixty children: 30 with GHD, 30 healthy children. The samples were collected from Maysan Hospital for Child & Brith. we founded highly significant between children with GHD and healthy children for all parameters except BMI and T3. from the current study We concluded that compared to leptin and neuropeptide Y, chromogranin A was found to have a better diagnostic capacity for differentiating between children with growth hormone deficit and healthy children. These theories imply the existence of overlapping metabolic, neurological, and hormonal changes associated with growth hormone shortage, necessitating additional investigation to validate these markers' function and their possible application as supplementary biomarkers.

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INTRODUCTION

Magnetic iron oxide nanoparticles (Fe_3O_4 NPs) have gained considerable attention in biomedical research because of their strong magnetic properties, large surface area, and ability to undergo surface modification for different biological applications. These characteristics make them promising materials for the separation and detection of biological molecules [1-3]. In addition to explored their influence on lipid metabolism, glucose regulation, insulin, and leptin in experimental obesity models [4-6]. Beyond these applications, appropriately functionalized Fe_3O_4 nanoparticles may provide a useful platform for isolating specific proteins from biological samples

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through magnetic separation [7]. This possibility is particularly relevant to endocrine research, where identifying changes in circulating biomarkers may improve our understanding of hormonal disorders. Accordingly, the potential use of magnetic Fe_3O_4 nanoparticles for detecting chromogranin A (CgA) in serum deserves investigation, particularly in children with growth hormone deficiency (GHD).

The granin family includes the acid glycoprotein chromogranin A (CgA). Chromogranin is mostly stored in dense secretory vesicles in neuroendocrine cells and neurons, and its production is coordinated with a variety of hormones, neuropeptides, and neurotransmitters [8,9]. It serves as a precursor to numerous significant and active peptides, including catestatin, vasostatin, and pancreastatin, which contribute to diverse inflammatory and vascular functions and control metabolism, in addition to its essential role as a biomarker of brain activity [10]. Chromogranin A (CgA) is a neuroendocrine biomarker with clinical significance, especially in the assessment of neuroendocrine tumors. However, because it is influenced by numerous pathological states, drugs, and physiological factors, its concentration in the blood is not entirely specific. Additionally, when comparing study results, variations in measurement techniques play a significant role. To properly comprehend chromogranin A's (CgA) clinical value, it is necessary to correlate it with other indicators [11].

Since appropriate growth depends on the careful integration of the central nervous system, the neuroendocrine system, and the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, research on CgA is crucial during childhood and early childhood development. Some children experience delayed growth and short stature, which may stem from a disorder in growth hormone secretion or a dysfunction in its neurological regulation. Children with slow growth characterized by low levels of insulin-like growth factor-1 (IGF-1) and reduced spontaneous growth hormone secretion have been diagnosed with a neurosecretory growth hormone dysfunction, even though they may show a satisfactory response to certain standard stimulation tests. When examining chromogranin A in children, it is crucial to use age-specific reference intervals (ARIs) instead of adult reference values, as demonstrated by well-established reference values for children and adolescents [9]. One of the primary reasons that children must be sent to an endocrinologist is because of their small size. Malnutrition, certain medical disorders, and hereditary reasons are the primary culprits. One of the key elements controlling linear growth is Growth Hormone/Insulin-like Growth Factor-1 (GH/IGF-1). However, children without a definite GH shortage may also exhibit short stature. Thus, distinguishing between idiopathic short stature (ISS) and growth hormone deficiency (GHD) is a significant clinical and research problem [12,13].

Neuropeptide Y (NPY) is a significant neuropeptide that is essential for controlling hunger, energy balance, and neurological and endocrine system communication. Given that CgA is found in a collection of neurosecretory granules that secrete and store a variety of peptides and neurotransmitters, its association with CgA offers a chance to investigate the neuroendocrine component of growth control. This explains the functional relationship between neuropeptides involved in growth and energy balance and CgA [14,15]. A hormone called leptin connects brain and glandular processes to energy and nutritional condition. Higher leptin levels were linked to catch-up growth after controlling for age, sex, BMI, and IGF-1, according to earlier research that supported the crucial link between the appetite hormone and growth. This lends credence to the theory that leptin regulates children's growth [16,17]. The majority of earlier research has concentrated on the role of each marker, such as chromogranin A, leptin, and neuropeptide Y, as controllers of energy balance and neuronal processes, despite the growing interest in elucidating the crucial role of neuroendocrine and metabolic markers in regulating growth.

The integrated link between chromogranin A, neuropeptide Y, leptin, and the GH/IGF-1 axis in children with short stature, however, has not been emphasized in prior research. To completely clarify this relationship, more investigation is necessary. The understanding that growth disorders include a range of illnesses, such as growth hormone insufficiency and idiopathic short stature, which may overlap in their clinical and laboratory characteristics, highlights the significance of this gap. The purpose of the current study is to compare the serum levels of leptin, neuropeptide Y, and chromogranin A in children with GHD to those in healthy children. Additionally, it looks at how these markers specifically, GH, IGF-1, and

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BMI relate to the growth hormone/IGF-1 axis. Additionally, the study intends to evaluate how these markers might be used separately or in combination to distinguish between children who have GHD that may lead to short stature in the future. Although conventional immunoassays are widely used to measure circulating biomarkers, magnetic iron oxide nanoparticles may offer an additional approach for improving the selective separation and detection of specific proteins in biological samples. However, their potential application in detecting chromogranin A in children with growth hormone deficiency remains insufficiently explored. Therefore, alongside the evaluation of hormonal and metabolic alterations, the present study also aims to investigate the feasibility of using Fe_3O_4 magnetic nanoparticles for CgA detection in the same serum samples and to assess their potential as a supplementary analytical tool.

MATERIALS AND METHODS

Study Design and Sample Collection

The sixty children in this study, ages five to eleven, were split into two groups: thirty children in good health and thirty children who had been diagnosed with growth hormone deficit by a specialist. Before the kids were given 1 milliliter of synthetic growth hormone, samples were taken. The samples were collected between April to July 2025 at Maysan Hospital for Child & Brith.

Synthesis of Fe_3O_4 Magnetic Nanoparticles

Magnetic iron oxide nanoparticles (Fe_3O_4 NPs) were synthesized using a chemical co-precipitation method involving ferric and ferrous iron salts. Briefly, 1.6220 g of anhydrous ferric chloride ($FeCl_3$) in addition to use 1.9608 g of ferrous ammonium sulfate hexahydrate [$(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$]. These were dissolved in 150 ml of deionized distilled water in a 500 ml flask. The resulting solution was placed on a hot plate and heated to approximately 90 °C under continuous magnetic stirring. During this process, ammonium hydroxide solution (NH_4OH, 32%; approximately 50 ml) was gradually introduced dropwise until the pH reached 10.0-10.5. The formation of a dark black precipitate indicated the production of magnetic iron oxide particles. The precipitate was subsequently collected using an external magnet allowing the supernatant to be carefully removed. The recovered nanoparticles were washed repeatedly with deionized water and ethanol to eliminate residual reagents and soluble impurities. The purified material was separated using filter paper and dried in an oven at 50 °C for 4 h.

Physicochemical Characterization of Fe_3O_4 Nanoparticles

The synthesized Fe_3O_4 nanoparticles were examined using several complementary analytical techniques to evaluate their structural, morphological, surface, and magnetic properties. X-ray diffraction (XRD) was employed to investigate the crystalline structure and identify diffraction peaks associated with spinel-type iron oxide. Fourier-transform infrared spectroscopy (FTIR) was performed to examine characteristic chemical bonds and surface functional groups, with particular attention to the vibrational bands associated with iron oxygen bonding. Transmission electron microscopy (TEM) was used to visualize particle morphology and assess their physical dimensions and size distribution. The magnetic behavior of the nanoparticles was investigated using vibrating sample magnetometry (VSM), allowing evaluation of the magnetization response under an applied magnetic field. Together, these techniques provided complementary information on the physicochemical characteristics of the prepared magnetic nanoparticles.

Biochemical and Hormonal Measurements

The youngsters were fasting when the venous blood samples were taken, height and weight were measured to find the body mass index. Chromogranin A, insulin, T3, T4, and TSH were assessed using an ELISA Cusabio China kit; growth hormone and neuropeptide Y were detected using an ELISA kit from Shanghai China. Additionally, growth hormone was measured using an ELSA kit from the USA, and insulin-like growth hormone was measured using an ELSA kit from Germany. A spectrophotometer was used to measure the levels of glucose.

Fe_3O_4 Nanoparticle-Assisted Detection of Chromogranin A in Serum

The potential application of the synthesized Fe_3O_4 nanoparticles for chromogranin A (CgA) detection was investigated. The magnetic nanoparticles were initially functionalized with carboxyl-containing surface groups, which were subsequently activated using 1-ethyl-3-(3-

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dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to facilitate the immobilization of anti-CgA antibodies. Serum aliquots were then incubated with the antibody-functionalized nanoparticles under optimized experimental conditions to promote selective binding of CgA. Following incubation, the resulting nanoparticle-antibody-antigen complexes were isolated using an external magnetic field and washed to remove unbound proteins. The recovered CgA was subsequently quantified using a validated CgA-specific enzyme-linked immunosorbent assay (ELISA), with calibration standards used for quantitative determination.

Statically analysis

This analysis makes use of the study's mean values and standard deviations. A student's t-test was also used to determine the significance of the difference between the means of the individual groups. The ROC curve and data correlations for the Jamovi program (2025) were calculated using Jamovi software (version 2.7). A significant *P-value* was defined as less than 0.05 [18-21].

RESULTS AND DISCUSSION

Clinical and Biochemical Characteristics of the Study Groups

As indicated in Table 1, the study's findings revealed variations in body mass index, thyroid hormone and biomarker levels between children with growth hormone insufficiency and a group of healthy kids. Leptin, growth hormone, thyroid hormones, chromocranin A, and neuropeptide Y levels were compared between the two groups. Children with growth hormone insufficiency had a body mass index (BMI) that was significantly higher than that of healthy children (18.7 ± 2.8 and 15.5 ± 0.9). When comparing the levels of threonine in children with GHD (1.8±0.3) and healthy children (2.0±0.3), there was no discernible difference. However, we found a highly significant drop in T4 (85.3±8.0) in GHD children compared to 96.4±7.3 in healthy children. Thyroid-stimulating hormone (TSH) levels were found to be significantly higher in children with growth hormone deficit (8.0±1.3) than in healthy children (2.8±0.8). Children with growth hormone insufficiency had significantly lower levels of GH, IGF-1, and chromogranin (0.1±0.08), (169±12.4), and (216±38.8), respectively, than healthy children (0.3±0.1), (195±16.2), and (270±61.9). On the other hand, children with growth hormone insufficiency showed significantly higher levels of leptin and neuropeptide Y (5.7±1.2, 49.4±5) than children in the healthy group (4.1±1.0, 43.5±5.7).

Children with growth hormone deficiency have higher body mass indices (BMI) because of increased fat formation and decreased lean mass, which lessens growth hormone's regulatory effects on lipid metabolism and body composition. According to other research, growth hormone treatment improves body composition and lowers fat mass.[22]

The current study found that children with growth hormone deficiency had lower levels of total thyroid hormone (T4) and higher levels of thyroid-stimulating hormone (TSH) when compared to healthy children. However, there was no statistically significant difference in the levels of total thyroid hormone (T3) between the two groups. These results may point to a reversible overlap between the hypothalamic-pituitary-thyroid axis and the growth hormone axis. Research has shown that children with growth hormone deficit may experience changes in thyroid hormone levels, varying in both the direction and degree of these changes. This study's decreasing T4 level may have caused an increase in TSH, which could be a compensatory reaction to the lower thyroid hormone levels.

Its relative maintenance through peripheral conversion of T4 to T3 may account for the lack of a considerable change in overall T3 levels. Studies that have demonstrated lower thyroxine levels in children with growth hormone insufficiency or during growth hormone therapy are somewhat compatible with these theories. The disparity between the findings of this study and some earlier research, however, may be explained by a recent review that showed variations in T3, T4, and TSH levels between trials [23,24]. This study explains why children with GHD may have lower amounts of insulin-like growth factor-1 (IGF-1) and growth hormone (GH) than children in good health [25]. The direct functional correlation between the two hormones within the GH/IGF-1 axis plays a bio-vital role in this discovery.

Growth hormone mainly stimulated the liver's production of IGF-1, which is thought to be a key mediator in the stimulation of linear growth and tissue development. so that children's growth and development are adversely affected by decreased GH secretion as a result of decreased

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IGF-1 synthesis. Given that GH secretion is pulsatile and variable whereas the blood level of IGF-1 is relatively steady, a reduction in IGF-1 is thus a crucial signal for evaluating GH/IGF-1 axis functioning. However, other research suggests that since IGF-1 levels can be impacted by age and nutritional state, they cannot be used as a reliable predictor of GH deficit [26]. Since leptin concentration is correlated with body fat percentage, it has been discovered that children with growth hormone shortage have elevated leptin concentrations as a result of changes in adipose tissue composition and an increase in adipose tissue linked to growth hormone deficiency. Elevated leptin concentrations are linked to severe growth hormone shortage, according to a study conducted on children with this condition [27].

Diagnostic Performance of Circulating Biomarkers

Table 2 and Fig. 1 shows that the chromogranin A had higher sensitivity (0.931) than neuropeptide Y and leptin, according to ROC curve values. It also attained the greatest value for the area under the curve (0.791), showing its effectiveness in differentiating between the two study groups. As an appetite-stimulating peptide, neuropeptide Y (NPY) plays a critical role in energy balance and appetite regulation even if high levels may be seen. Since leptin typically suppresses the NPY pathway, elevated leptin and NPY synthesis may suggest a breakdown in the control of central appetite signaling. Therefore, rather than necessarily being a direct result of growth hormone deficit on NPY, their simultaneous rise may indicate a modified response to leptin signaling [28]. Children with growth hormone shortage had lower amounts of chromocranin A (CgA) than healthy children, as the current study found.

This is probably because chromocranin A is linked to neuroendocrine cells and secretory granules that store and release peptides and hormones. CgA is essential for the production of secretory granules as well as the control of their release and storage. Therefore, in children with growth hormone deficit, a reduction in its level may indicate a change in the activity or secretory efficiency of neuroendocrine cells. Nevertheless, more direct research is needed to validate this association [29]. This study contradicts a recent study that found children with growth hormone insufficiency had higher levels of CgA than children in good health. Variations in sample characteristics, the degree of growth hormone deficit, treatment status, and various techniques for assessing CgA could all contribute to the disparity. Therefore, we propose that the decline in CgA may be a consequence of altered neuroendocrine function linked to growth hormone deficiency; however, due to the small number of studies and the inconsistent findings, it cannot be regarded as a qualitative indicator of

Table 1. Control children and children with GHD for all parameters (Mean± SD).

Parameters	Mean± SD Control N.30	Mean ±SD Children with GHDN.30	P value
Age	7.4±1.8	7.71.9±	<.201
BMI kg/m^2	15.5±0.9	18.7±2.8	<.001
T3ng/ml	2.0±0.3	1.8±0.3	<.111
T4ng/ml	96.4±7.3	85.3±8.0	<.001
TSHμIU/mL	2.8±0.8	8.0±1.3	<.001
GH ng/ml	0.3±0.1	0.1±0.1	<.001
Leptin ng/ml	4.1±1.0	5.7±1.2	<.001
IGF-1ng/ml	195±16.2	169±12.4	<.001
NPY Pg/ml	43.5±5.7	49.4±5	<.001
CgAPg/ml	270±61.9	216±38.8	<.001

[a] Levene's test is significant ($p < .05$), suggesting a violation of the assumption of equal variances

Table 2. ROC analysis for Children with GHD and children healthy.

	Optimal Cutoff Analysis					
Predictor	Optimal Cutoff	AUC	Sensitivity	Specificity	Accuracy	Clinical Recommendation
CgA	214.150	0.791	0.931	0.552	0.741	Moderate discriminatory performance
NPY	48.350	0.776	0.828	0.621	0.724	Moderate discriminatory performance
Leptin	5.300	0.825	0.862	0.655	0.759	Moderate discriminatory performance

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growth hormone deficiency in isolation [30]. To the best of the researchers' knowledge, this study is one of the few that looked at the levels of leptin, neuropeptide Y, and Cg A in kids with growth hormone insufficiency. The association between these biomarkers and the neurological, glandular, and altered metabolic processes linked to growth hormone insufficiency may be strengthened by this work. The interpretation of biochemical markers also depends on the reliability of the methods used for their detection. Sahira and Al-Abboodi (2024) emphasized the limitations of conventional diagnostic techniques and the importance of exploring alternative approaches in malaria diagnosis. Although addressing a different disease, their review highlights the broader need to evaluate analytical accuracy before introducing new diagnostic technologies into clinical practice

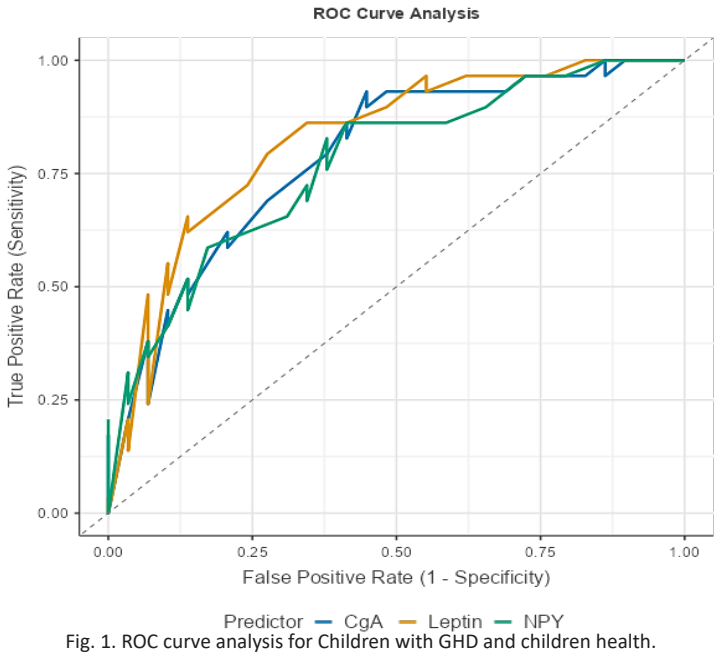


Fig. 1. ROC curve analysis for Children with GHD and children health.

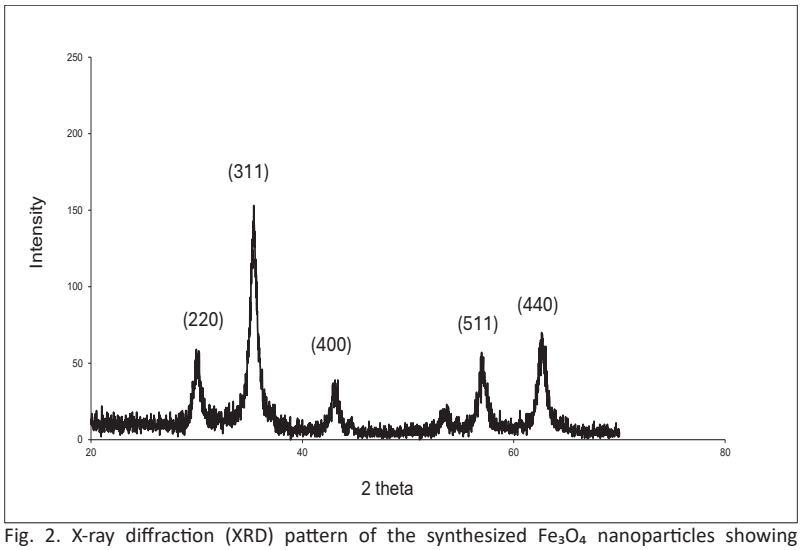


Fig. 2. X-ray diffraction (XRD) pattern of the synthesized Fe_3O_4 nanoparticles showing characteristic reflections of the spinel iron oxide structure.

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[31].

Structural and Morphological Characterization of Fe_3O_4 Nanoparticles

The XRD pattern revealed distinct diffraction peaks corresponding to the (311), (400), (511), and (440) crystallographic planes (Fig. 2). These reflections are consistent with the cubic spinel structure of iron oxide, indicating the formation of crystalline nanoparticles. The prominent (311) peak further supports the presence of a well-developed crystalline phase.

The FTIR spectra showed differences between bare Fe_3O_4 nanoparticles and the surface-modified Fe_3O_4–COOH sample (Fig. 3). Changes in the absorption bands suggest alterations in the chemical environment of the nanoparticle surface following functionalization. These findings provide preliminary evidence of surface modification, although confirmation of specific functional groups requires careful assignment of the characteristic bands. The structural and surface properties of nanoparticles are important considerations when evaluating their biomedical applications. Similarly,

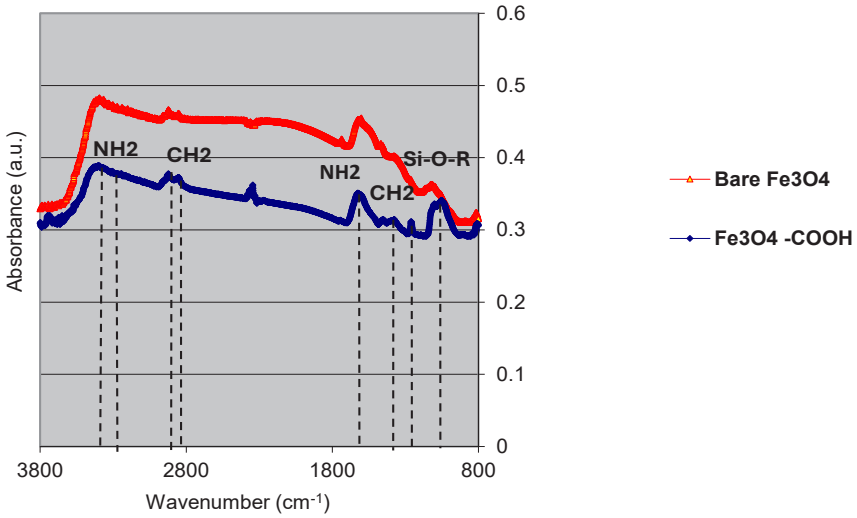


Fig. 3. FTIR spectra of bare Fe_3O_4 and Fe_3O_4–COOH nanoparticles illustrating differences in their surface chemical characteristics.

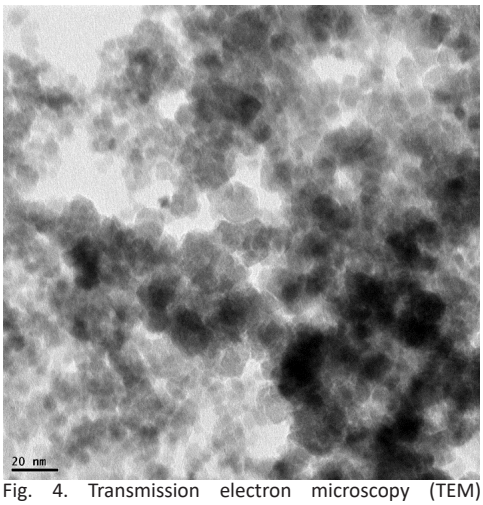


Fig. 4. Transmission electron microscopy (TEM) micrographs of synthesized Fe_3O_4 nanoparticles showing their morphology and particle size distribution.

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some studiess used XRD and FTIR to characterize silver oxide nanoparticles and their nanohybrid formulation [32-34]. Although the materials differ, both studies highlight the value of physicochemical characterization before investigating potential biological applications.

TEM examination was performed to assess the morphology and primary particle size of the prepared nanoparticles (Fig. 4). The micrographs should be used to report the observed particle shape, mean diameter, and extent of aggregation.

The VSM analysis demonstrated a clear increase in magnetization with increasing applied magnetic field (Fig. 5). The S-shaped magnetization profile indicates that the prepared nanoparticles respond strongly to an external magnetic field. This magnetic responsiveness supports their potential use in magnetic separation procedures, although their precise magnetic classification requires evaluation of coercivity and remanent magnetization.

In Silico Modelling of CgA Capture Using Fe_3O_4 Nanoparticles

A computational model was developed to explore the possible binding of chromogranin A (CgA) to antibody-functionalized Fe_3O_4 nanoparticles. The simulation predicted a gradual increase in CgA capture with longer incubation periods (Fig. 6). Under the selected baseline assumptions, approximately 36.1% of CgA was captured after 15 min, increasing to 82.4% after 60 min and 95.5% after 120 min. The predicted capture rate was also influenced by the assumed availability of antibody-binding sites, with higher binding capacities producing faster antigen capture. When the original mean serum CgA concentrations were incorporated into the model, the predicted recovered concentrations after 60 min were approximately 170.2 pg/mL for healthy controls and 136.2 pg/mL for children with GHD. These values included assumed magnetic separation and protein recovery efficiencies. Sensitivity analysis further demonstrated that changes in antibody-binding capacity and association kinetics substantially influenced the predicted capture efficiency (Fig. 7). These findings illustrate the theoretical potential of functionalized Fe_3O_4 nanoparticles as a platform for CgA isolation. However, the model remains exploratory, and its predicted performance requires experimental validation before any improvement in analytical or diagnostic sensitivity can be established. Computational approaches provide useful opportunities to explore nanoparticle behaviour before conducting further laboratory experiments. Hassan et al. (2023) discussed the growing contribution of artificial intelligence to nanotechnology, while Rasheed et al. (2026) demonstrated the usefulness of machine learning in nanostructure characterization. Although the present study uses a mathematical binding model rather than machine learning, these investigations support

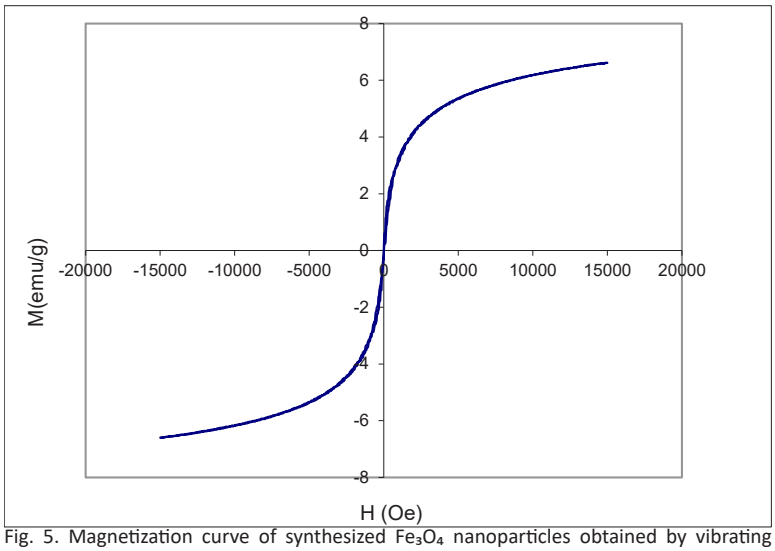


Fig. 5. Magnetization curve of synthesized Fe_3O_4 nanoparticles obtained by vibrating sample magnetometry (VSM).

the broader value of integrating computational approaches with experimental nanomaterial research [35- 37]. The cellular microenvironment and the properties of biomaterials play important roles in regulating biological responses. Al-Abboodi et al. (2012) developed a hydrogel-based microfluidic system to investigate cell behaviour under controlled conditions. Similarly, Kim et al. (2024, 2026) demonstrated the potential of engineered hydrogel coatings for controlled drug delivery. Although these studies addressed different applications, they highlight the versatility

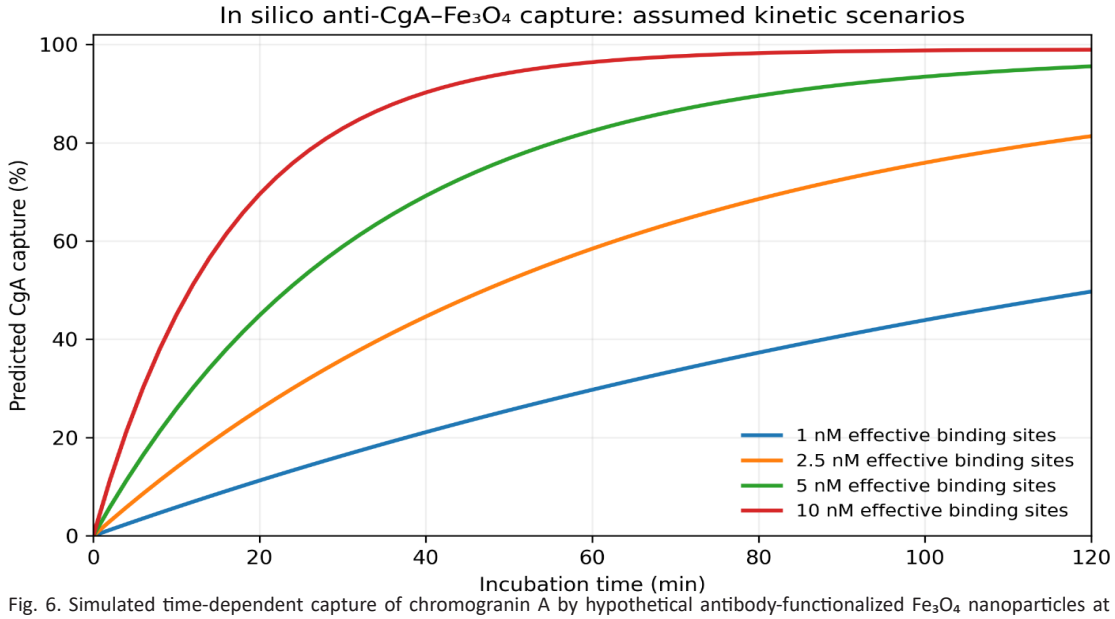


Fig. 6. Simulated time-dependent capture of chromogranin A by hypothetical antibody-functionalized Fe_3O_4 nanoparticles at different assumed effective binding-site concentrations. All curves represent computational predictions rather than experimental measurements.

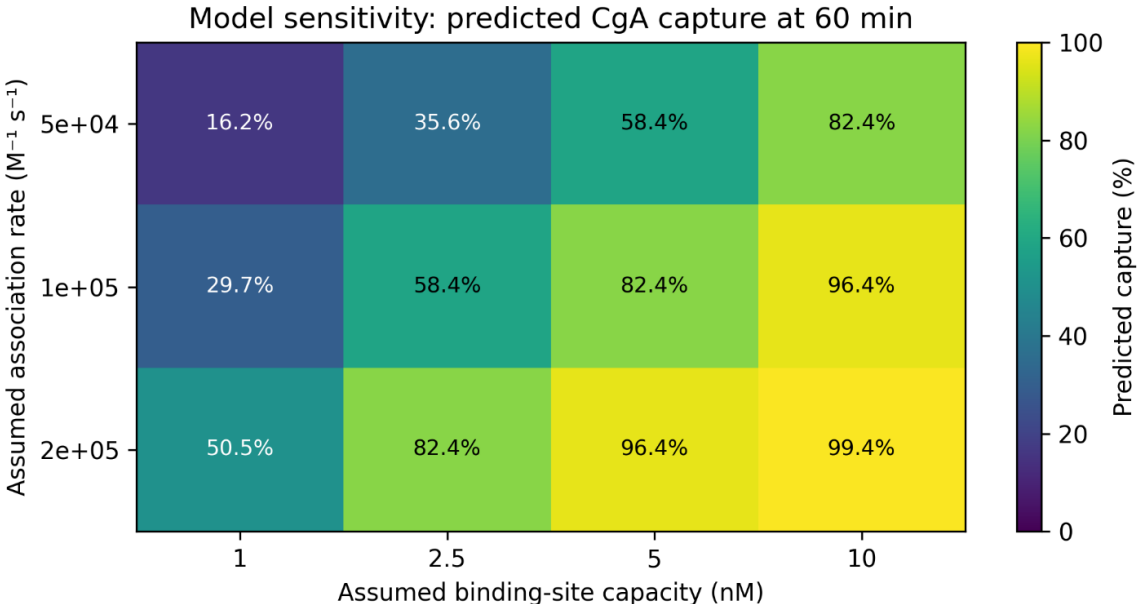


Fig. 7. Computational sensitivity analysis showing the influence of assumed antibody association rates and effective binding-site concentrations on CgA capture after 60 min of incubation with Fe_3O_4 nanoparticles.

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of biomaterial-based platforms. Such approaches may provide a foundation for future integration of Fe_3O_4 nanoparticles with cell-based systems to investigate neuroendocrine activity and CgA secretion in relation to growth hormone deficiency [38-42].

CONCLUSION

The results of this study showed that children with growth hormone insufficiency differed from healthy children in a number of hormonal and metabolic indicators. Growth hormone, IGF-1, chromocranin A, and total T4 levels declined, but body mass index (BMI), leptin, neuropeptide Y, and TSH levels increased. On the other hand, total T3 did not significantly differ between the two groups. Compared to leptin and neuropeptide Y, chromocrin A was found to have a better diagnostic capacity for differentiating between children with growth hormone deficit and healthy children. These theories imply the existence of overlapping metabolic, neurological, and hormonal changes associated with growth hormone shortage, necessitating additional investigation to validate these markers' function and their possible application as supplementary biomarkers. The synthesized Fe_3O_4 nanoparticles demonstrated structural and magnetic properties that support their potential use in biomedical applications. Furthermore, the computational model predicted time-dependent CgA capture, with an estimated efficiency of 95.5% after 120 min under the assumed conditions. These findings suggest that antibody-functionalized Fe_3O_4 nanoparticles could provide a promising approach for CgA isolation and detection. However, the simulated predictions require experimental validation before their analytical or clinical usefulness can be established.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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