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SPEXIN LEVEL IN GROWTH HORMONE DEFICIENCY IRAQI CHILDREN

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Spexin (SPX) is a newly discovered brain adipokine implicated in various homeostatic functions including metabolism, energy balance, endocrine processes and growth hormone (GH) production in particular. At the same time, the growth-promoting effects of GH are influenced by Insulin-like growth factor-1 (IGF-1) and vitamin D3. The aim of this study was to investigate the possible involvement of SPX in growth hormone deficiency (GHD) in children. The research involved 90 children (40 with growth hormone deficiency and 50 healthy controls aged 5-14). Serum levels of GH, IGF and vitamin D_3 were tested using a chemiluminescent immunoassay, that of SPX - by Elabscience ELISA Kit. The results revealed that children with GHD had significantly higher SPX levels compared to the control group. No significant difference in IGF-1 and vitamin D_3 levels between patients and control groups was observed. In the GHD group, we found a significant negative correlation between SPX and GH levels; at the same time, there was no correlation between SPX and D_3 levels. These findings suggest that the changes in SPX levels may contribute to growth hormone deficiency.

K e y w o r d s: growth hormone deficiency, spexin, IGf-1, vitamin D_3, Iraqi children.

Growth hormone deficiency (GHD) occurs when the body doesn't produce enough growth hormone (GH) [1], resulting in abnormally short stature with balanced body proportions. Growth hormone deficiency can be present at birth (congenital) or occur later in life (acquired). Some factors that can cause acquired GHD include tumors in the hypothalamus or pituitary gland located in the brain, injury to the head such as trauma, and radiation therapy for cancer treatment, specifically when it targets the hypothalamus and pituitary [2].

Lack of GH in children can lead to short height and delayed puberty. Adults with growth hormone deficiency, even with treatment, are at higher risk for heart problems and stroke. Therefore, finding new biomarkers could be effective in monitoring GHD. This disease is typically diagnosed in two main age groups: around age 4 when children start attending school and developmental differences become more noticeable. Around 10-13 years for girls and 12-16 years for boys, coinciding with a delay in the typical growth spurt associated with puberty. It's important to note that the prevalence of GHD does not appear to vary significantly based on race or ethnicity [3, 4].

Insulin-like growth factor-1 (IGF-1) is produced by the liver in response to growth hormone (GH). It plays a key role in the growth-promoting effects of GH [5]. The level of IGF-1 can provide information about the body's natural production of GH. IGF-1 levels are relatively stable throughout the day [6]. They can be used to assess the status of GH in children [7, 8], as low IGF-1 levels may indicate a lack of GH production. However, IGF-1 levels can be affected by various factors, including age, puberty, chronic illnesses, malnutrition, and GH deficiency. In most cases, low IGF-1 levels are a strong indication of GH deficiency [9, 10].

Spexin (SPX) is a newly identified peptide released by hypothalamic neurons, comprising 14 amino acids. Along with kisspeptin (KISS) and galanin (GAL), SPX belongs to a common gene family. SPX interacts with GAL2 and GAL3 receptors to carry out its functions [11]. The SPX sequence is similar across different animal species, from vertebrates to invertebrates. Both SPX mRNA and protein are abundant in mammals, avian, and fish tissues, including the brain and body [12]. Various biological functions of SPX have been identified in non-mammalian and mammalian organisms, such as food

consumption, metabolism, reproduction, pain perception, digestive tract movements, stress responses, and hormonal regulation [12-14].

The natural hormone SPX was discovered in 2007 [15]. The gene that produces it, C12orf39, is found on chromosome 12 and encodes for prepro-spexin [16]. A series of protein production steps convert the inactive preproSPX into the active form of SPX. Spexin is thought to be vital for survival and has been found in various body tissues and organs. In fact, SPX messenger RNA and protein are present in many body systems, including the heart, bones, digestion, urinary tract, reproductive organs, hormones, and brain [17].

SPX is widely distributed and found in various tissues throughout the body of many organisms, indicating its significance in diverse biological processes. Research has demonstrated its involvement in regulating food intake [14, 18, 19 and 20], glucose and fat metabolism [20, 21], gastrointestinal function [22], pain perception [23, 24], endocrine activity [19, 20, 24], reproduction [26, 27], cardiovascular health [28], and conditions such as obesity, anorexia, diabetes, anxiety, and depression [29-32].

Research indicates that vitamin D_3 influences the production of adipokines, primarily from adipose tissue. These adipokines [33], including SPX, have significant roles in various bodily functions. SPX which specifically helps regulate the absorption of long-chain fatty acids, body fat storage, controlling food intake and energy balance [34].

The endocrine effects of SPX have been identified in various hormone-producing tissues, such as the hypothalamus, thyroid, adrenals, reproductive organs, pancreas, and fat cells [35]. In studies involving rat cells, SPX has been shown to promote hormone release in the adrenal glands. Specifically, it has been found to increase aldosterone secretion in cells from the outer region of the adrenal cortex and induce corticosterone secretion in rats *in vitro* [35]. These findings suggest that SPX plays a role in controlling the adrenocortical secretory function [35].

SPX inhibits the release of luteinizing hormone (LH) [23]. Studies have shown that treatment with estrogen reduces SPX expression in hypothalamic nuclei of spotted scat [19], while direct administration of SPX increases the production of other hormones (gonadotropin-inhibitory hormone (GnIH) and gonadotropin-releasing hormone (GnRH)) in the brain but decreases the production of others (growth hormone and follicular stimulating hormone (FSH)). This suggests that SPX has hormonal effects on reproduction [18]. Therefore, the current study aims to explore the role of SPX in children's GHD.

SPX has been found to reduce both the production of insulin and a key factor involved in its regulation (insulin promoter factor 1). It also suppresses insulin release in isolated pancreatic cells (islets) when stimulated by glucose [36]. In obese rats, SPX treatment lowers levels of ghrelin, leptin, and corticosterone while raising levels of T3 and glucagon in the blood [21]. These findings indicate that SPX may act as a hormone, regulating various endocrine processes.

Materials and Methods

Patients. From October 2023 to December 2023, 40 children, male and female with GHD, were enrolled at the National Diabetes Center/ Mustansiriyah University in Baghdad, Iraq, and were compared to 50 healthy children as a control group and considering age and sex. Patients diagnosed with GHD (before treatment) ranged in age from 5 to 14 years. Before the commencement of this trial, all patients' parents provided their written and dated consent for their participation. Furthermore, this study received ethical approval from the ethics committee of the National Diabetes Center at Mustansiriyah University in (September 2023), ensuring compliance with the principles outlined in the 1964 Declaration of Helsinki and any subsequent revisions or comparable ethical standards.

Methods. By using a disposable plastic syringe with a (10 ml) capacity, 10 ml of venous blood were collected. Then, let it clot in a glass gel tube, and, later, centrifuged for 10 min at 3000 rpm to obtain serum. We asked the patients about their health and did a checkup (measuring height and weight). Calculated their body mass index using the formula BMI = weight (kg)/height^2 (m^2) [37]. The clinicians used all this information, along with X-rays, growth velocity, and bone age, to diagnose GHD. Tools for the diagnosis of GHD include measurement of IGF-1 and provocative GH. GH and IGF-1 were tested by DiaSorin analyzer device (Liaison hGH kit- Italy) and (Liaison IGF-1 kit, Italy), respectively. Vitamin D_3 was tested by VIDAS device (VIDAS VITD kit, France). The level of serum SPX in the patients and control groups was determined by using an elabscience (USA) enzyme-linked immunosorbent assay (ELISA) kit and following the manufacturer's instructions.

Exclusion criteria. The following were the exclusion criteria: - any subject with diabetes mellitus or other chronic diseases; - any subject with autoimmune diseases; - any subject with other endocrinopathy.

Statistical analysis. Statistical data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0 (SPSS Inc, Chicago, IL, USA) software program. The one-sample Kolmogorov-Smirnov test was used to determine parametric or non-parametric analyses, all variables (except IGF-1) were determined to be non-parametric distribution. Thus, Mann-Whitney U test was used to evaluate the level of significance between the patient and control groups. Results are presented as median, minimum, and maximum. Spearman test was used for correlation. A result was considered statistically significant if the *P*-value was below 0.05.

Results and Discussion

The ages of the patients and control group included in this study were between 5 and 14 years old, and the mean ages was 11.06 ± 2.89 (mean ± SD, n = 40) compared to control – 9.40 ± 2.27 (mean ± SD, n = 50). The gender of the subjects was roughly distributed (Fig. 1).

In this study, anthropometric measurements showed no significant differences among the two groups (except in height: P = 0.022) (Table 1).

Also, in our study, levels of SPX were significantly higher in patients compared to the control group (P = 0.000). Medians are shown in (Table 2).

Also, results show a significant negative correlation between SPX and GH (P = 0.000) with a correlation coefficient (r = -0.767) (Fig. 2).

We found no significant correlation between SPX and D_3 (P = 0.084) with a correlation coefficient (r = -0.183) (Fig. 3).

Body mass index (BMI), a ratio of weight to height, is widely used to assess overweight and obesity. In 2006, the WHO developed a growth standard for young children under the age of five, based on measurements from healthy children globally [38].

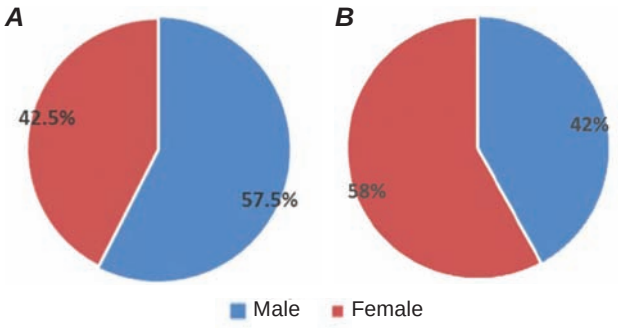

Fig. 1. Gender distribution: ***A*** *– in the patient group (57.5% male, 42.5% female) and* ***B*** *– control group (42% male, 58% female)*

In this study, BMI measurements showed no significant differences among the patients and control groups (P = 0.075) (Table 1). However, there was a significant decrease in patients' heights compared to the control group (P = 0.022). In conclusion, no correlations have been found between weight, height, or BMI and the SPX in the study group samples. Therefore, we suggest these biometric data may not be considered when using this neuropeptide as a biomarker. Also, the two study groups were well-matched regarding gender. This study finds that more males than females were referred to endocrine clinics (Fig. 1) due to growth disturbances but without statistical significance. Short stature in females is often reported lately due to the parent's belief that it is normal because it is a female.

Study results revealed that there is no significant difference in IGF-1 between patients and control groups (P = 0.760) (Table 2). Despite multiple past studies suggesting that IGF-1 levels are strongly indicative of GHD [10, 11], this study has demonstrated that IGF-1 levels have poor diagnostic accuracy due to its normal levels in both GHD children and control groups. Hence, IGF-1 should not be used alone for GHD screening.

This study revealed that levels of SPX were significantly higher in the patient group [1211.5 (1017-2577)] compared to the control group [762.5 (714-870)] (P = 0.000) (Table 2). This result gives

Table 1. Anthropometric measurements of GHD in patients and control

Metabolite	Control (median, min-max)	Patient (median, min-max)	*P* Value
BMI, kg/m^2	18.01 (12.25-21.33)	16.56 (9.6-31.64)	0.075
Height, m	1.38 (0.97-1.3)	1.33 (0.8-1.59)	0.022
Weight, kg	27 (13-50)	30 (11-80)	0.328

Table 2. Levels of studied parameters in patient and control groups

Metabolite	Control (median, min-max)	Patient (median, min-max)	*P* Value
SPX, (pg/ml)	762.5 (714-870)	1411.5 (1017-2577)	0.000
GH, ng/ml	7.95 (6.9-19.1)	0.28 (0.1-3.5)	0.000
IGF-1, ng/ml	183 (25.38-541)	196 (47-396)	0.760
D_3, ng/ml	11.4 (8.3-17.8)	10.65 (8.1-17.64)	0.015

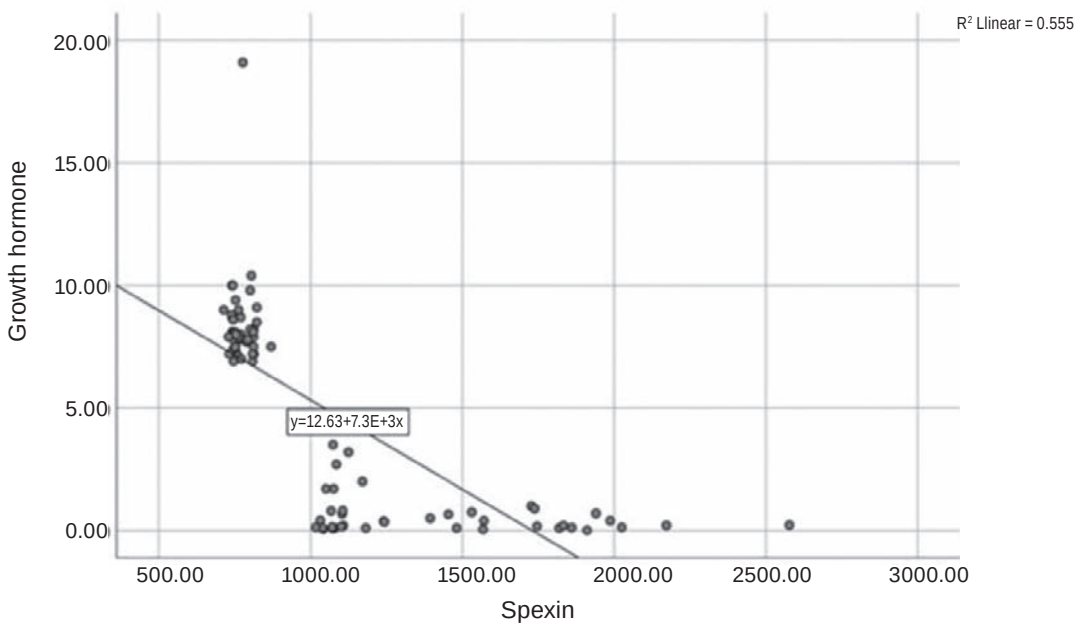

Fig. 2. Correlation between SPX and GH

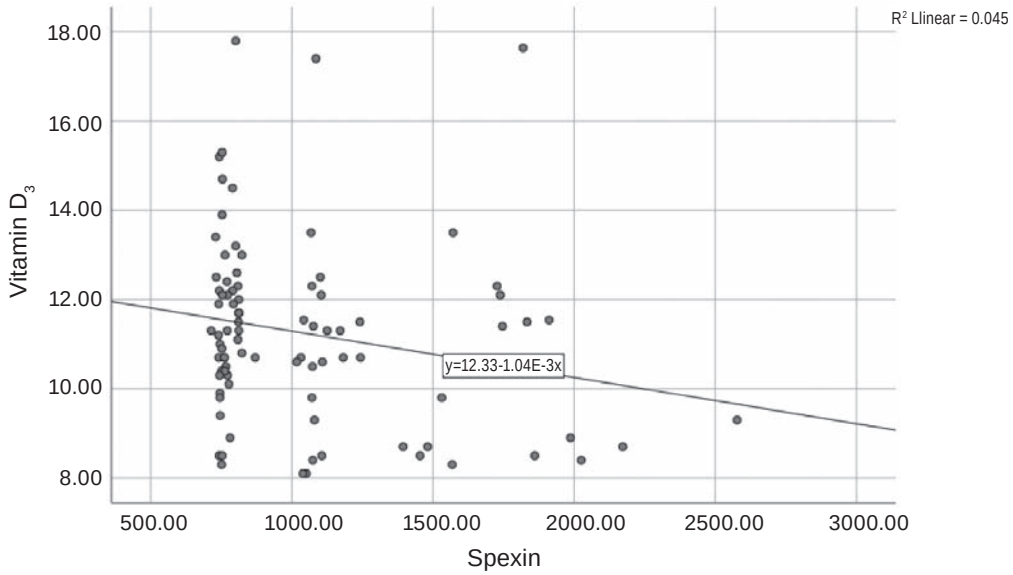

Fig. 3. Correlation between SPX and D3

a strong negative correlation between SPX and GH (r = -0.767, P = 0.000) (Fig. 2). In 2018, a study (Wang et al., 2018) proved that intraperitoneal injection of SPX led to enhanced expression of GniH and GNrH hormones in the brain's hypothalamus region, which led to suppression in GH. This means that when we try to optimize SPX levels it may improve GH secretion avoiding the complications of the GH deficiency.

As far as we know, this study is the first to examine SPX and vitamin D_3 levels in children with GHD compared to healthy children. In 2020 (Cheshmazar E. et al.) studied the effect of the daily administration of vitamin D_3 in obese participants with vitamin D_3 deficiency (≤ 20 nmol/l) on SPX levels and found that it did not alter serum SPX concentrations [39].

Correlation results between SPX and vitamin D_3 showed no significant correlation between them (P = 0.084) with a correlation coefficient (r = -0.183) (Fig. 3). Recent findings indicate that vitamin D_3 plays a regulatory role in controlling the production and release of various signaling molecules called adipokines [33], but, we cannot be sure that vitamin D_3 correlates to SPX levels related to GHD in children untill now. Hence, the endocrine effects of SPX are not fully known.

Conclusion. As far as our current research has shown, this study is the first published to assess SPX levels in children with GHD. A significant difference (high levels) of SPX hormone in children with GHD (without treatment) compared to healthy children was observed. These differences may play a role in the etiology of GHD as SPX is truly proven to be expressed in all of the endocrine glands and the highest levels of expression in the adrenal gland. Also, we found no significant correlation between SPX and vitamin D_3 levels in GHD children; hence, further studies are needed to show vitamin D_3 effects on this adipokine and confirm results. We recommend more comprehensive studies about SPX hormone in children treated and not treated with GH drug, making subgroups considering their bone age and fast glucose level.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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РІВЕНЬ СПЕКСИНУ В ІРАКСЬКИХ ДІТЕЙ З ДЕФІЦИТОМ ГОРМОНУ РОСТУ

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Спексин (SPX) — це нещодавно відкритий адипокін мозку, який бере участь у різних гомеостатичних функціях, включаючи метаболізм, енергетичний баланс, ендокринні процеси та, зокрема, виробництво гормону росту (GH). У той же час на стимулюючі ефекти GH впливають інсуліноподібний фактор росту-1 (IGF-1) і вітамін D_3. У роботі досліджували можливу участь SPX у розвитку дефіциту GHD у дітей. У дослідженні взяли участь 90 дітей (40 з дефіцитом гормону росту та 50 здорових дітей віком 5-14 років). Рівні GH, IGF і вітаміну D_3 у сироватці крові визначали хемілюмінесцентним імуноаналізом, а SPX – за допомогою Elabscience ELISA Kit. Результати показали, що у дітей з дефіцитом GH рівень SPX був значно вищим порівняно з контрольною групою. Істотної різниці в рівнях IGF-1 і вітаміну D_3 між пацієнтами та контрольною групою не виявлено. У групі з дефіцитом GH спостерігалася значна негативна кореляція між рівнями SPX і GH, при цьому не було кореляції між рівнями SPX і D_3. Ці результати свідчать, що зміна рівнів SPX можуть бути пов'язані з дефіцитом гормону росту.

Ключові слова: дефіцит гормону росту, спексин, IGf-1, вітамін D_3, іракські діти.

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