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Serum Asprosin and Ceramide Level in Pediatric Isolated Growth Hormone Deficiency in Babylon Province

A Thesis

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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

"صدق الله العلي العظيم"

سورة البقرة (٣٢)

Dedication

I dedicate my work to my loving parents for their encouragement and their patience

I dedicate my work to my wife for her tolerance and endless support.

I also dedicate my work to my lovely brother and sister

I also dedicate my work to my lovely daughter Narjis

To all colleagues who helped me

To all patients

Muntadher

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Muntadher

Supervisor Certification

We certify that this thesis entitled " **Serum Asprosin and Ceramide Level in Pediatric Isolated Growth Hormone Deficiency in Babylon Province** "Was carried under our supervision at the College of Medicine, University of Babylon, as a partial fulfillment for the requirement of the degree of Master of science in Clinical Biochemistry.

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In view of the available recommendation, I forward the present thesis for debate by the examining committee.

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Summary

Growth hormone deficiency is classically defined as insufficient growth hormone (GH) secretion that results in a decrease in the production of GH-dependent hormones and growth factors, such as insulin- like growth Factor-I (IGF-I), IGF-II and their binding proteins (IGFBPs) . The primary function of GH is to promote postnatal longitudinal growth. When the pituitary gland does not secrete enough growth hormone to promote the body's growth, it usually appears as a slow or flat rate of growth in both early and late childhood. When compared to peers of the same age and gender, children with growth hormone deficiency have appropriate body proportions, although they are generally chubbier, shorter, and appear younger than their age.

The present study was designed to investigate the level of asprosin, ceramide, malondialdehyde and glucose in patients with growth hormone deficiency, as well as to study the correlation between these biochemical parameters.

This study was designed as a case-control study includes 103 individuals but after some mathematic calculation need to exclude 15 individuals and also to match between patients and control in age and gender the number was 88 individuals conducted a forty-three patient with idiopathic isolated growth hormone deficiency (24 males and 19 female), aged (4-14 years) with mean ± standard deviation of (10.4 ± 3.0) years. The patients group include twenty-nine child treated with recombinant human growth hormone (rhGH) and fourteen children without treatment. And forty-five apparently healthy individuals matched with patients in age and gender.

The serum samples were used to measure biochemical parameters include asprosin, ceramide, malondialdehyde and glucose.

Results of the present study revealed that there was a significant ($P<0.05$) decrease in the levels of asprosin, ceramides and glucose in patients with growth hormone deficiency compared with their control group. While a non-significant ($P>0.05$) difference in malondialdehyde levels between the two groups.

In this study significant positive correlation between asprosin with ceramide and glucose, and there was significant positive correlation between ceramide and glucose.

In conclusion, Asprosin may be one of underlying cause of GHD through its indirect role in releasing of GH. GHD lead to decrease level of ceramide by the effect of GH on lipid metabolism. Presence of positive correlation between serum asprosin, ceramide and glucose strength its relation to GHD.

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List of Abbreviations

Abbreviations	Details
AC	Adenylyl cyclase
AD	Autosomal dominant
AgRP	Agouti-related peptide
AR	Autosomal recessive
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
cAMP	Cyclic Adenosine Monophosphate
CDC	Centers for Disease Control
Cer	Ceramides
CPHD	Combined pituitary hormone deficiency
CRE	cAMP response element
CREB	cAMP-responsive element-binding protein
CST	Clonidine stimulation test
DSS	Disproportionate Short Stature
DW	Distilled water
ELISA	Enzyme linked immunosorbent assay
FBN1	Fibrillin 1 gene
FFA	Free fatty acid
GalCer	Galactosylceramide

GH	Growth hormone
GHBP	Growth hormone binding proteins
GHD	Growth hormone deficiency
GHR	Growth hormone receptor
GHRH	Growth hormone-releasing hormone
GHRHR	Growth hormone-releasing hormone receptor
GHS	Growth Hormone Secretagogue
GHS-R	Growth Hormone Secretagogue - receptor
GlcCer	Glucosylceramide
GPCR	G-protein coupled receptors
GST	Glucagon stimulation test
GTP	Guanosine triphosphate
HRP	Streptavidin- Horseradish Peroxidase
IGFBP-3	IGF-binding protein 3
IGF-I	Insulin-like growth factor-I
ITT	Insulin tolerance test
IUGR	Intrauterine growth retardation
JAK	Janus kinase
LCB	long-chain base
MDA	Malondialdehyde
NPV	Negative predictive value
NPY	Neuropeptide Y

OD	Optical density
Olfr734	Olfactory 734
p. Value	Probability
pit-1	Pituitary-specific positive transcription factor 1
PKA	Protein kinase A
PPV	Positive predictive value
PSS	Proportionate Short Stature
r	Pearson correlation coefficient
rhGH	Recombinant human Growth hormone
ROC	Receiver operating characteristic curve
ROS	Reactive oxygen species
SD	Standard deviation
SM	Sphingomyelin
SRIF	Somatotropin releasing inhibiting factor
SS	Short Stature
STAT	Signal transducer and activator of transcription
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
WHO	World Health Organization
xg	Gravitational force

1. Introduction

Growth hormone deficiency (GHD) is an endocrine condition which defined as a reduction in growth hormone (GH) synthesis that results in a reduce in the production of GH-dependent hormones and growth factors, such as insulin- like growth factor-I (IGF-I), IGF-II and their binding proteins (IGFBPs) (1). Children with GHD have appropriate body proportions when compared to peers of the same age and gender, although they are generally chubbier, shorter, and appear younger than their age (2,3)

Growth hormone , known as "somatotropin," is an anabolic hormone produced and secreted from somatotroph cells which is found in the anterior lobe of the pituitary gland (4) ; GH regulates various metabolic aspects, including glucose homeostasis, fat mobilization, and oxidation; it also promotes protein synthesis and cell proliferation, as well as tissue and body growth(5).

Growth hormone deficiency is a relatively rare cause of short stature. Before evaluating a short child for GHD, commoner causes such as physiological (familial short stature or constitutional delay of growth and puberty), hypothyroidism, small for gestational age , chronic systemic disease, celiac disease, Turner syndrome, or skeletal dysplasia need to be considered and appropriately ruled out (6).

Asprosin is a fasting-induced gluconeogenic protein hormone mainly synthesized and released by white adipose tissue, that was discovered and first identified as a novel glucogenic protein adipokine by Romere *et al*. in a study of Neonatal progeroid syndrome patients in 2016 (7). The central nervous system, peripheral tissues, and organs all have a complex role for asprosin.

Appetite, glucose metabolism, insulin resistance, cell death, and other processes are all affected by it (8).

Ceramide (Cer) is a hydrophobic backbone and the precursor for all complex sphingolipids (9). Sphingolipids play a role in a number of physiological processes, including skin barrier formation, myelin maintenance, immunity, blood vessel stabilization, recognition of bacteria, bacterial toxins, and viruses, insulin resistance, spermatogenesis, and auditory sense formation (10–15).

Malondialdehyde is a highly reactive three-carbon dialdehyde formed as a result of the depletion of antioxidant systems during the peroxidation of polyunsaturated fatty acids by reactive oxygen species. MDA comes in two forms: endogenous (from lipid peroxidation) and exogenous (from diet) (16–18).

1.1 short stature

Short stature (SS) in children is defined as a height of less than two standard deviations (-2SD) or less than the third percentile for age, gender, and appropriate population reference from pubertal stage. This is not to be confused with poor growth, which is defined as a height velocity (cm/year) that is less than two standard deviations below the predicted velocity for the age (19).

Short stature is a common problem in children around the world, particularly in developing countries, when compared with well-fed and genetically relevant population (20).

There are two types of short stature, proportionate short stature (PSS) and disproportionate short stature (DSS). PSS is identified when a person's limbs and trunk heights are in proportion, whereas DSS is diagnosed when this proportion is missing and the person's sitting and standing heights are vastly different.

Depending on the cause, different terms are used to characterize short stature. These include idiopathic short stature, familial short stature, “constitutional short stature, constitutional delay of growth adolescence , etc. (21).

1.1.1 Etiology of short stature

There are many causes of SS including renal disease, cancer, pulmonary disease, cystic fibrosis, heart disease, and other chronic diseases can all cause short stature. Coeliac disease is an excellent example of a treatable cause of short stature, particularly in younger children. Malnutrition and therapies like glucocorticoids, chemotherapeutic drugs, radiotherapy can result in short stature.

Common endocrinological causes of short stature include hypothyroidism, hypopituitarism (isolated growth hormone deficiency or multiple anterior pituitary hormone deficiencies), hypercortisolism and classical Laron syndrome (20).

1.1.2 Epidemiology of Short Stature

According to the definition, 97.5 % of the population is of normal or tall stature, while just -2 SD, or 2.5 % of the population has short stature. The global prevalence of SS is 3% (22). The SS prevalence in Saudi children and adolescents is considered to be similar to the global prevalence, but the exact prevalence is not known (23).

The prevalence of short stature in Jordan was 4.9% (24); and the prevalence of growth hormone deficiency in children with short stature was 32.2% (25).

By using the 1978 World Health Organization (WHO), the 2000 Centers for Disease Control (CDC), and the 2007 WHO growth references, the prevalence of moderate short stature was 12.1%, 11%, and 11.3% in boys and 10.9%, 11.3%, and 10.5% in girls, respectively.

In West Bank, Palestine stated that by studying the prevalence of SS in school children aged 13–15 years in Ramallah, it was found that 9.2% of boys and 7.3% of girls were suffering from SS; and in Hebron, 9.4% and 4.2%, in boys and girls, respectively (26).

In China the prevalence of SS was 2.23% in urban versus 5.12% in rural areas; 2.60% in developed, 3.72% in intermediately developed, and 4.69% in underdeveloped regions (27).

The prevalence of SS in a rural region of South Africa was between 5% and 7% in children and adolescents aged 5–20 years (28). Turkey study reported a prevalence of 5.7 % in school children aged 6–16 years (29). According to a Pakistani study, the prevalence of SS was 16.5% between the ages of 6 and 12 years (30).

In South India a study recorded the prevalence rate of 2.86% in children in school. The study examined 15644 children, out of which 448 (2.86%) had short stature. Only 9.20% of children were reported to have short stature caused by growth hormone deficiency (31).

1.2 Growth Hormone

Growth hormone (GH), also known as "somatotropin," is an anabolic hormone produced and secreted by the anterior lobe of the pituitary gland's somatotroph cells (4); GH regulates various metabolic aspects, including glucose homeostasis, fat mobilization, and oxidation; it also promotes protein synthesis and cell proliferation, as well as tissue and body growth (5).

1.2.1 Growth Hormone Chemistry

Growth hormone is a 191 amino acids , single polypeptide and there are two disulfide bridges (amino acids 54 and 165, and amino acids 182 and 189) (32). Growth hormone is made up of four main α-helices and three mini-helices within the connecting loops .Two circulating forms of GH are present: a 22-kDa form that is a 191 amino acid chain (full-length GH) that represents 85% to 90% of circulating GH, and a 20-kDa GH that lacks amino acids 32 through 46 (33),as in Figure 1-1

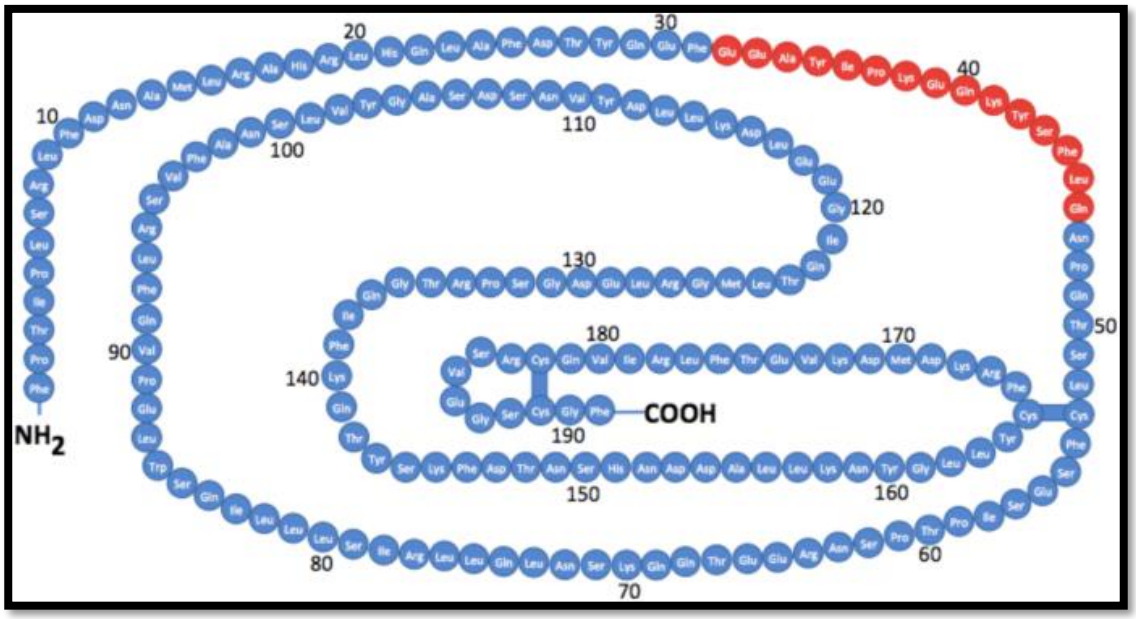


Figure 1-1 The 22 KDa-GH isoform molecule with 191 amino acids (34).

1.2.2 Growth Hormone Gene

In humans, the growth hormone (GH) family is located in the chromosome 17 includes the pituitary HGH (referred as HGH- N), the placental HGH (named HGH-V) and the chorionic somatomammotropin CSH; along with prolactin which are collectively referred as somatolactogens (35,36), as in Figure 1-2.

HGH-N has been attributed metabolic and somatogenic functions after birth, HGH-V has been attributed metabolic and somatogenic functions during pregnancy, and CSH is considered a lactogenic hormone (36).

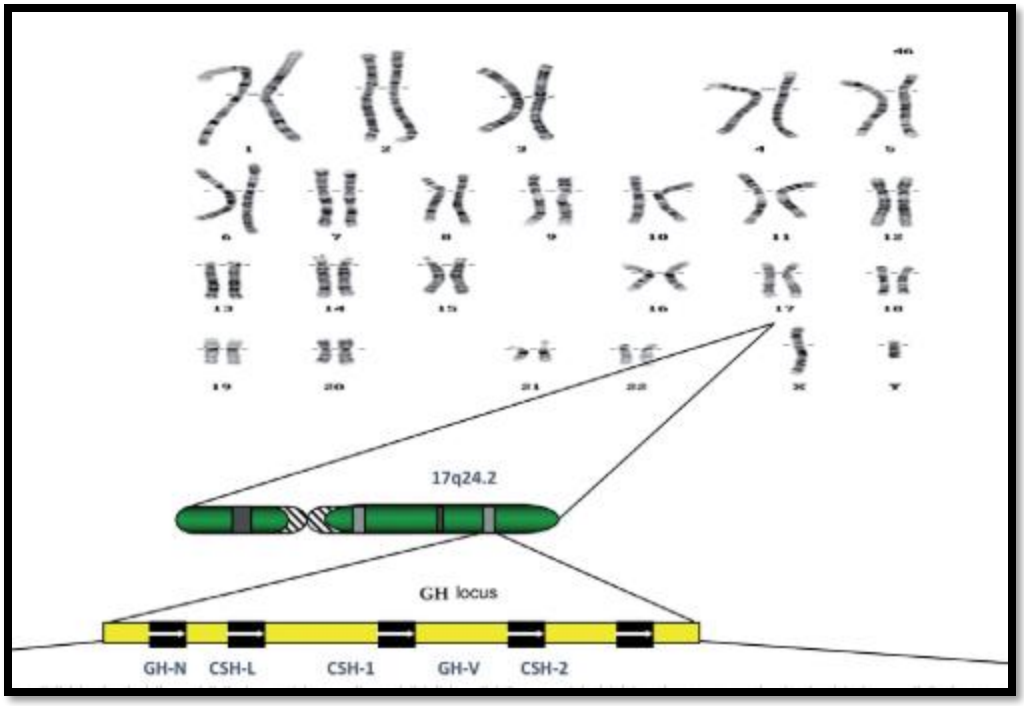


Figure 1-2 The location and arrangement of growth hormone gene family (36).

1.2.3 Growth Hormone Biosynthesis

The somatotrophs of the adenohypophysis (anterior pituitary) are the major producers and secretors of GH. The activation of G-protein coupled receptors (GPCRs) in somatotrophs is usually the first step in modulating these processes (37–39), as in figure 1-3.

The intracellular attachment of a heterotrimeric G protein (composed by α, β and γ subunits) to a transmembrane GPCR is induced by the extracellular binding of hypothalamic growth hormone-releasing hormone (GHRH) to a transmembrane GPCR (37–39). The dissociation of the G protein and GPCR is induced by the binding of guanosine triphosphate (GTP) to the G protein. As a result, Gα and Gβγ-subunits become disconnected (40).

In the case of a growth hormone stimulator, the activated Gα-subunit (Gα), in turn, stimulates the adenylyl cyclase (AC) activity (41). So the subunit involved is recognized as a stimulatory Gα (Gαs). The binding of a GH suppressor (somatostatin), on the other hand, activates an inhibitory Gα-subunit (Gαi), which reduces AC activity (39,41).

Adenylyl cyclase catalyzes the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP) (42). The rise in cAMP levels after AC activation allows cAMP to bind to the two regulatory subunits of the tetrameric protein kinase A (PKA), allowing dissociation and activation of the two PKA-catabolic subunits (38).

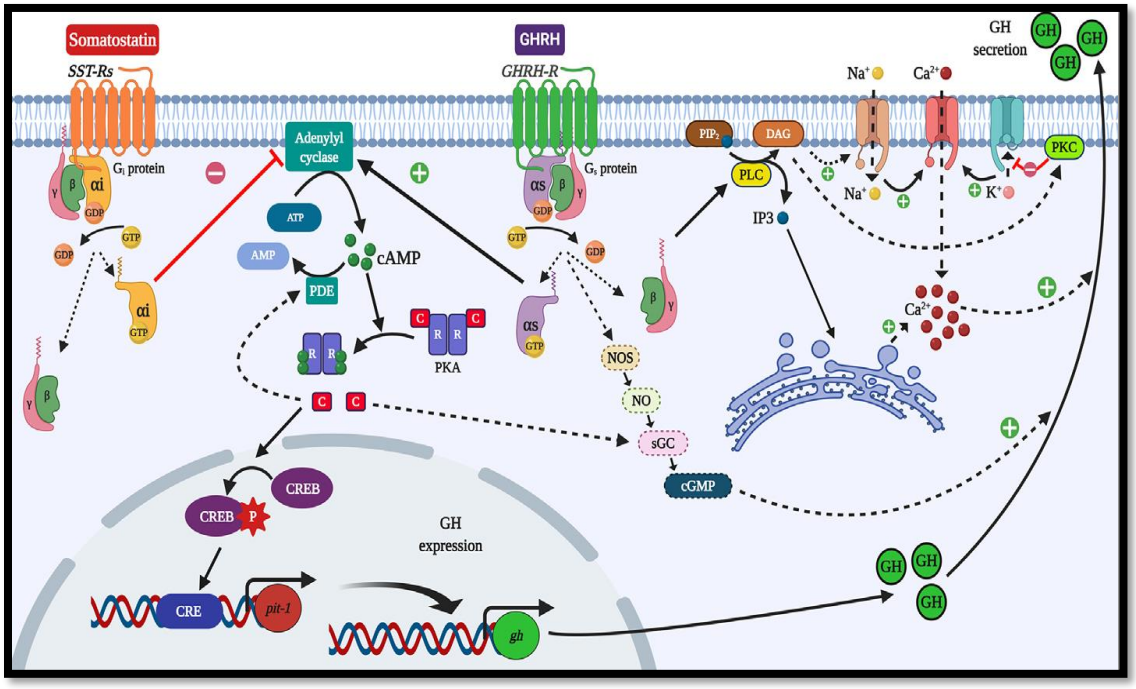


Figure 1-3 A simplified review of the cAMP/PKA/CREB pathway's role in somatotroph GH production and secretion (43). Protein kinase A (PKA), Protein Kinase C, (PKC) Protein lipase C (PLC), Nitric oxide synthesis (NOS).

These activated catabolic subunits can then act as serine-threonine kinases, phosphorylating a variety of substrates such as membrane, cytosolic,

and nuclear proteins (38,42). The cAMP-responsive element-binding protein CREB stands out as a major modulator of cAMP-PKA-dependent transcriptional regulation in somatotrophs among these target substrates (39,44). PKA phosphorylates CREB at Ser-133, allowing it to attach to the cAMP response element (CRE) (38).

The cAMP-responsive element increases the expression of the GH gene by acting as a transcription factor for a variety of cAMP-regulated genes, including pituitary-specific positive transcription factor 1 (pit-1) (39,45).

1.2.4 Regulation of Growth Hormone Secretion

The somatotroph cells of the anterior pituitary release GH in a pulsatile way. The hypothalamic hormones growth hormone-releasing hormone (GHRH; positive regulation) and somatostatin (somatotropin releasing inhibiting factor; SRIF; negative regulation) are the primary regulators of release (46).

Growth hormone releasing hormone is a peptide hormone that activates the cAMP signaling pathway in somatotroph cells by interacting with a G protein-coupled receptor (GHRHR). This results in increased GH mRNA transcription and release. The influx of extracellular Ca^{+2} causes the release of GH from secretory vesicles when GHRHR signaling is activated in somatotroph cells (46).

Growth hormone secretion is negatively regulated by a complex series of short and long feedback loops. The key regulator is insulin-like growth factor 1 (IGF1), which is secreted from target tissues, particularly the liver. Increased serum GH and IGF1 trigger feedback loops that inhibit

GHRH, release somatostatin and as a result inhibit pituitary GH secretion (47,48).

Ghrelin, a GH secretagogue produced primarily by the stomach's endocrine cells, and also by the gastrointestinal tract and the hypothalamus, influences GH secretion (49), as in figure 1-4.

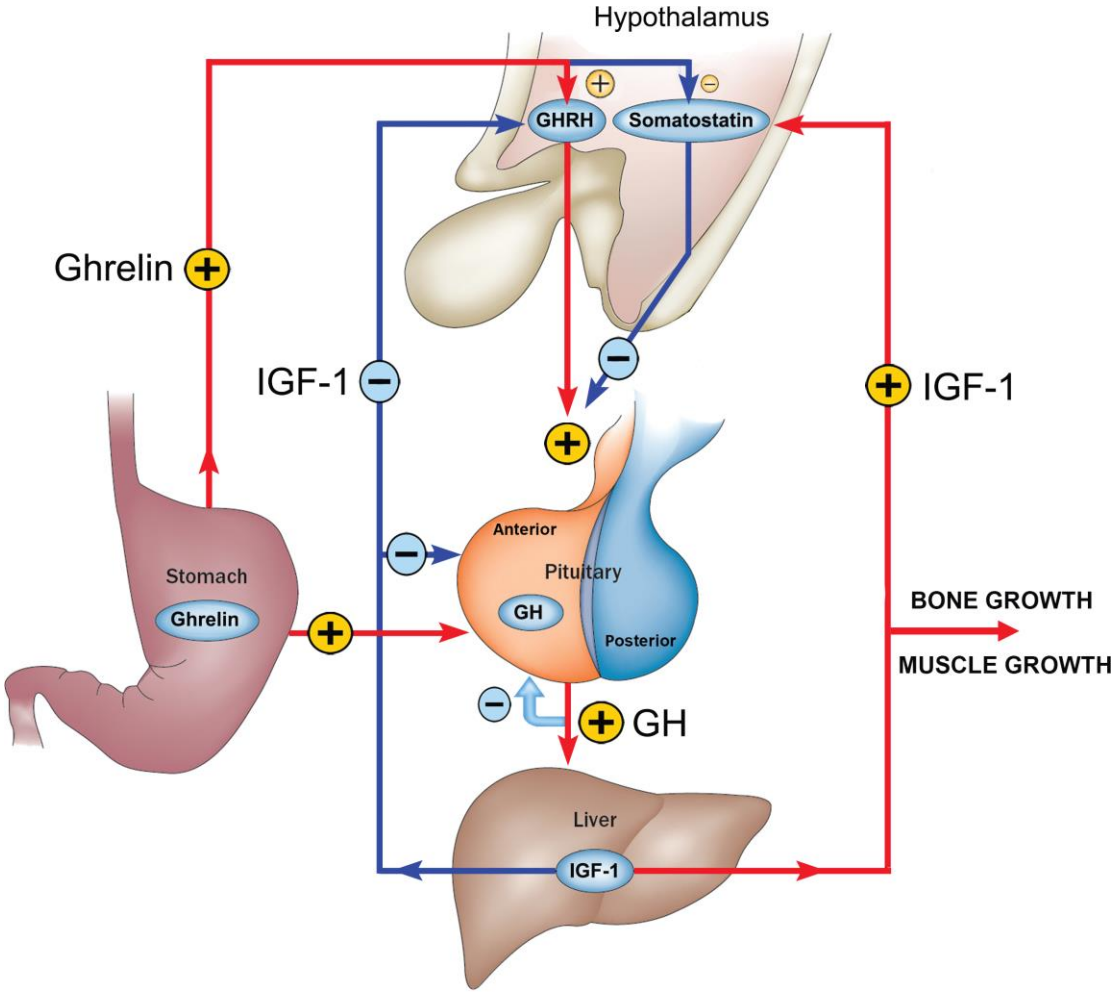


Figure 1-4 Regulation of growth hormone secretion (50).

Furthermore, leptin, an anorexigenic peptide found in adipocytes, the pituitary, and other brain regions, has been identified as an important modulator of GH secretion. Depending on whether the effect is short-term or long-term, leptin has varied effects on GH secretion (51). Short-term effects

of leptin enhance GH secretion, mostly through an increase in GHRH and a decrease in significantly SRIF expression, whereas long-term effects reduce GH secretion, probably due to a reduced response to GHRH (33).

Further endogenous and exogenous factors that influence the secretion of GH have been identified. Examples of such hypoglycemia, insulin, clonidine, arginine and levodopa , some of which have been used clinically to identify potential pituitary insufficiency (33).Other key stimuli for secretion include nutrition, exercise and the onset of deep sleep (52,53).

1.2.5 Growth hormone binding protein

In humans, slightly less than half of the GH in circulation is linked to high molecular weight carrier proteins. To date, two GH binding proteins have been found: one with a high affinity that contains the GH receptor's extracellular domain, and another with a low affinity that has been identified as α-2 macroglobulin and may be of less importance (54). Although the function of GHBP is unclear, it is thought to be involved in adjusting the availability and activity of circulating GH. The results indicate that GHBP blocks GH from binding to the GHR and consequently inhibits GH's biological function. GHBP, on the other hand, significantly extends the half-life of GH in plasma and has even been shown to improve GH activity in some circumstances (55).

1.2.6 Growth hormone receptor signal transduction

The GHR is a type I cytokine receptor that lacks intrinsic kinase activity and requires the activation of Janus kinase 2 (JAK2), a non-receptor tyrosine kinase (56,57). as in figure 1-5.

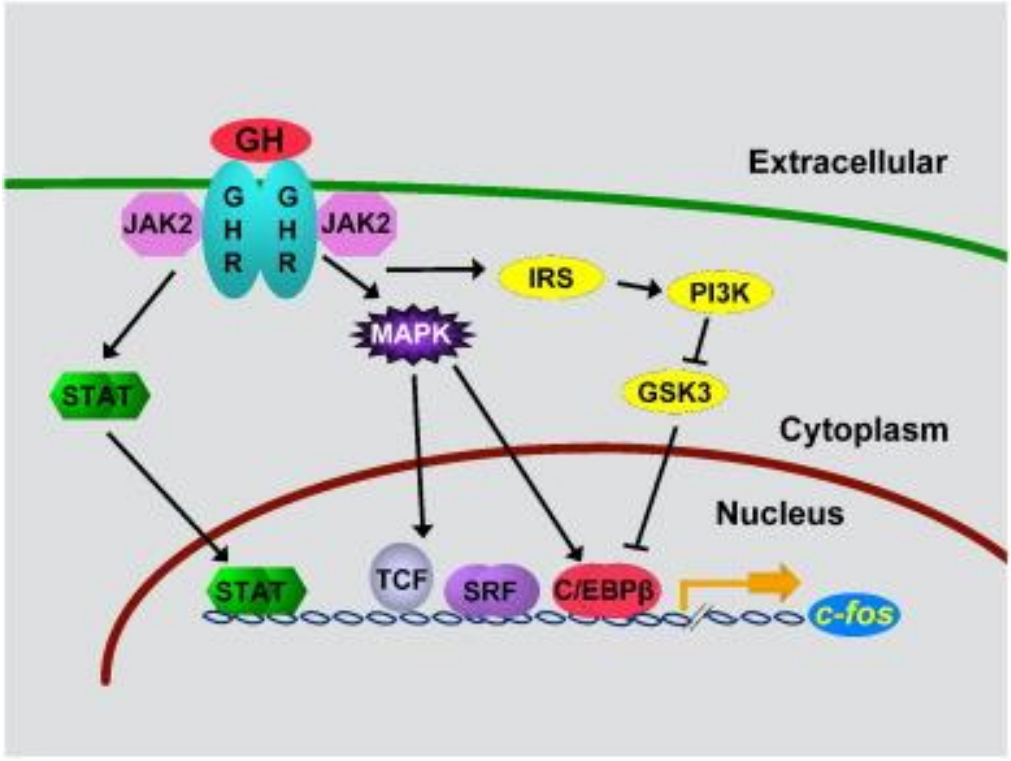


Figure 1-5 Growth hormone receptor signal transduction (58).

GHR signal transduction is mediated by these kinases (56). The GH ligand binds with a predimerized GHR homodimer via two binding sites with differing affinity for the receptor. Binding causes a rotational change in the receptor's transmembrane domain, which causes two JAK2 molecules linked with the receptor's cytoplasmic domain to be transphosphorylated and activated (59,60). Phosphorylated JAK2 then phosphorylates tyrosines in GHR's cytoplasmic domain, allowing signaling molecules to be recruited to the receptor. The JAK-STAT (signal transducer and activator of transcription) pathway is the principal signaling pathway triggered by GH. STAT1, 3, 5a, and 5b are the STAT molecules that are activated by GH signaling. Phosphatidylinositol 3-kinase (PI3 K) and mitogen-activated protein kinase (MAPK) signaling pathways, as well as Src homology 2 (SH2B1), a scaffold

protein that interacts with JAK2 and causes GH-induced cytoskeleton alterations, are also used (57).

Following activation, the GHR has been seen to rapidly translocate to the nucleus, but its function there is unclear. GHR signaling is suppressed by suppressor of cytokine signaling proteins 1–3 (SOCS1–3), which are negative regulators that aid in the ubiquitination and destruction of the receptor after it has been activated. Dephosphorylation of the receptor by numerous protein tyrosine phosphatases and protein inhibitors of active STATs (PIAS) also results in its downregulation (56).

1.2.7 Growth hormone physiological function

The primary function of GH is to promote postnatal longitudinal growth. The main action of pituitary-derived GH is the stimulation of hepatic insulin-like growth factors (IGFs) (61).

The pulsatile secretion pattern of GH is the most important regulator of circulating insulin-like growth factor 1 (IGF-1), whose bioavailability is more stable due to the fact that most of the circulating IGF-1 is bound with IGF-1 binding proteins (62). The liver is mainly responsible for maintaining circulating IGF-1 levels. As a result, activation of the GH receptor (GHR) in hepatocytes induces IGF-1 synthesis and secretion, as well as raising serum levels of the hormone (62,63)

The GH/IGF axis regulates physiological processes such as growth stimulation, protein synthesis, cell proliferation, and metabolism in a variety of target tissues, including muscle and adipose tissue (61), as in figure 1-6 .

It promotes bone formation and regulates lipid, carbohydrate, nitrogen, and mineral metabolism as well as electrolyte homeostasis (46,64).

The immune system, cardiovascular system, neurogenesis, and central nervous system, as well as aging, are all affected by GH (65–68).

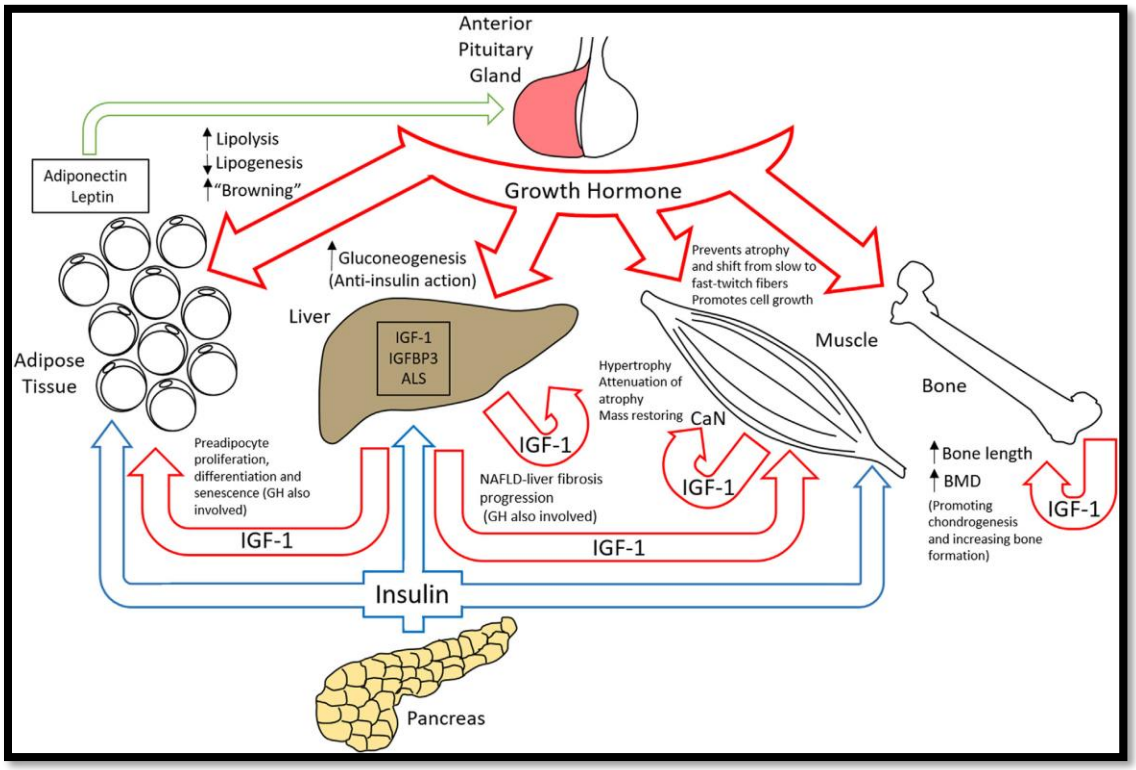


Figure 1-6 Organ-specific roles of the GH/IGF axis (69).

The sites of action of GH include the following:

- Epiphysis: Stimulation of epiphyseal growth.
- Bone: Stimulation of osteoclast differentiation and activity, stimulation of osteoblast activity, and increase of bone mass by endochondral bone formation (70).
- Adipose tissue: Acute insulin-like effects, followed by increased lipolysis, inhibition of lipoprotein lipase, stimulation of hormone sensitive lipase, decreased glucose transport, and decreased lipogenesis (71).

- Muscle: Increased amino acid transport, increased nitrogen retention, increased lean tissue, and increased energy expenditure (71).

1.2.7.1 Insulin-like growth factor 1 (IGF-I)

IGF-I initially known as somatomedin C, since it was ‘mediating the effects of somatotropin’(33). IGF-I is a single-chain polypeptide with 70 amino acids. IGF-II is made up of 67 amino acids and has a similar structure to IGF-I. IGFs are comparable to insulin in that they have A, B, and C domains, just like proinsulin. The C domain of IGFs is active, unlike the C domain of insulin, which is eliminated during the processing of proinsulin. A carboxyterminal D domain is also present in IGF-I and -II (72).

About half of the amino acid sequences of IGF-I and IGF- II are shared with proinsulin (72). They are ligands for the same receptors, but with different affinities, and share some similarities in their actions. Insulin's metabolic function is mediated by the insulin receptor (IR), whereas IGFs' mitogenic effects are mediated by the IGF-I receptor (IGF-IR). Both the IR and the IGF-IR are tyrosine kinase glycoproteins found on the cell surface. IGF-I binds with high affinity to the IGF- IR, and with low affinity to the IGF-IIR. It also binds with low affinity to the IR, thus inducing insulin-like actions (73).

IGF-I and IGF-II are growth factors produced by a variety of tissues. The liver is the primary source, but synthesis has also been found in the kidneys, ovaries, testes, placenta, pancreas, skin, and lungs. They are endocrine polypeptide hormones that have local autocrine and paracrine actions in IGF-producing tissues. IGF-I in the free circulation mediates several of the effects of pituitary GH, which in turn controls IGF-I production

and release (72). More than 75% of IGF1 binds to the IGF binding protein 3 (IGFBP3) with high affinity, and this complex is then maintained by Acid Labile Subunit (ALS), leads to an increased half-life of 16 hours (74).

1.3 Growth Hormone Deficiency

Growth hormone deficiency (GHD) is classically defined as insufficient GH secretion that results in a decrease in the production of GH-dependent hormones and growth factors, such as insulin- like growth factor-I (IGF-I), IGF-II and their binding proteins (IGFBPs) (1). When the pituitary gland does not secrete enough growth hormone to promote the body's growth, it usually appears as a slow or flat rate of growth in both early and late childhood (75). When compared to peers of the same age and gender, children with GHD have appropriate body proportions, although they are generally chubbier, shorter, and appear younger than their age (2,3).

1.3.1 Classification and Etiology

Growth hormone deficiency may be isolated or combined with other pituitary hormone deficiencies (CPHD, combined pituitary hormone deficiency) and may be congenital or acquired (76). As shown in Table 1-1.

Acquired GHD may be secondary to hypothalamic-pituitary damage at birth or intracranial neoplasm (i.e. craniopharyngioma), infiltrative diseases (i.e. Langerhans cell histiocytosis), infections (i.e. tuberculosis, HIV), trauma, cranial or total body irradiation (TBI) and chemotherapy.

Table 1-1 Etiology of growth hormone deficiency (6).

Congenital
Genetic • Multiple pituitary hormone deficiencies: Mutations in *HESX1*, *LHX3*, *LHX4*, *SOX3*, *GLI2*, *PROP1*, *PITX2* and *PIT1*genes. • Isolated GH deficiency: Mutations in *GH1*, *GHRH* and *GHRHR* genes **Congenital cranial malformations** • Holoprosencephaly, schizencephaly, septo optic dysplasia • Syndromic: Pallister Hall syndrome, Rieger syndrome, Prader Willi syndrome
Acquired
Tumors • Benign: Craniopharyngioma, arachnoid cyst, pituitary adenoma, Rathke's cleft cyst • Malignant: Dysgerminoma, meningioma, glioma, metastatic Hodgkin's Disease **Trauma:** • Surgical, skull fracture, birth injury **Inflammation:**

- Histiocytosis, sarcoidosis, tuberculosis, meningitis, hemochromatosis, autoimmune hypophysitis, Pituitary apoplexy Irradiation.

The term “idiopathic” which mean a clear cause for pituitary GHD is often not identified, although some of these patients may actually have undiagnosed gene defects in GH production/secretion or have first manifestations of combined pituitary hormone deficiencies (77).

In most cases, GHD is “idiopathic” and only in 20% of patients an organic cause is identified. Among idiopathic cases, abnormalities in magnetic resonance imaging (MRI) of hypothalamic- pituitary region are frequent (pituitary hypoplesia, lack of pituitary stalk, ectopic posterior pituitary) (78).

In some cases of GHD, an autoimmune origin may be hypothesized based on the detection of circulating anti-pituitary antibodies directed against GH-secreting cells. Anti- pituitary antibodies have also been detected in some patients with idiopathic short stature, who subsequently showed impaired GH secretion suggestive of a particular type of acquired GHD (79).

1.3.1.1 Genetic Defect in Isolated GHD

Gene defects have been associated with GHD in Table 1-2. GH (GH1) and GH releasing hormone receptor(GHRHR) genes have been reported to have mutations. Classic GHD (types IA, IB, and II) or bio-inactive GH syndrome (normal or increased circulating not active GH levels) can be caused by GH1 mutations (80). Anti-GH antibodies can form during GH therapy in patients with homozygous GH1 deletions, which are a prevalent

cause of type IA GHD. Type IB GHD is a less severe form caused by GH1 or GHRHR mutations, whereas GHD type II is a dominant form caused by exon 3 skipping, resulting in the secretion of a GH isoform (17.5 kDa) with a dominant negative effect (81).

Furthermore, X-linked type III GHD is linked to agammaglobulinemia and has been linked to BTK and *SOX3* gene mutations (82). Mutations in the gene encoding the ghrelin receptor (GHSR), which decrease GH production, could also be a cause of GHD (83).

Finally, a number of studies have linked isolated GHD to congenital syndromes caused by mutations and/or deletions in genes that aren't involved in growth (i.e. biallelic mutations in RNPC3, compound heterozygosity for *IFT172* or mutation of *ALMS1*) (84–86).

Table 1-2 Gene defects associated with isolated GHD.

Disorder	Gene(s)	Inheritance	Clinical Features/GH levels	References
Isolated GHD type IB	GHRHR	AR	Low serum GH	(87,88)
Isolated GHD type IA	GH1	AR	No serum GH	(87,88)
Isolated GHD type IB	GH1	AR	Low serum GH	(87,88)
Isolated GHD type II	GH1	AD	Variable height deficit and pituitary size; low GH levels; other pituitary deficits can develop	(87,88)
Isolated GHD type III	BTK, SOX3	XLR	Low GH levels with agammaglobulinaemia	(87,88)
Isolated partial GHD	GHSR	AR, AD	Variable serum GH and IGF-I	(83,89)
Almstrom syndrome	ALMS1 RNPC3 IFT172	AR AR AR	50% of cases are GHD. Severe GHD, hypoplasia anterior Pituitary. Functional GHD, retinopathy,	(84) (85) (86)

			metaphyseal dysplasia, hypertension.	

AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive.

1.3.2 Clinical Features of Growth Hormone Deficiency

In patients with deficient GH production (GHD), growth velocity is often slow in childhood. There is conflicting research about whether a child with GHD has a normal birth length. The length standard deviation (SD) score of 46 babies with congenital GHD was normal at delivery but decelerated in infancy, according to a study done by (Pena-Almazan *et al.)* in New York (90).

Patients with an ectopic posterior pituitary with a thin or interrupted pituitary stalk have been reported to have increased jaundice, hypoxia, hypoglycemia, and breech births (91).

Males with microphallus may also have gonadotropin insufficiency; however, even without gonadotropin deficiency, the penis may be small (92).

In congenital GHD, the patient's bone age is often delayed compared to his or her chronological age. The proportions of the patient's skeleton will be related to their bone age rather than their chronological age. When comparing linear growth to weight, patients tend to be in the lower percentiles for age. They may appear ‘chubby’ or have a ‘cherubic’ facial appearance with flat nasal bridge and midface hypoplasia. The patient's voice may sound younger than his or her chronological age, and the patient may have sparse/thin hair, delayed closure of the anterior fontanelle, delayed dentition, and delayed puberty. Midline defects might include a single central incisor, lip or palate malformations, corpus callosum absence, or heart issues. Gross motor milestones may be delayed as a result of GH's effect on muscle strength. These

patients have severe short stature (-4.7 to -3.1 SD below mean) when they do not receive GH replacement (93).

1.3.3 Diagnosis of GHD

Clinical and auxological assessment, radiological evaluation, and biochemical tests for the pituitary-IGF axis should all be used to diagnose GHD in children. Genetic testing is sometimes required (1).

1.3.3.1 Clinical and Auxological Assessment Growth

Growth hormone deficiency can strike at any age (from infancy to puberty), with a wide range of symptoms. Males with isolated GHD typically have hypoglycemia, persistent jaundice, and microphallus and/or cryptorchidism (1).

The most prevalent symptoms of idiopathic GHD in pre-pubertal short children include SS and slow growth for their age. Immature faces, large foreheads, depressed midline development, single central incisor, optic nerve hypoplasia, thin and sparse hair, sluggish nail growth, high-pitched voice, low muscular bulk, increased subcutaneous fat, and low-density lipoprotein cholesterol are phenotypic markers of severe GHD (1).

Before beginning endocrinological assessment in a child with short stature, other causes of growth failure should be ruled out, such as hypothyroidism, chronic systemic diseases, Turner syndrome, malnutrition, or skeletal problems. According to the GH Research Society's Consensus Guidelines for Diagnosis and Treatment of GHD in Childhood and Adolescence from the 2000 meeting, statement criteria to start evaluation for GHD are as follows: (94),

- Severe SS (height <3 SD below the mean);

- Height less than -1.5 SD below the mid-parental height;
- Height less than -2 SD below mean and either height velocity less than -1 SD below mean over the past year or decrease in height SD of more than 0.5 SD over the past year;
- In the absence of short stature, height velocity less than -2 SD below mean over 1 year or less than -1.5 SD below mean over 2 years;
- Signs of intracranial lesion;
- Signs of combined pituitary hormone deficiency; and
- Neonatal signs and symptoms of GHD, including hypoglycaemia, prolonged jaundice, microphallus and/or cryptorchidism, cranial midline abnormalities.

Reduced bone mineralization and decreased bone density are common in growth hormone-deficient patients, resulting in delayed skeletal maturation. However, according to a study by Hogler and collaborators, cortical and trabecular densities in GHD children are normal, and the risk of fracture is not raised (95).

1.3.3.2 Radiological Investigations

An x-ray of the left wrist and hand is used to evaluate bone age in children above the age of one year (94). Although the results may not always be correct in 2- or 3-year-old children, bone age can be determined more precisely from X-rays of the knee and foot, as in the neonate. The severity and duration of GHD affects delayed bone maturation (96).

The most common method for visualizing hypothalamic-pituitary anatomy is magnetic resonance imaging. In short children, neuroimaging may be used to rule out a tumor, such as a craniopharyngioma or an optic nerve

glioma. An ectopic posterior pituitary gland, anterior pituitary hypoplasia, and a thin pituitary stalk are the most common radiological findings in GHD children. Many children with idiopathic GHD, on the other hand, have no pituitary problems (97).

1.3.3.3 biochemical Tests

Due to the pulsatile nature of growth hormone release in the body, diagnosing GHD can be difficult. A single measurement of growth hormone taken at random is rarely diagnostic. Due to overlapping results in healthy and GHD patients, biochemical growth hormone markers such as insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) have limited diagnostic utility (98).

The insulin tolerance test (ITT), the growth hormone-releasing hormone (GHRH), arginine, glucagon, levodopa, and clonidine stimulation tests are some of the GH stimulation tests (Dynamic Tests) used to identify GH deficiency (99),see Table (1-3) .One reliable growth hormone stimulation test is enough to make the diagnosis, according to the Growth Hormone Research Society (23).

In the United States, an insulin tolerance test (ITT) is the gold standard stimulation test for adults with GHD (98). The cut-off point for the diagnosis is a GH level of 3 to 5 ng/mL during acute hypoglycemia, (i.e., glucose less than 40 mg/dL). However, ITT is not recommended for patients with coronary artery disease or epilepsy. One of the most significant disadvantages of this test is that it necessitates continuous monitoring due to potential side effects such as seizures and loss of consciousness.

The glucagon stimulation test (GST) is an acceptable alternative test to ITT. GST stimulates GH more effectively than other stimulation tests, such as clonidine. Furthermore, intramuscular or subcutaneous administration of GST is more effective than intravenous route. GST's key benefits are reproducibility and safety. The procedure unusually extensive duration, up to four hours, is considered a disadvantage of the test (98).

The clonidine stimulation test (CST) is the test of choice for identifying GHD in children (100). Because the GH response peak time might be early or late, there is no fixed peak time with CST in clinical practice. The GH response is monitored at intervals of 0, 30, 45, 60, and 90 minutes after an oral dose of 0.15 mg/m^2 of clonidine is given. It can be costly to measure the GH response at different periods. According to Thakur et al., the 60-minute post-CST assessment is the most important for excluding GHD (79.5% specificity). The 90-minute addition increases the test's specificity to 92.3% (100).

Somatostatin release is inhibited by arginine, which promotes GH secretion. Arginine HCl (0.5 g/kg to 40 g) is given intravenously during a 30-minute period. At 0 (baseline), 30, 60, 90, and 120 minutes, blood samples for GH measurement should be obtained. At 60 minutes after starting the arginine infusion, the maximum GH peak should occur. The adverse effects of nausea and vomiting are very common (1).

Table 1-3 Dynamic Tests for Diagnosing GH deficiency Hormone (101).

GHD stimulation test	GH measurements	Administration	Observations
Insulin tolerance test	5 GH measurements between 0-120 min	Intravenous	Associated with a high rate of false positive when used as a first test; risk of hypoglycemia
Clonidine	4 GH measurements between 0-90 min	Oral	Hypotension and drowsiness
Glucagon	5 GH measurements between 0-3 h	Intramuscular	
Levodopa	5 GH measurements between 0-120 min	Subcutaneous/ Intramuscular	Vomiting and headache
Arginine	5 GH measurements between 0-120 min	Intravenous	Suggested to be a better option compared with ITT (lower rate of false positives when used as a first test). Not widely available
GHRH	5 GH measurements between 0-60 min	Intravenous	Not widely available

GHRH, however, stimulates the pituitary gland directly and may result in false negatives in patients with disorders in the hypothalamus or a history of radiation treatment (99).

Levodopa stimulates GH through dopamine receptors in the hypothalamus. Macimorelin an oral GH secretagogue receptor-1a agonist that was recently approved in the United States and Europe, stimulates GH release with similar sensitivity (92%) and specificity (96%) to ITT, and has fewer side effects(102). although a severe side effect reported was long QT interval on electrocardiography. Macimorelin is not yet introduced in South Korea (99).Conventionally peak GH values below 7 µg/L or 10 µg/L after stimulation tests are considered as indicative of GH deficiency (6).

IGFs are peptide factors that mediate the activities of GH and are GH-dependent. Because the levels of IGFs remain constant throughout the day, it

may be examined with a single measurement. However, IGF-1 lacks specificity in the pediatric age group, as a lower level could indicate other endocrine and nutritional problems. Furthermore, the patient's age and pubertal status have a considerable impact on it, as it may overlap with normality. A number of studies have found that IGF-1 level in children do not correlate with GH levels. In practice, a single IGF-1 and IGFBP-3 measurement, together with a single GH stimulation test, yields more information about the situation. A second stimulation test confirms the distinction between GHD and a normal short stature in the case of a subnormal GH stimulation test result (23).

1.3.3.4 Genetic Screening

Between 3-30% of the cases with GHD are suggested to have a genetic cause, and it might be even higher, since imagistic methods, such as magnetic resonance, roughly detect 12-20% of the lesions of the hypothalamus and pituitary gland. At least four Mendelian disorders of familial isolated growth hormone deficiency have been described (type IA, IB, which are autosomal recessive, type II, which is autosomal dominant, and type III, which is X-linked) (103).

Numerous mutations in the GH gene and GHRH receptor gene have been reported in patients with congenital isolated GHD(104). A recent study published in February 2018 describes a novel mutation (c.97C>T) of the GH releasing hormone receptor gene that causes isolated GHD type IB. The variant was identified at the evaluation of a pediatric patient with severe growth failure, who expressed low GH in response to 2 stimulation tests(105). Wit et al. recommends genetic screening for GH and GHRHR mutations in children with severe isolated GHD and a family history of GHD (106).

Other genetic causes incriminated to have a role in the GHD pathology are the mutations in the Gs alpha gene leading to GHRH resistance and mutations in the gene encoding the growth hormone (GH) secretagogue receptor (GHSR)(107). Also, a wide variety of mutations in genes encoding transcription factors involved in pituitary development were reported, such as*: HESX1, OTX2, SOX2, SOX3, LHX3, PITX2, PROP1, POU1F1* and *TCF7L1*. Additionally, two *KCNQ*1 mutations have been recently described to be involved in growth hormone deficiency pathology(108). Genetic screening could obviously bring a great insight in the GHD pathology, but it remains an open question if it would be also cost effective to include it in the routine evaluation of the patients with GHD. Although major progress has been made in this area, genetic testing continues to be difficult to access, mostly because of its high costs, especially in the low-income and middle-income countries (109).

1.3.4 Growth Hormone Deficiency Treatment

Recombinant human GH (rhGH) has been used to treat children with short stature due to GHD for 35 years, resulting in a wealth of experience around the world (110–112) . The key goals are to increase height velocity and achieve adult height that is acceptable for genetic potential, as evidenced by numerous articles (110). There is a significant improvement in length and weight in children diagnosed between the ages of 0 and 3 years, with the improvement being greater in those under the age of 12 months and those with CPHD (113). Weight-based or body surface-based dosing is currently recommended at the start of treatment, followed by personalized doses depending on responsiveness, with a goal of lower doses for individuals with more severe GHD. Dose adjustments should be made every 6–12 months

based on the patient's growth response. Body surface- dosing is suggested for patients with obesity. IGF-1 levels can be measured once a year or after a dose change to track compliance, efficacy, and safety (114). For safety reasons, GH dose should be reduced if IGF-1 values exceed the upper limit of the reference range (110,115). Re-evaluation of additional etiologies of short stature, concomitant diseases, and adherence are recommended if treatment does not work (116).

A recent study found that non-adherence to GH treatment ranged from 7 to 71 percent based on a thorough review of electronic databases. Non-adherence was caused by a variety of factors, including the treatment's length, discomfort with daily injections, and forgetting to take the medication, among others (117) .

1.4 Asprosin

Asprosin is a fasting-induced gluconeogenic protein hormone mainly synthesized and released by white adipose tissue, that was discovered and first identified as a novel glucogenic protein adipokine by Romere *et al*. in a study of Neonatal progeroid syndrome patients in 2016 (7). The central nervous system, peripheral tissues, and organs all have a complex role for asprosin. Appetite, glucose metabolism, insulin resistance, cell death, and other processes are all affected by it (8).

1.4.1 Synthesis of Asprosin

Asprosin, encoded by two exons (exon 65 and exon 66) of the gene Fibrillin 1 (FBN1)(8). FBN1 is found on chromosome 15q21.1, with a length of 235 kb and a total of 66 exons (118). These exons code for a proprotein with a length of 2871 amino acids. Following that, activated protease furin

cleaves the translated proprotein at its C terminus, resulting in mature fibrillin-1 and 140-amino-acid long asprosin (8). As shown in figure 1-7.

Fibrillin-1 is a large extracellular matrix protein (350 KDa) that forms bead-on-a-string microfibrils in a periodic manner by interacting with other proteins, notably microfibril-associated glycoproteins. One of the most important roles of fibrillin-containing microfibrils is to provide a scaffold for the production of elastic fibers in elastic tissues. The C-terminal Asprosin is released into the circulation(119).Since FBN1is expressed in a variety of human tissues, white adipose is unlikely to be the only source of plasma Asprosin. Asprosin secretion from dermal fibroblasts has been documented by Romere *et al*. (7).Lee *et al*. discovered that pancreatic cells secrete Asprosin(120). Asprosin can be created by skin and pancreatic cells, according to these studies. Ugur and Aydin recently discovered asprosin in human saliva samples (121).

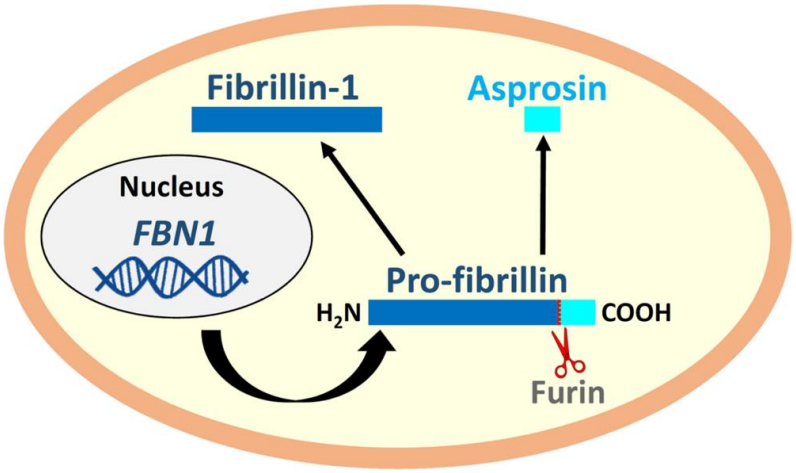


Figure 1-7 Cleavage of pro-fibrillin to produce asprosin (122).

1.4.2 Metabolic Effects of Asprosin

1.4.2.1 Glucogenic Action of Asprosin

The liver is asprosin's primary target organ, which it activates to create and release glucose (7). Asprosin stimulates glucose synthesis in the liver via the Olfactory 734 (Olfr734) receptor in both fasting and obese people, according to Li *et al.* in 2019 (123). By activating protein kinase A (PKA) in the liver via the G protein and cyclic AMP (cAMP) messenger pathway, asprosin promotes glucose release from hepatocytes (124). Furthermore, Asprosin's activity is independent of glucagon and catecholamine axis activation, which is also implicated in glucose release, according to Romere et al (7). Figure 1-8 show the action of asprosin in the liver and brain.

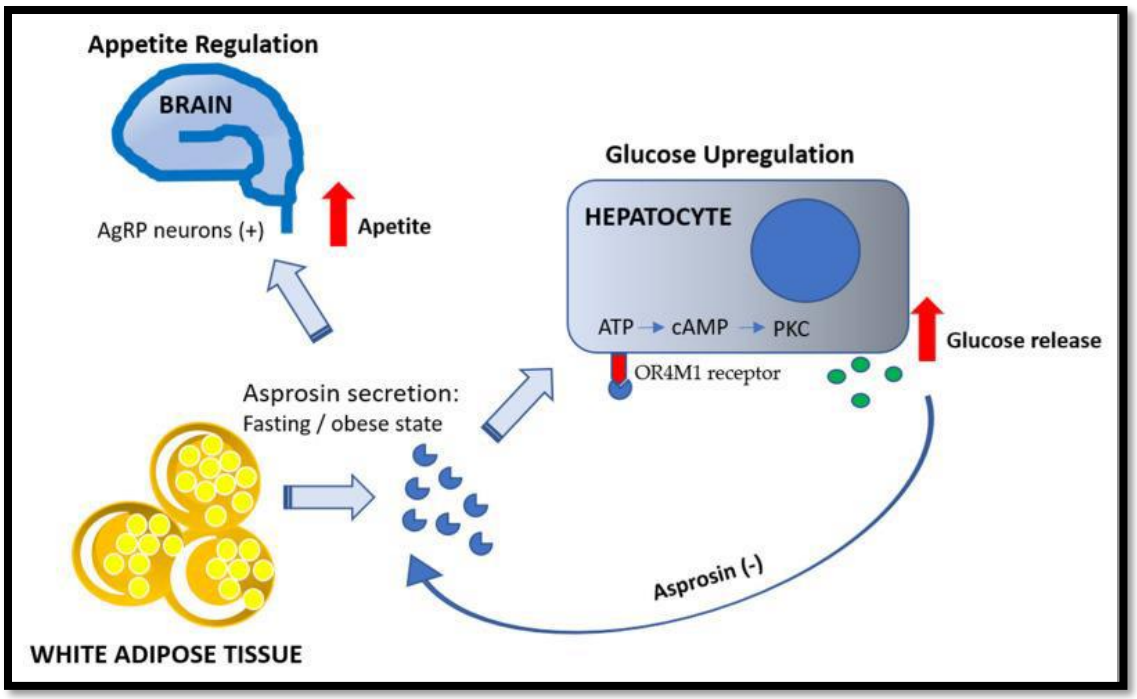


Figure 1-8 Mechanisms of asprosin action (125).

High insulin levels block asprosin's function, preventing asprosin-mediated increases in PKA activity and glucose release. Asprosin appears to

be functionally opposed to insulin, based on these findings. Furthermore, asprosin levels are inversely proportional to glucose levels, with low glucose levels (fasting state) boosting asprosin production and high glucose levels inhibiting it (feeding state) (125).

1.4.2.2 Orexigenic Action of Asprosin:

Asprosin is a hormone that has a role in appetite regulation. According to Duerrschmid *et al*. , Asprosin penetrates the blood–brain barrier, activating orexigenic agouti-related peptide (AgRP) neurons in the hypothalamus and suppressing arcuate proopiomelanocortin (POMC) anorexigenic neurons indirectly(126). The activation of the Gs–cAMP–PKA axis appears to be linked to asprosin's action. Food intake and energy homeostasis are controlled by AgRP neurons. The orexigenic neuropeptide AgRP, as well as neuropeptide Y (NPY) and GABA transmitters, are all secreted by this tiny fraction of arcuate nucleus neurons, and are all necessary for eating promotion (127).

1.4.2.3 Pathological action of asprosin

Insulin-resistant individuals and mice both have pathologically high asprosin levels (128). Besides, asprosin leads to islet β-cell secretion dysfunction and apoptosis (120) Through inflammatory mechanisms, it lowers skeletal muscle insulin sensitivity (129,130).

1.5 Ceramides

1.5.1 Ceramide Structure

Ceramide (Cer) is a hydrophobic backbone and the precursor for all complex sphingolipids (9). Cer is made up of two hydrophobic chains, a long-chain base (LCB) and a fatty acid (FA), linked by an amide bond, see Figure 1-8. The addition of a polar head group to the C1 hydroxyl group of Cer's LCB section confers amphipathic characteristics to the resulting sphingolipid. Different species have different in vivo head group kinds (10,131,132). It's either phosphocholine [in sphingomyelin (SM)] or sugar chains in mammals (in glycosphingolipids) (10,133).

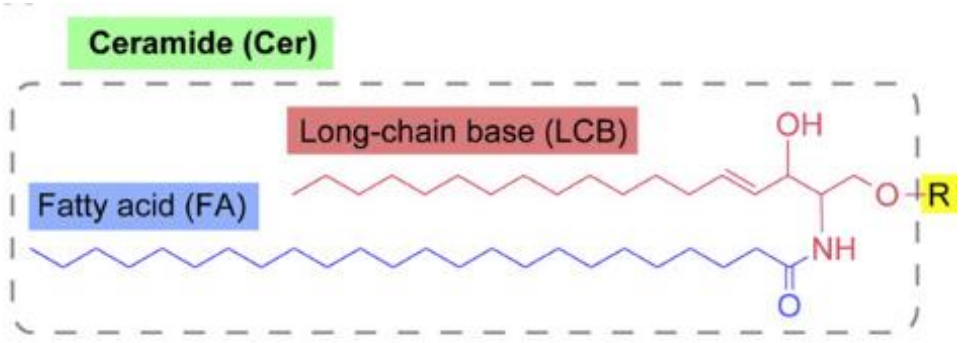


Figure 1-9. Structures of sphingolipids, R represents a polar head group: phosphocholine in SM or sugar chains in glycosphingolipids (134).

At the C1 and C3 positions, all LCBs have two hydroxyl groups, and one amino group at the C2 position. Dihydrosphingosine (dihydro-Sph; sphinganine), sphingosine (Sph), phytosphingosine (phyto-Sph), and 6-hydroxysphingosine LCBs (6-OH Sph) are the principal mammalian LCBs (135).

Ceramides have a wide range of fatty acid compositions, but they are usually lengthy. Their acyl chain lengths range from 14 to 26 carbon atoms (or greater), although the most common fatty acids are palmitic (C16:0) and stearic (C18:0) non-hydroxy fatty acids. The fatty acids are commonly saturated or mono-unsaturated (9).

1.5.2 Ceramide as Precursor for All Complex Sphingolipids

1.5.2.1 Sphingomyelin

Sphingomyelin (SM) is the most abundant eukaryotic sphingolipid and one of the plasma membrane's primary components. The chemical structure of a phospholipid determines the structural function of SM in a cell.

SM (N-acyl-sphingosine-1-phosphorylcholine) is composed of ceramide which comprises a sphingoid backbone, an 18-carbon aminoalcohol with one trans-double bond in position 4 (136), with an attached hydrophobic fatty acid chain, and polar phosphocholine or, less frequently, phosphoethanolamine residues (137).

SM levels are necessary for cell function because it is an essential modulator of plasma membrane characteristics that regulates the membrane gathering of proteins involved in cellular proliferation, growth, and apoptosis, as well as being an important source of ceramide (138,139).

1.5.2.2Glycosphingolipids

There are hundreds of glycosphingolipids with different sugar classes and/or linking modalities. Glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, fucose, and sialic acid are sugar residues found in mammalian sphingolipids (10,11,140). Glucosylceramide (GlcCer) and

galactosylceramide (GalCer) are the simplest glycosphingolipids, each of which has a glucose or galactose residue attached to Cer via β- linkage. Cerebroside, also known as mono-hexosylceramide, is made up of GlcCer and GalCer. Sulfatide is a sulfated GalCer derivative. Glycosphingolipids containing sialic acid (s) are known as gangliosides.

In mammals, intracellular sphingolipids make up about 10% of total lipids(141), Despite the fact that their concentrations differ between tissues. The plasma membrane, particularly the outer leaflet, is rich in sphingolipids (142). Sphingolipids make up 20–30% of the total lipids in the plasma membrane (141).

1.5.3 Pathways for Ceramides Synthesis

Ceramides are produced in three different ways;

- Sphingomyelinase pathway: This is a catabolic process that involves enzymes that hydrolyze sphingomyelin in the cell membrane to produce ceramides and phosphocholine, and is considered a "fast" approach to make ceramides (143–145).
- The de novo pathway: This route occurs in the endoplasmic reticulum, where serine palmitoyltransferase catalyzes the condensation of palmitate and serine to generate 3-keto-dihydrosphingosine (the rate-limiting step of the pathway). Then, sphinganine is created by 3-keto-dihydrosphingosine reductase, and dihydroceramide is produced by the action of different isoforms of ceramide synthase, which enhance the reactivity of Acyl-CoA of different chain lengths to the molecule of sphinganine. Dihydroceramide desaturase completes the process by forming ceramides by introducing a double bond in position 4–5 trans

of dihydroceramide. Ceramides are then carried to the Golgi apparatus, where they can be converted to various sphingolipids (e.g., sphingomyelin) (143–145).

- Salvage pathway: These are found in endo-lysosomes and allow sphingomyelin to be transformed into ceramides after being broken down into sphingosine by ceramide synthase.

Ceramides can be transferred to the Golgi apparatus and converted to additional sphingolipid groups in addition to these main pathways (143–145).

1.5.4 Physiological Functions

Sphingolipids play a role in a number of physiological processes, including skin barrier formation, myelin maintenance, immunity, blood vessel stabilization, recognition of bacteria, bacterial toxins, and viruses, insulin resistance, spermatogenesis, and auditory sense formation (10–15). They perform these functions through influencing cellular processes like lipid microdomain formation, apoptosis, organelle and membrane structural organization, survival, migration, signaling, intracellular protein trafficking, autophagy, adhesion, stress response, and metabolic control (13,146). Among them, Cer plays important roles in skin barrier formation by forming multi-lamellar lipids (lipid lamella) in the stratum corneum of epidermis (147). GalCer and sulfatide are enriched in myelin and are important for myelin function and maintenance (148).

1.6 Malondialdehyde (MDA)

Malondialdehyde is a highly reactive three-carbon dialdehyde formed as a result of the depletion of antioxidant systems during the peroxidation of polyunsaturated fatty acids by reactive oxygen species. MDA comes in two forms: endogenous (from lipid peroxidation) and exogenous (from diet) (16–18).

The state of imbalance between reactive oxygen species (ROS) and a biological system's ability to eliminate reactive intermediates quickly is known as "oxidative stress." The pathophysiology of many diseases has been linked to the development of oxidative stress as a result of free oxygen radical production (149).

The reaction of ROS with lipids is known as "Lipid peroxidation," which is a biological lipid oxidation, chain, and free radical process that produces peroxides of omega-3 and omega-6 fatty acids. Initiation, propagation, and termination are the three stages of this reaction, which are typical of free radical reactions and reinitiation. Reactive aldehydes such as malondialdehyde and 4-hydroxynonenal are produced by lipid peroxidation (150).

Malondialdehyde alters the physical structure of cell membranes and is involved in the creation of protein, DNA, and RNA in an indirect manner. It also has carcinogenic and mutagenic effects. For many years, MDA has been utilized as a biomarker for lipid peroxidation and oxidative stress (151).

The ability of MDA to condense with two equivalents of thiobarbituric acid to produce a fluorescent red derivative that can be measured by spectrophotometry is used in laboratory detection and quantification (18).

Aims of the study

1. Demonstrate the association of growth hormone deficiency with asprosin and ceramide level.

2. Estimate the correlation between asprosin and ceramide.

3. Assess the oxidative stress state in GHD through the level of MDA.

4. Estimate the correlation between ceramide and MDA.

2. Material and methods

2.1 Subjects

The present case-control study was conducted between 1st of October 2021 until the 1st of May 2022. The practical side of the study was performed at the laboratory of the biochemistry department in the faculty of the medicine / University of Babylon.

2.1.1 Sample Size

The sample size was determined according to the Fisher formula for sample size (152) which is:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n= sample size

Z= Z statistic for the level of confidence interval 95% which = 1.96.

P= prevalence

The global prevalence of SS is 3% (22).

d= precision (in proportion of one; if 5%, d = 0.05).

n= $(1.96)^2$ *0.03*0.97 / 0.0025 = 44

2.1.2 Study population

This study includes 103 individuals but after some mathematic calculation need to exclude 15 individuals and also to match between patients and control in age and gender the number was 88 individuals; 43 patients with Idiopathic isolated GHD (Diagnosis was made by specialist pediatric endocrinologist depending on patient's history, examination, clonidine stimulation test and pituitary MRI) and 45 apparently healthy children.

2.1.2.1 Patient Group

Forty-three patients with GHD who attend pediatric endocrine clinic in Babylon teaching hospital for maternity and children in Hilla city during the study period and met the inclusion and exclusion criteria were involved in this study. The patients group include twenty-nine child treated with recombinant human growth hormone (rhGH) and fourteen children without treatment.

Inclusion criteria

- IGHD
- Prepubertal children (as determined by specialist endocrinologist)
- Age range between (4-14) years.

Exclusion criteria

- Any subject with diabetes mellitus or other chronic diseases.
- Any subject with autoimmune diseases.
- Any subject with other endocrinopathy.

The following information were achieved through face to face interview with patients and/or their relatives: name, date of birth, sex and duration of disease. While medical record was used to get the height and weight of patients.

Body mass index (BMI) was calculated using the formula [BMI = Weight (kg) / [height]^2 (m)] and then BMI percentile, BMI Z-score and height Z-score achieved according to Centers for Disease Control chart using digital calculator (example of growth chart in appendix). A BMI that is less than the 5th percentile is considered underweight and above the 95th percentile is considered obese. Children with a BMI between the 85th and 95th percentile are considered to be overweight (example of BMI chart in appendix) (153). Height Z-score < -2 SD short; -2 SD – +2SD Normal; > +2 SD tall.

2.1.2.2 Control Group

Forty-five control subjects were involved in this study. These subjects were relatives to patients attend to the hospital were apparently healthy height , weight where checked and BMI was calculated.

All control subjects are match with patients in the age, sex, and BMI.

2.1.3 Ethical Issues

In the current study the ethical issues are based on:

1. Approval by a scientific committee of Babylon Medical College (University of Babylon, Iraq) and the Biochemistry Department in the same college.
2. Approval of the scientific committee of Babylon teaching hospital for maternity and children in Hilla city , Babylon province.

The objectives and methodology of this study were explained to all participants in the current study to gain their verbal acceptance.

2.2 Chemicals

All the chemicals and kits that are used in the present study are listed in Table 2-1.

Table 2-1 Chemicals and kits that used in the study

No	Chemicals	Origin
1	Human Asprosin ELISA Kit	Bioassay Technology Laboratory (China)
2	Ceramide ELISA Kit	Bioassay Technology Laboratory (China)
3	Trichloroacetic acid (TCA)	Thomas Baker (India)
4	Thiobarbituric acid (TBA)	BDH (England)
5	Glucose Kit	Biolabo (France)

2.3 Instruments and Equipment

All the instruments and equipment that were used in the work are listed in Table 2-2

Table 2-2 The Instruments and Equipment Used

No.	Instruments and Materials	Company, Origin
1	Deep Freeze	GFL, (Germany)
2	Centrifuge	Hettich, (Germany)
3	ELISA Reader	Biotek, (USA)
4	ELISA Washer	Biotek, (USA)
5	Distiller	GFL, (Germany)
6	Spectrophotometer	CECIL,(England)
7	Blue and yellow tips	JRL, (Lebanon)
8	Incubator	Fisher Cient, (Germany)
9	Water bath	Grant, (Germany)
10	Micropipettes (5-50 µL), (2-20 µL),(20-200 µL) , (100-1000 µL)	Slamed, (Germany)
11	Multichannel micropipette(0-250 µL)	Slamed, (Germany)
12	Printer	Epson, (Indonesia)
13	Test tube with Separating gel	AFCO, (Jordan)
14	Plain tube	ASL, Jorden
15	Eppendorf tube (1.5mL)	ASL, Jorden
16	Sensitive electronic balance	Grant, (Germany)
17	Filter papers	AFCO , Jorden
18	Disposable syringes	Universal ,(china)
19	Volumetric flask, funnel ,beaker	Schoot,(Germany)

2.4 Methods

2.4.1 Collection of Samples

Using a disposable syringe (5 mL), venous blood samples were obtained from control and patients. Subjects were asked to come in for a blood sample. Five milliliters of blood were obtained through vein puncture and progressively pumped into disposable tubes containing separating gel. The blood in the gel-containing tubes was allowed to clot for 10 minutes at room temperature before being centrifuged for 10 minutes at 2000 xg, then the serum separated into small volumes eppendorf and kept in a deep freezer (-20 C) to carry out the assay. The blood samples obtained from the groups were used to estimate asprosin, ceramide, MDA and blood glucose.

2.4.2 Determination of Serum Asprosin levels

Asprosin level was measured by enzyme linked immunosorbent assay kit. Components of asprosin ELISA kits in Table 2-3.

Principle:

The Sandwich-ELISA technique is used in this ELISA kit. The plate has been pre-coated with human asprosin antibody. Asprosin present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human asprosin Antibody is added and binds to asprosin in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated asprosin antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human asprosin. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. (154) .

Table 2-3 Components of asprosin ELISA kits

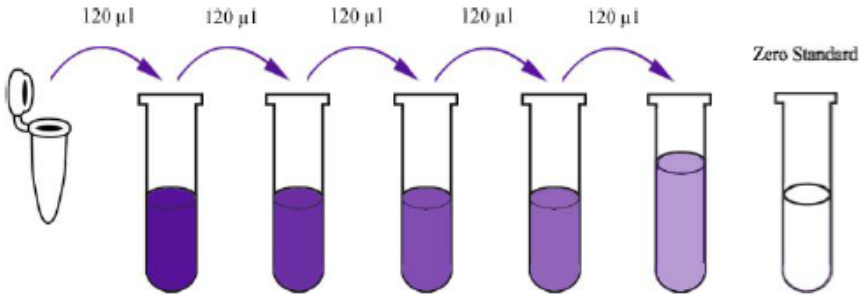
Components	Quantity (96T)
Standard solution (128 ng/mL)	0.5ml x1
Pre-coated ELISA plate	12 * 8 well strips x1
Standard diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop solution	6ml x1
Substrate solution A	6ml x1
Substrate solution B	6ml x1
Wash buffer Concentrate (25x)	20ml x1
Biotinylated Human Asprosin antibody	1ml x1
User instruction	1
Plate sealer	2 pics

Reagent preparation

A- All reagents before used were elevated to room temperature.

B-The original standard sample was diluted as the following:

64ng/mL	Standard No 5	120µL Original Standard + 120µL Standard Diluent
32ng/mL	Standard No.4	120µL Standard No.5 + 120µL Standard Diluent
16ng/mL	Standard No.3	120µL Standard No.4 + 120µL Standard Diluent
8ng/mL	Standard No.2	120µL Standard No.3 + 120µL Standard Diluent
4ng/mL	Standard No.1	120µL Standard No.2 + 120µL Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
128ng/ml	64ng/ml	32ng/ml	16ng/ml	8ng/ml	4ng/ml

Figure 2-1 Concentration of standards of asprosin

C- A volume of 20 mL of concentrated (1x) Wash Buffer were diluted into 500 mL of Wash Buffer concentrate (25x) with distilled water. When crystals have made in the concentrates, solution was mixed gently until the crystals have fully dissolved.

Assay Procedure:

1. The assay was performed at room temperature.

2. A volume of 50 µL of standard was added to well standard.

3. A volume of 40 µL of sample were added to sample well and then added 10 µL of anti- Asprosin antibody, 50µL of Streptavidin-HRP was added to both sample and standard wells. Mixed well. The over plate covered with sealer and incubated for 60 min at 37 °c.

4.The sealer was eliminated and the plate washed 5 times over with a wash buffer. For each wash, wells were soaked for 30 sec. with at least 0.35 mL wash buffer. Aspire for automatic washing of all wells, by washing with

wash buffer 5 times, filling wells with wash buffer. The plate was blotted into paper towels or other absorbing material.

5. A volume of 50µL substrate solution A was added and then 50µL substrate solution B for each wells. The coated plate incubated with new sealer for 10 min. at 37°c in dark media.

6. To each well 50µL of stop solution was added, and the color change from the blue to yellow immediately.

7. The optical density (OD value) of each well identified directly after applying the stop solution by utilize a microplate reader set at 450 nm within 10 min.

Detection range: 0.5 – 100 ng/mL

Sensitivity: 0.23 ng/mL

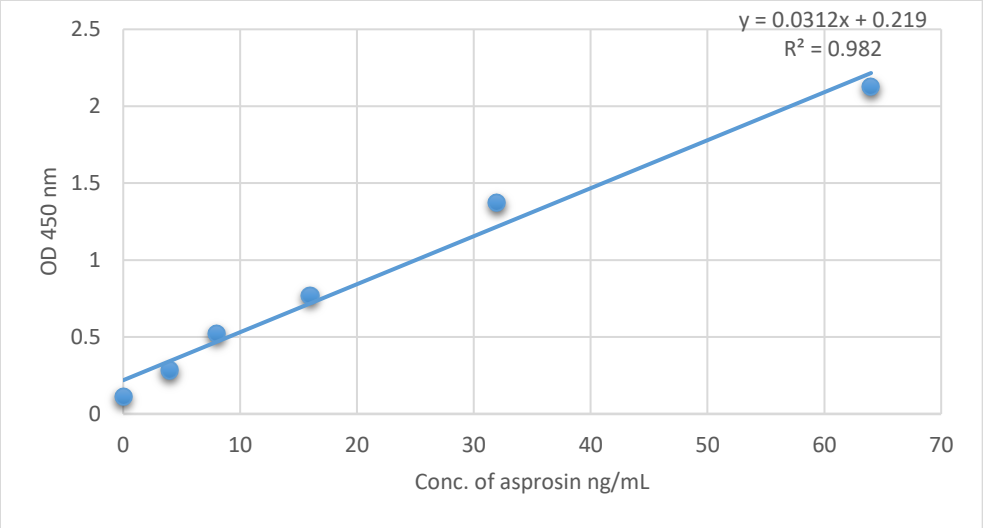


Figure 2-2 Standard curve for human asprosin

2.4.3 Determination of serum Ceramide levels

Ceramide level was measured by enzyme linked immunosorbent assay kit. Components of Ceramide ELISA kits in Table 2-4.

Principle:

The Sandwich-ELISA technique is used in this ELISA kit. The plate has been pre-coated with human CER antibody. Ceramide present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human CER antibody is added and binds to ceramide in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated CER antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human asprosin. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm (155).

Table 2-4 Components of Ceramide ELISA kits

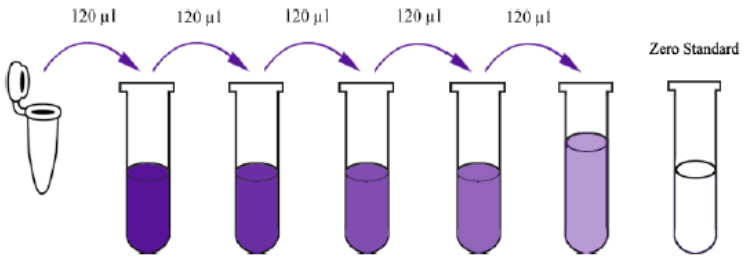
Components	Quantity (96T)
Standard solution (480ng/mL)	0.5ml x1
Pre-coated ELISA plate	12 * 8 well strips x1
Standard diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop solution	6ml x1
Substrate solution A	6ml x1
Substrate solution B	6ml x1
Wash buffer Concentrate (25x)	20ml x1
Biotinylated Human Ceramide antibody	1ml x1
User instruction	1
Plate sealer	2 pics

Reagent preparation

A- All reagents before used were elevated to room temperature.

B-The original standard sample was diluted as the following:

240ng/ml	Standard No 5	120µl Original Standard + 120µl Standard Diluent
120ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
60ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
30ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
15ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
480ng/ml	240ng/ml	120ng/ml	60ng/ml	30ng/ml	15ng/ml

Figure 2-3 Concentration of standards of ceramides

C- A volume of 20 mL of concentrated (1x) Wash Buffer were diluted into 500 mL of Wash Buffer concentrate (25x) with distilled water. When crystals have made in the concentrates, solution was mixed gently until the crystals have fully dissolved.

Assay Procedure:

1. The assay was performed at room temperature.

2. A volume of 50 µL of standard was added to well standard.

3. A volume of 40 µL of sample were added to sample well and then added 10 µL of anti-ceramide antibody, 50µL of Streptavidin-HRP was added to both sample and standard wells. Mixed well. The over plate covered with sealer and incubated for 60 min at 37 °c.

4.The sealer was eliminated and the plate washed 5 times over with a wash buffer. For each wash, wells were soaked for 30 sec. with at least 0.35 mL wash buffer. Aspire for automatic washing of all wells, by washing with wash buffer 5 times, filling wells with wash buffer. The plate was blotted into paper towels or other absorbing material.

5. A volume of 50µL substrate solution A was added and then 50µL substrate solution B for each wells. The coated plate incubated with new sealer for 10 min. at 37°c in dark media.

6. To each well 50µl of stop solution was added, and the color change from the blue to yellow immediately.

7. The optical density (OD value) of each well identified directly after applying the stop solution by utilize a microplate reader set at 450 nm within 10 min.

Detection range: 1 – 400 ng/mL

Sensitivity: 0.62 ng/mL

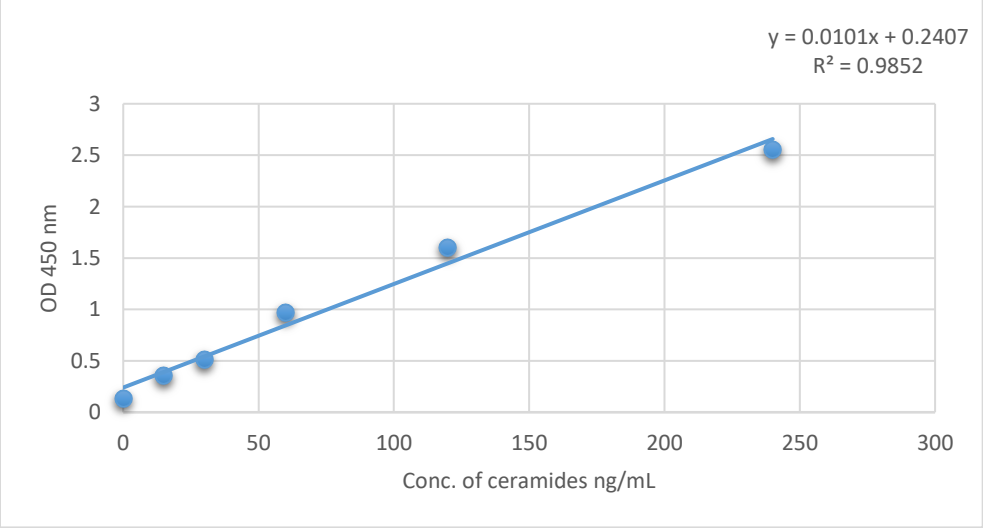
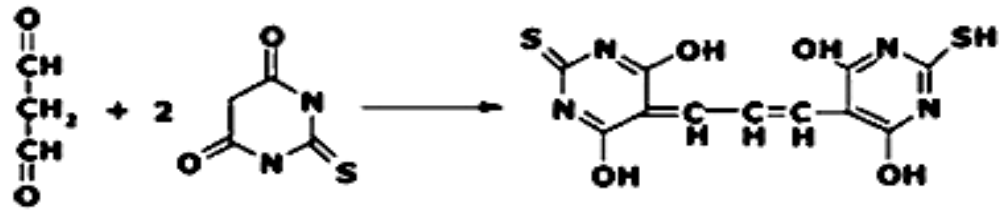


Figure 2-4 Standard curve for ceramide

2.4.4 Determination of Serum Malondialdehyde (MDA) Level

Principle:

Serum MDA was determined by condensation with thiobarbituric acid forming MDA-TBA_2 product as shown below. this complex is a pink chromogen that absorbs strongly at 532 nm (156).



MDA TBA MDA-TBA_2

Preparation of Reagents:

1.Trichloroacetic acid (TCA) reagent (70 %):

Seventy gram (70 g) of TCA was taken and dissolved in a final volume of 100 ml of distilled water (DW).

2.Trichloroacetic acid (TCA) reagent (17.5%):

Seventeen and half gram (17.5 g) of TCA was taken and the volume was completed to 100 ml with DW.

3.Thiobarbituric acid(TBA) reagent (0.6 %):

Sixty hundred mg (0.6 g) of TBA was dissolved in a final volume of 100 ml of DW using a water bath to complete dissolving of TBA.

Procedure:

1- Two set of tubes have been prepared as shown in Table 2-5:

Table 2-5 Procedure of MDA measurement

Reagent	Sample	Blank
Serum	0.15 mL	-
TCA 17.5%	1 mL	1 mL
TBA 0.6%	1 mL	1 mL
All tubes have been mixed well by vortex, incubated in boiling water bath for 15 minutes then allowed to cool.		
TCA 70%	1 mL	1 mL
H_2O	-	0.15 mL

2. The mixture was left to stand at room temperature for 20 minutes.

3. The mixture was centrifuged at 2000 xg for 15 minutes, and the supernatant was taken out for measuring the absorbance at 532 nm. (156)

Calculations:

$$\text{Concentration of MDA (µmol/l)} = \frac{\text{A. of sample}}{L \times E_o} \times D$$

L: Light path (1cm)

E_o: Extinction coefficient = $1.56 \times 10^5\ M^{-1}cm^{-1}$

D: Dilution factor

$$\text{D: Dilution factor} = \frac{\text{total volume}}{\text{Sample volume}} = \frac{3.15\ mL}{0.15\ mL} = 21$$

2.4.5 Determination of Blood Glucose

Principle

Glucose is oxidised by Glucose oxidase to gluconic acid and hydrogen peroxide which in conjunction with Peroxidase, reacts with choro- 4-phenol and PAP to form a red quinoneimine. The absorbance of the colored complex, proportional to the concentration of glucose in the specimen is measured at 500 nm (157).

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} H_2O_2 + \text{Gluconate}$$

$$2H_2O_2 + \text{Phenol} + \text{4-AA} \xrightarrow{\text{POD}} 4H_2O + \text{Quinonimine}$$

Reagents Composition

Table 2-6 Reagents composition of glucose Kit

Reagent 1 (enzyme buffer)	R1	Phosphate Buffer 150 mmol/L Glucose oxidase (GOD) ≥ 20 000 UI/L Peroxidase (POD) ≥1000 UI/L 4-Amino-antipyrine (PAP) 0.8 mmol/L
Reagent 2 (Chromogen)	R2	Chloro-4-phenol 2 mmol/L
Reagent 3 (Standard)	R3	Glucose 100 mg/dL (5.55 mmol/L)

Procedure

Reagents and specimens were allowed at room temperature before using.

Table 2-7 procedure of blood glucose

Pipette into test tubes	**Blank**	**Standard**	**Sample**
Working Reagent (R1+R2)	1mL	1mL	1mL
Distilled water	10 µL		
Standard		10 µL	
Serum			10 µL
The tubes were mixed, then left stand for 10 minutes at 37˚C. The absorbance was read at 500 nm, by using cuvette of 1 cm light path.			

Calculations

The result was calculated as following:

$$\text{Glucose concentration} = \frac{\text{Abs(Assay)}}{\text{Abs(standard)}} \text{ x Standard concentration (100 mg/dL).}$$

2.5 Statistical Analysis

Statistical analysis was carried out using SPSS version 21. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. ANOVA test was used to compare means among three groups or more. Pearson correlation coefficient was used to assess the relationship between two continuous variables. A p-value of ≤ 0.05 was considered as significant.

Receiver operating characteristic(ROC) curve was used to evaluate the diagnostic value of asprosin and ceramide in GHD. The sensitivity and specificity of biochemical parameter were measured and the optimal cutoff was calculated according to "Youden Index" by select the point that is closest to the top-left corner of the ROC curve giving equal weight to sensitivity and specificity when picking a cut-off point is a typical practice. This idea is often referred to as the Youden Index (158).The area under the curve (AUC) provides a useful tool to compare different biomarkers as Table 2- 8.

Table 2-8 List of AUC ranges and their classification levels (159).

AUC Range	Classification Level
0.90 - 1.00	Excellent
0.80 - 0.90	Good
0.70 - 0.80	Fair
0.60 - 0.70	Poor
0.50 - 0.60	Failure

3. Results and Discussion

3.1 Demographic Characteristics in Patients and Control

3.1.1 Age

The means and standard deviation of age in patients and control were shown in Table 3-1. Patients included in this study had age range (4-14) years; with mean±SD (10.4 ± 3.0) years. Control group was with an age range (4-14) years and mean±SD (9.3 ± 2.6) years.

Table 3-1 Means ± standard deviation of age in patients and control

	Group	**N**	**Mean ± SD**	**P-value**
Age (year)	**Patients**	43	10.4 ± 3.0	0.071
	control	45	9.3 ± 2.6	

P value < 0.05 was significant

A non-significant difference in the mean of age between GHD patients and the control group was belong to the matching of the age range between the subjects of the two studied groups to eliminate its effect on other parameters.

The age range used in this study is similar to the study conducted by Aldabagh *et al.* and Tahasalih *et al.* , the age range used in these two studies were (3 -15) years, (4 -12) years respectively (160,161).

The purpose of selection this age range is to reduce age effects on studied variables.

3.1.2 Gender Distribution in Patients and Control

Among forty-three patients with GHD who contributed to this study, there were 24 (56%) males and 19 (44%) females, as shown in figure 3-1.

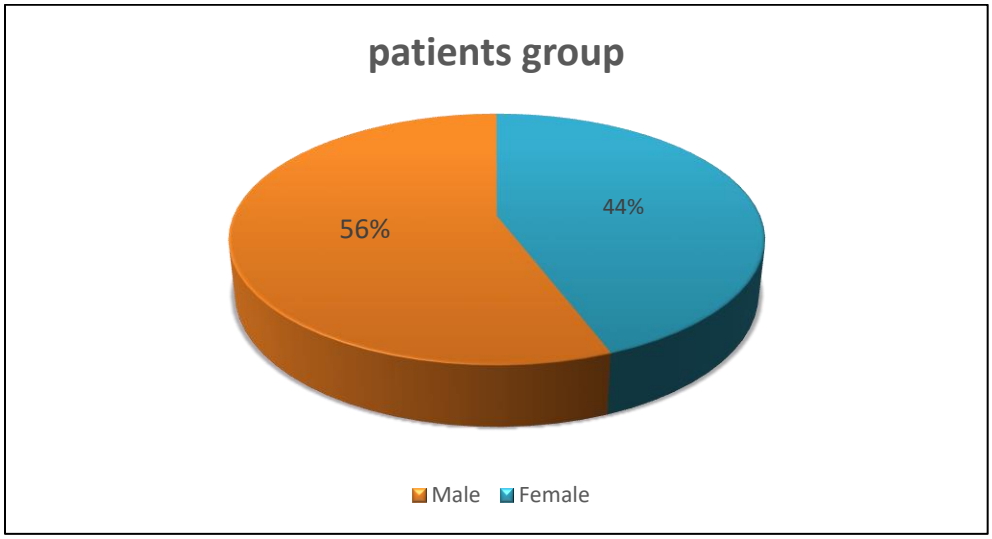


Figure 3-1 Rate of male and female among patients

Among forty-five control who contributed to this study, there were 26 (58%) males and 19 (42%) females, as shown in figure 3-2.

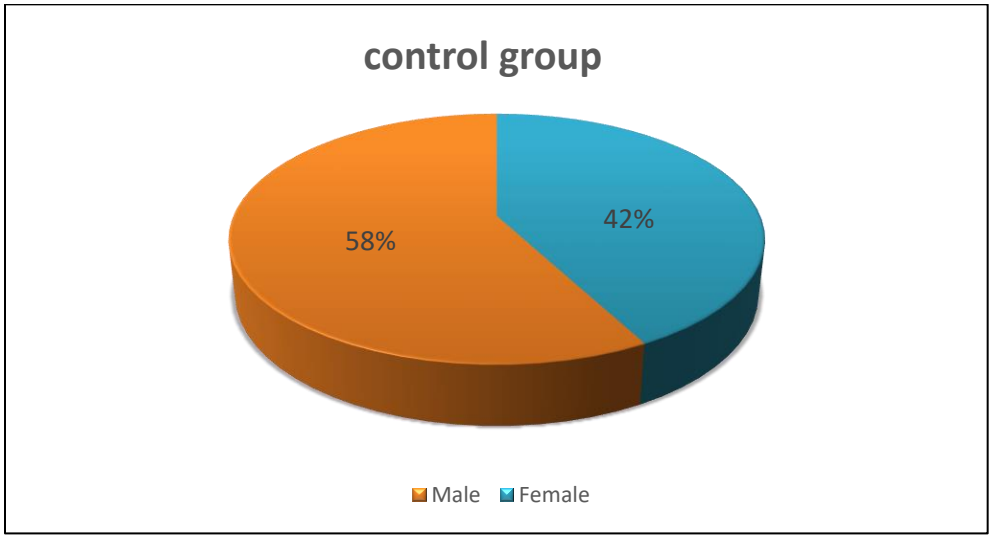


Figure 3-2 Rate of male and female among control group

The two studied group were well matched regarding gender distribution (P-value = 0.855).

It has been noticed that more males than females were referred to endocrine clinics due to growth disturbances. Short stature in female is often not detected, or it is reported late which may be due to bad social habits, or the parents' belief that it is normal and perhaps shortened because it is a female. For the correct care of short girls, a worldwide understanding of gender biases is essential (162).

A similar result has also been observed in a review by Ranke *et al*. in Europe, USA, and Japan (162). In Iraq a similar result has also been observed by Aldabagh *et al*. (160).

3.1.3 Anthropometric Measurements

The data presented in Table 3-2 show the anthropometric measurements of GHD patients and the control.

The results revealed a significant decrease in the height and height Z-score of the patients (121.9±19.32cm) (-2.9±1.48) compared with the control (131.4±14.65cm)(-0.2±1.27) ($P<0.05$) , this is due to the fact that the GHD is one of the reasons for short stature (163).

There were non-significant differences ($P>0.05$) in the weight of the patients (27.6±11.86 kg) compared with their values in the control (31.6±11.34 kg).

Table 3-2 Anthropometric measurements of GHD patients and the control

Anthropometric measurements	mean±SD		P-value
	Patients	control	
Height(cm)	121.9±19.32	131.4±14.65	0.011
Height Z score	-2.9±1.48	-0.2±1.27	<0.001
Weight(kg)	27.6±11.86	31.6±11.34	0.108
BMI	17.6±3.82	18.3±4.31	0.428
BMI percentile (%)	47.2±33.56	58.8±34.97	0.117
BMI Z score	-0.144±1.48	0.214±1.48	0.26

P value < 0.05 was significant

Body mass index is used differently for children. It is calculated in the same way as for adults but then compared to typical values for other children of the same age and gender. Instead of comparison against fixed thresholds for underweight and overweight, the BMI is compared against the percentiles for children of the same sex and age. A BMI that is less than the 5th percentile is considered underweight and above the 95th percentile is considered obese. Children with a BMI between the 85th and 95th percentile are considered to be overweight (153).

Regarding BMI, the results revealed non-significant difference between the patients (17.6±3.82) and the control (18.3±4.31) (P>0.05). Also non-

significant difference in BMI percentile (47.2±33.56%) and BMI Z-score (-0.144±1.48) in the patients compared with their values in the control (58.8±34.97% and 0.214±1.48) respectively.

3.2.4 The Distribution of Patients According to Residence

The distribution of patients according to residence showed that the majority (67%) of patient with GHD came from rural area. While only (33%) of patient with GHD came from urban area. As shown in figure 3-3.

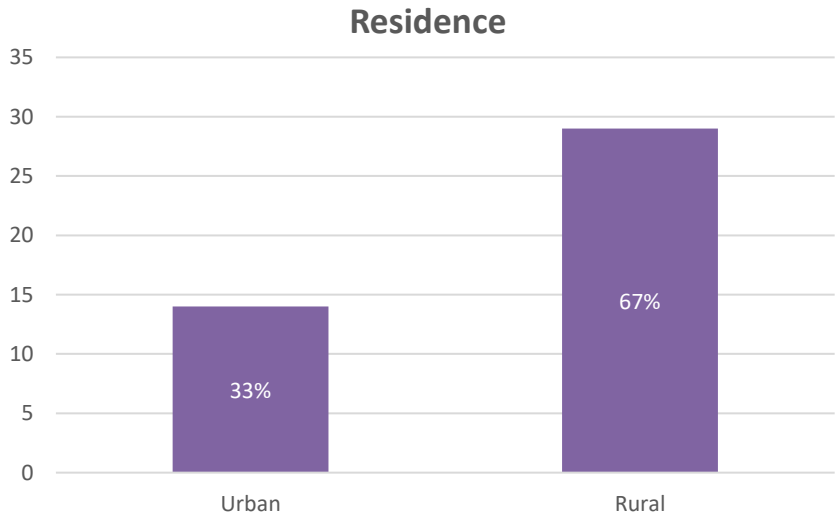


Figure 3-3 Distribution of patients according to residence

Several of inherited and gene-related disorders have been reported among births of consanguineous marriages as reported by Al-Samarrai *et al.* (164); and as 3-30% of the causes of GHD are suggested to have a genetic cause (103).The finding of present study that the majority of GHD patients (67%) came from rural area may be attributed to the increase consanguineous marriages in this area.

3.2. Biochemical Parameters

3.2.1 Asprosin

The findings demonstrated a significant decrease in the asprosin level of the patients (27.7±18.81ng/ml) compared with the control (42.1±27.33 ng/ml), as in Table 3-3.

Table 3-3 Comparison of asprosin level in patients and control group

Study Group	N	Asprosin level (ng/ml) Mean ± SD	P-value
patients	43	27.7±18.81	**0.005**
control	45	42.1±27.33	

P value < 0.05 was significant

Asprosin is involved in appetite, glucose metabolism, insulin resistance, cell apoptosis, etc (8). Asprosin is an important hormone in appetite regulation. According to Duerrschmid *et al*. (126), asprosin crosses the blood–brain barrier and affects appetite stimulation directly by activating orexigenic agouti-related peptide (AgRP) neurons in the hypothalamus, while also inhibiting arcuate proopiomelanocortin (POMC) anorexigenic neurons indirectly. It has been shown that the effect of asprosin is linked with the activation of the Gαs–cAMP–PKA axis. Food intake and energy homeostasis are controlled by AgRP+ neurons. This small subset of arcuate nucleus neurons secretes the orexigenic neuropeptide AgRP, as well as neuropeptide

Y (NPY) and GABA transmitters, the last two are essential for feeding promotion (127).

Ghrelin , a type of Growth Hormone Secretagogue (GHS), is produced primarily by the stomach's endocrine cells, and also by the intestinal system and the hypothalamus and it acts in the arcuate nucleus of the hypothalamus and in somatotrophs in the pituitary to release growth hormone(49,165). GHSs act through the GHS- receptor (GHS-R) which is a G protein-coupled receptor (GPCR) and stimulates the release of GH(166). ghrelin and GHRH colocalize in the hypothalamic arcuate nucleus, where ghrelin can induce GHRH release directly (167).

Besides its ability to stimulate GH secretion, ghrelin is a well-known orexigenic hormone, the effects of which are mediated through activation of both hypothalamic and extra-hypothalamic regions involved in the regulation of homeostatic as well as hedonic feeding (168). Interestingly, ghrelin and asprosin, seem to activate a partially overlapping subset of AgRP neurons within the arcuate nucleus of the hypothalamus, and asprosin-deficiency makes these neurons less responsive to ghrelin-mediated activation (126,169).

In spite of most GHD is Idiopathic and between 3-30% of the cases of GHD are suggested to have a genetic cause (103);Present study suggest that may asprosin’s overlapping with AgRP neurons is the same in GHS- receptor (GHS-R) and decrease in asprosin level may lead to the deficiency of GH. These are just suggestions and still asprosin contribute to underling mechanism of GHD but may be as cause or result.

There is a case-control study done by Baykus *et al.* that found a significant decrease in asprosin levels in pregnant women with intrauterine growth retardation (IUGR) in both cord blood and maternal blood (170).

Also there is non-significant differences (P>0.05) in asprosin level between patients treated with recombinant GH and patient without rhGH. as Table 3-4 show. This strengthen that asprosin more likely to be a cause for GHD than to be a result.

Table 3-4 Comparison of asprosin level in patients treated with rhGH and patients without rhGH treatment

Subgroup	N	Asprosin level (ng/ml) Mean ± SD	P-value
With rhGH	29	29.8±18.65	0.298
Without rhGH	14	26.4±19.09	

P value< 0.05 was significant
recombinant human growth hormone (rhGH)

In addition to that study demonstrated non-significant difference (P>0.05) between male (24.1±16.50ng/ml) and female (32.4±20.92ng/ml). as Table 3-5 revealed.

Table 3-5 Comparison of Asprosin level in male and female groups

Subgroups	N	Asprosin level (ng/ml) Mean ± SD	P-value
Male Patients	24	24.1±16.50	0.153
Female Patients	19	32.4±20.92	

P value < 0.05 was significant

3.2.2 Ceramide

The results revealed a significant (P<0.05) decrease in the ceramide level of the patients (106.0±65.04ng/ml) compared with the control (149.3±91.88ng/ml), as in Table 3-6.

Table 3-6 Ceramide level in patients and control group.

Study Group	N	Ceramide level (ng/ml) Mean ± SD	P-value
patients	43	104.5±64.39	**0.01**
control	45	149.3±91.88	

P value < 0.05 was significant

This is may be due to the fact that GH stimulates lipolysis via activation of the hormone-sensitive lipase, primarily in the visceral adipose tissue, which results in free fatty acid (FFA) flux from adipose tissue to circulation (171).

In contrast to the GH effects on adipose tissue, GH promotes cellular uptake of FFA in skeletal muscle by increasing the activity of lipoprotein lipase (172). The re-esterification of triglycerides from FFA results in the accumulation of lipid intermediates such as diacylglycerol and ceramides in skeletal muscle (173,174). So, decrease in ceramide level in patient with GHD may due to deficiency of GH.

On the other hand, the mean of ceramide level was higher in patients treated with rhGH (110.5±65.57 ng/ml) than the other without rhGH (92.1±62.36 ng/ml) but this difference was statistically not significant (P-

value 0.385). This strengthen that ceramide more likely to be a result for GHD than to be a cause. As Table 3-7.

Table 3-7 Comparison of ceramide level in patients treated with rhGH and patients without rhGH treatment

Subgroup	N	Ceramide level (ng/ml) Mean ± SD	P-value
With rhGH	29	110.5±65.57	0.385
Without rhGH	14	92.1±65.36	

P value < 0.05 was significant
recombinant human growth hormone (rhGH)

Also, study revealed a non-significant (P>0.05) difference in ceramide level between male (94.9±60.99ng/ml) and female (120.0±68.89ng/ml), as Table 3-8.

Table 3-8 Ceramide level in male and female groups

Subgroups	N	Ceramide level (ng/ml) Mean ± SD	P-value
Male patients	24	92.2±59.17	0.162
Female patients	19	120.0±68.89	

P value < 0.05 was significant

3.3.3 Malondialdehyde

The findings demonstrated a non-significant(P>0.05) difference in MDA levels between patients and their control group. as shown in the Table 3-9.

Table 3-9 The means ± standard deviation of MDA level in patients and control group

Study Group	N	MDA level (µmol/l) Mean ± SD	P-value
patient	43	9.5±6.53	0. 216
control	45	7.9±5.75	

P value < 0.05 was significant

Also there is non-significant (P>0.05) differences in MDA level between patient treated with recombinant GH (9.5±6.69 µmol/l) and patient without rhGH (8.6±6.18 µmol/l), as Table 3-10.

Table 3-10 Comparison of MDA level in patients treated with rhGH and patients without rhGH treatment

Subgroup	N	MDA level (µmol/l) Mean ± SD	P-value
With rhGH	29	9.5±6.69	0.668
Without rhGH	14	8.6±6.18	

P value < 0.05 was significant
recombinant human growth hormone (rhGH)

Besides that, study revealed a non-significant (P>0.05) difference in MDA level between male (8.5±6.23 µmol/l) and female (10.8±6.84µmol/l).as Table 3-11.

Table 3-11 MDA level in male and female groups

Subgroups	N	MDA level (µmol/L) Mean ± SD	P-value
Male patients	24	8.5±6.23	0.258
Female patients	19	10.8±6.84	

P value < 0.05 was significant

3.3.4 Glucose

The findings demonstrated a significant difference in glucose levels between patients and their control group (P<0.05). as shown in the Table 3-12.

Table 3-12 Comparison of glucose level between patients and control group

Study Group	N	Glucose level (mg/dl) Mean ± SD	P-value
patients	43	79.2±19.8	**0.003**
control	45	91.8±18.9	

P value < 0.05 was significant

The results revealed a significant (P<0.05) decrease in the glucose level of the patients (79.2±19.8 mg/dl) compared with the control (91.8±18.9 mg/dl), this may be due to lower asprosin level in patients compared with the control which result in decrease blood glucose in patients.

Also, study revealed a significant difference in glucose level between patient treated with rhGH and with patient without rhGH. As Table 3-13.

Table 3-13 Comparison of glucose level in patients treated with rhGH and patients without rhGH treatment

Subgroup	N	Glucose level (mg/dl) Mean ± SD	P-value
With rhGH	29	87.9±29.93	**0.042**
Without rhGH	14	74.9±10.74	

P value < 0.05 was significant

recombinant human growth hormone (rhGH)

The results revealed a significant increase in the glucose level in patient treated with rhGH(87.9±29.93mg/dl), compared with patient without rhGH (74.9±10.74 mg/dl), this may be due to that GH is an insulin counter-regulatory hormone that increases glucose synthesis in the liver and kidneys while decreasing glucose uptake in peripheral tissues, making it a hyperglycemic hormone. Hypoglycemia is common in people with GHD, especially in early childhood (175).

3.4 Pearson Correlations of Asprosin and Ceramide, with other parameters.

Table (3-14) Correlations between biochemical markers.

		Asprosin	Ceramide
Age (years)	**R**	- 0.147	-0.116
	P	0.172	0.281
	N	88	88
BMI %	**R**	0.004	0.012
	P	0.969	0.911
	N	88	88
Asprosin (ng/ml)	**R**	1	0.975**
	P	----	0.000
	N	88	88
Ceramide (ng/ml)	**R**	0.975**	1
	P	0.000	----
	N	88	88
MDA (µmol/l)	**R**	0.033	0.045
	P	0.76	0.679
	N	88	88
Glucose (mg/dl)	**R**	0.260*	0.277**
	P	0.014	0.009
	N	88	88

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
r Pearson correlation coefficient

3.4.1 Correlations Between Asprosin and Ceramide

Serum asprosin showed a positive correlation with ceramide (r=0.975, P<0.001), this may be clarified through that asprosin may be have an effect on GH secretion by GHS- receptor (GHS-R), and GH have effect on lipid metabolism. So asprosin level indirectly effect ceramide level as figure 3-4.

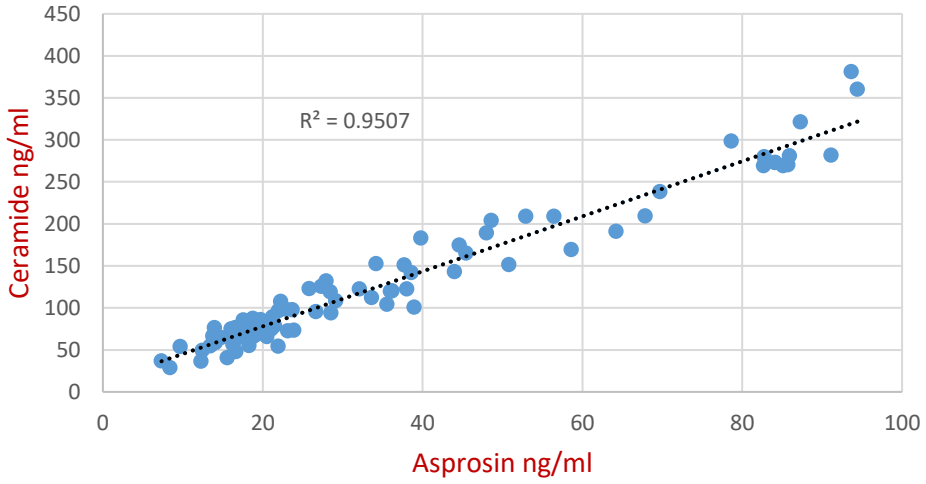


Figure 3-4 Correlations between asprosin and ceramide

3.4.2 Correlations between Asprosin and Glucose

Serum asprosin showed a positive correlation with glucose (r=0.260, P=0.014), as in figure 3-5. This correlation can be explain by that asprosin activating protein kinase A (PKA) in the liver via the Olfactory 734 (Olfr734) receptor promotes glucose release from hepatocytes (124). Also asprosin levels are inversely proportional to glucose levels, with low glucose levels (fasting state) boosting asprosin production and high glucose levels inhibiting

it (feeding state) (125),and in spite of a significant decrease in the glucose level of the patients (79.2±19.8 mg/dl) compared with the control group (91.8±18.9 mg/dl) asprosin level decrease in patients with GHD which may be due to genetic defect in asprosin gene, so in summary the initial problem is asprosin deficiency that lead to GH deficiency and both of them cause lower blood glucose.

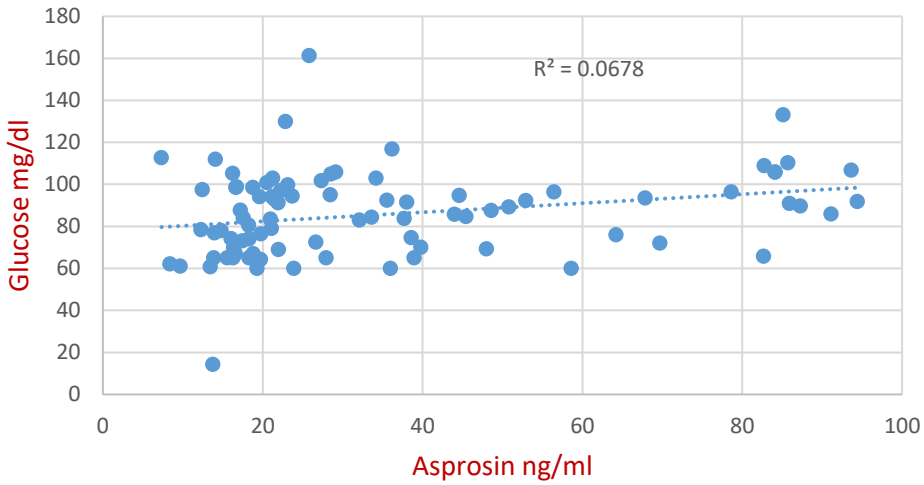


Figure 3-5 Correlations between asprosin and glucose

3.4.3 Correlations Between Ceramide and Glucose

Serum Ceramide showed a positive correlation with glucose (r=0.277, P=0.009), as in figure 3-6. GHD will cause decrease in both blood glucose and ceramide level so, the correlation between ceramide and blood glucose is related to GHD.

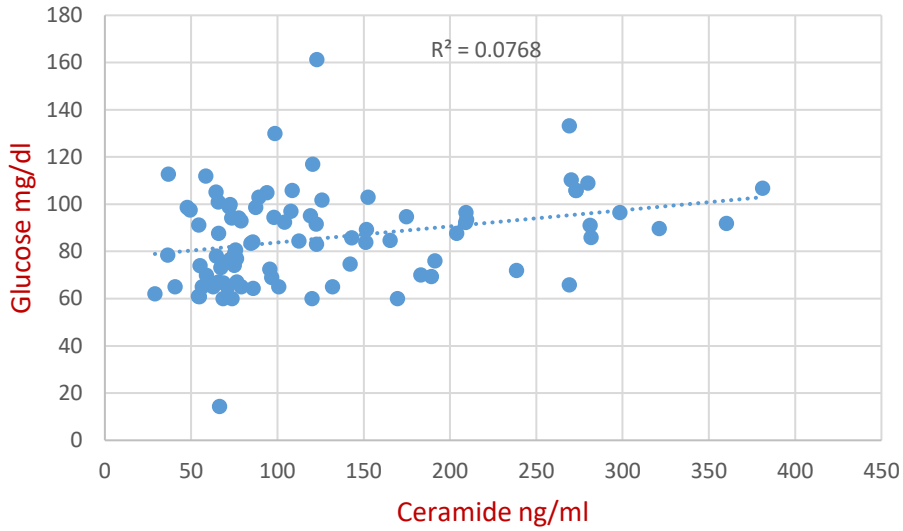


Figure 3-6 Correlations between ceramide and glucose

3.5 Diagnostic values for Serum Asprosin and Ceramide

Receiver operating characteristic curve (ROC) was used to assess the diagnostic values of Asprosin and Ceramide in identifying GHD patients, and which of them is more specific or sensitive in the diagnosis.

3.5.1 Receiver operating characteristic Curve for Asprosin

The area under the curve (AUC) was 0.686, 95%CI= (0.576 - 0.797), p-value =0.003. The sensitivity and specificity of the test at the cut-off value of asprosin 21.144 ng/ml were 58.1% and 75.6%, respectively. Positive predictive value (PPV) 65.3%, negative predictive value(NPV) 69.4%, As shown in figure 3-7.

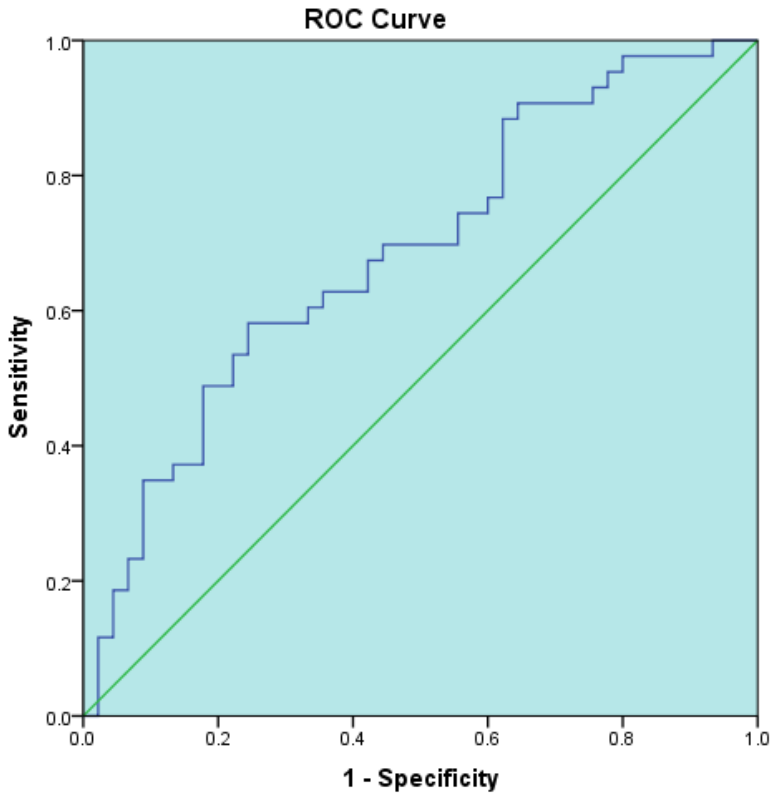


Figure 3-7 Receiver operating characteristic curve for asprosin

For asprosin, present results stated that it had poor diagnostic value in the diagnosis of GHD. As Table 3-15.

Table 3-15: Receiver operating characteristic curve analysis for asprosin

Variable	**AUC**	**Threshold**	**P-value**	**SN**	**SP**	**PPV**	**NPV**
Asprosin (ng/ml)	0.686	21.144	0.003	58.1%	75.6%	65.3%	69.4%

SN=sensitivity, SP=specificity, PPV= positive predictive value,

NPV= negative predictive value, AUC= area under the curve.

P value < 0.05 was significant

3.5.2 Receiver operating characteristic Curve for Ceramide

The area under the curve (AUC) was 0.656, 95%CI= (0.543 - 0.770), p-value=0.012. The sensitivity and specificity of the test at the cut-off value of ceramide 197.5 ng/ml were 93% and 31.1%, respectively. positive predictive value (PPV) 82.3%, negative predictive value(NPV) 49.3%, As shown in figure 3-8.

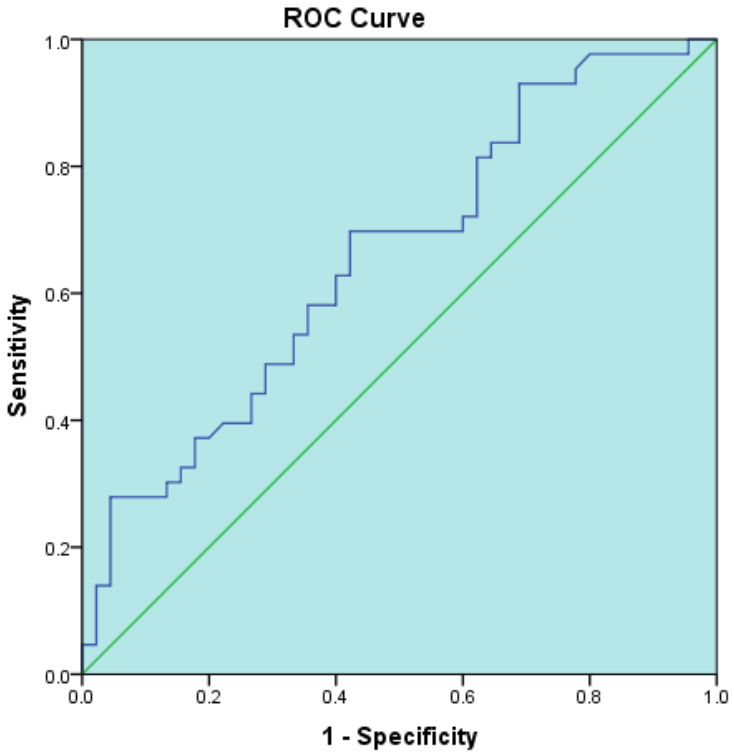


Figure 3-8 Receiver operating characteristic curve for ceramide

For ceramide, present results stated that it had poor diagnostic value in the diagnosis of GHD. As Table 3-16.

Table 3-16: Receiver operating characteristic curve analysis for ceramide

Variable	AUC	Threshold	P-value	SN	SP	PPV	NPV
Ceramide (ng/ml)	0.656	197.5	0.012	93%	31.1%	82.3%	49.3%

SN=sensitivity, SP=specificity, PPV= positive predictive value,

NPV= negative predictive value, AUC= area under the curve.

P value < 0.05 was significant

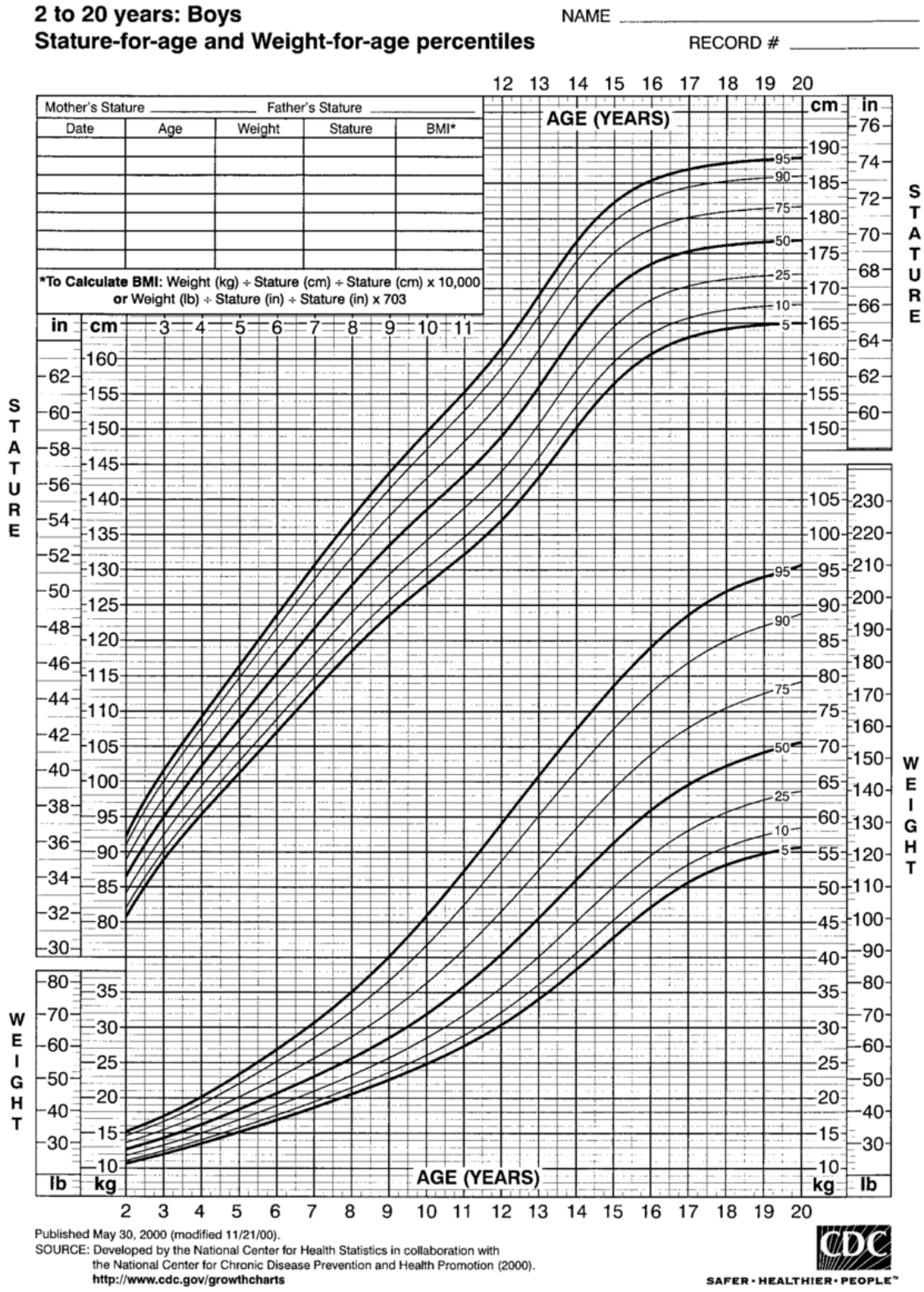
Conclusion

- Asprosin may be one of underlying cause of GHD through its indirect role in releasing of GH.
- GHD lead to decrease level of ceramide by the effect of GH on lipid metabolism.
- Presence of positive correlation between serum asprosin, ceramide and glucose strength its relation to GHD.
- According to the sensitivity and specificity, asprosin and ceramide have poor diagnostic value for GHD.

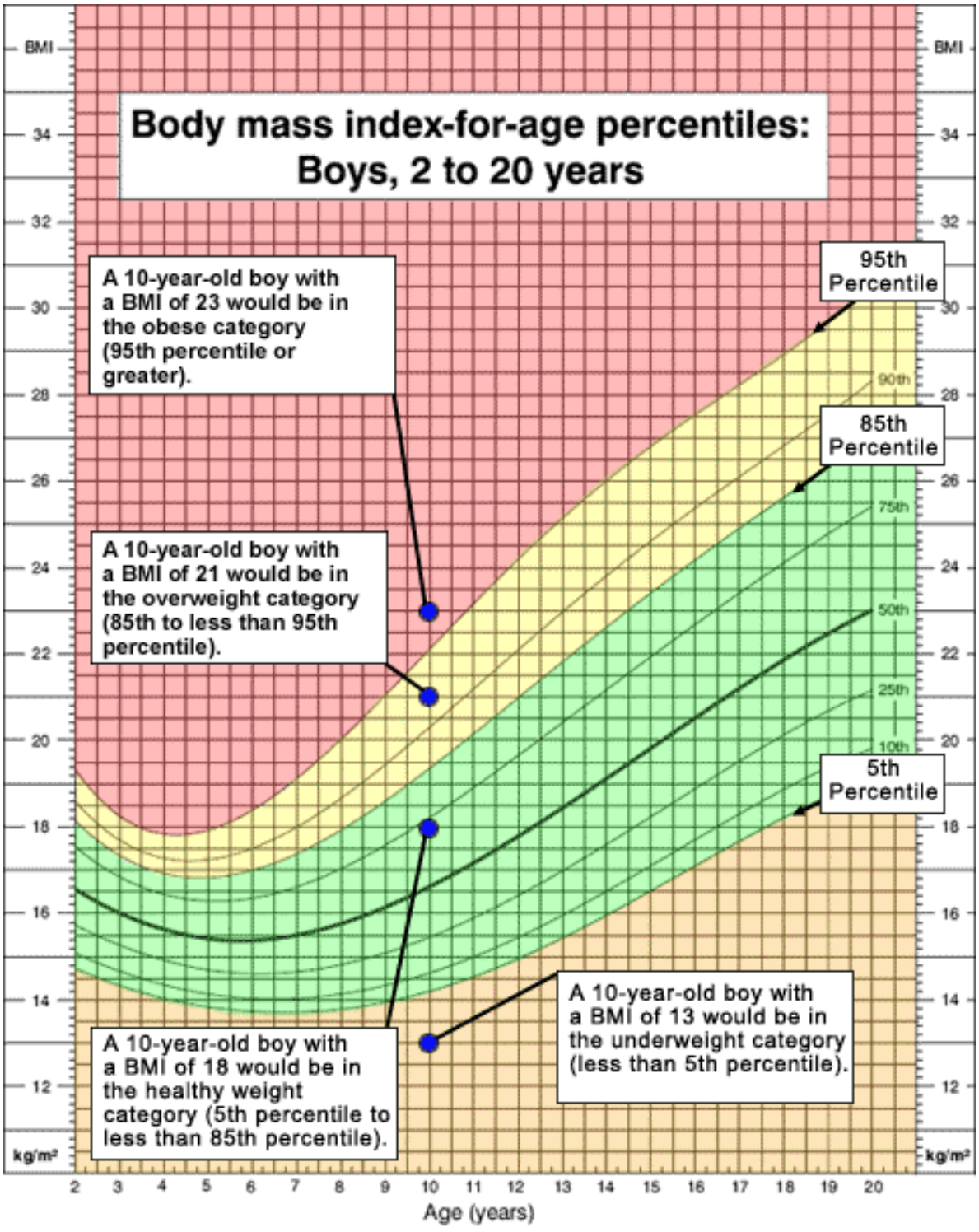
Recommendations

- Genetic study for asprosin gene in GHD Patients to find the cause of its low level in these patient.
- Estimate ghrelin level and its correlation with asprosin level in GHD patients.
- pharmacological study to applicate the administration of recombinant asprosin as treatment for GHD.

Appendix


2 to 20 years: Boys
Stature-for-age and Weight-for-age percentiles
NAME
RECORD #
Mother's Stature
Father's Stature
Date
Age
Weight
Stature
BMI*
*To Calculate BMI: Weight (kg) ÷ Stature (cm) ÷ Stature (cm) x 10,000
or Weight (lb) ÷ Stature (in) ÷ Stature (in) x 703
AGE (YEARS)
STATURE
WEIGHT
95
90
75
50
25
10
5
Published May 30, 2000 (modified 11/21/00).
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).
http://www.cdc.gov/growthcharts
SAFER · HEALTHIER · PEOPLE™

References


Body mass index-for-age percentiles:
Boys, 2 to 20 years
BMI
kg/m²
Age (years)
95th Percentile
90th
85th Percentile
75th
50th
25th
10th
5th Percentile
A 10-year-old boy with a BMI of 23 would be in the obese category (95th percentile or greater).
A 10-year-old boy with a BMI of 21 would be in the overweight category (85th to less than 95th percentile).
A 10-year-old boy with a BMI of 18 would be in the healthy weight category (5th percentile to less than 85th percentile).
A 10-year-old boy with a BMI of 13 would be in the underweight category (less than 5th percentile).

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نستنتج ان من المرجح ان يكون الاسبروسين هو أحد الأسباب الكامنة وراء نقص هرمون النمو من خلال دوره غير المباشر في إطلاق الهرمون. يؤدي نقص هرمون النمو إلى خفض مستوى السيراميد من خلال تأثير هرمون النمو على التمثيل الغذائي للدهون. وجود علاقة ايجابية بين الاسبروسين و السيراميد والجلوكوز عند مرضى نقص هرمون النمو.

الخلاصة

يُعرَّف نقص هرمون النمو بشكل كلاسيكي بأنه إفراز غير كافٍ لهرمون النمو مما يؤدي إلى انخفاض في إنتاج الهرمونات المعتمدة على هرمون النمو وعوامل النمو ، مثل عامل النمو الشبيه بالأنسولين -١ (IGF-I) و IGF-II والبروتينات الملزمة (IGFBPs) .تتمثل الوظيفة الأساسية لـهرمون النمو في تعزيز النمو الطولي بعد الولادة. عندما لا تفرز الغدة النخامية ما يكفي من هرمون النمو لتعزيز نمو الجسم ، فإنها تظهر عادةً كبطئ في معدل النمو في كل من الطفولة المبكرة والمتأخرة بالمقارنة مع أقرانهم من نفس العمر والجنس ، فإن الأطفال الذين يعانون من نقص هرمون النمو لديهم جسم ذو ابعاد متناسقة، على الرغم من أنهم عمومًا ممتلئون ، وأقصر ، ويبدون أصغر من سنهم.

صممت الدراسة الحالية للتحقق من مستوى الأسبروسين والسيراميد والمالونديالديهيد والجلوكوز في المرضى ، وكذلك لدراسة الارتباط بين هذه المعايير البيوكيميائية.

تم تصميم هذه الدراسة كدراسة بين مرضى واصحاء يشمل ١٠٣ أفراد ولكن بعد بعض الحسابات الرياضية يلزم استبعاد ١٥ فردًا وأيضًا للمطابقة بين المرضى والاصحاء في العمر والجنس كان العدد ٨٨ فردًا حيث أجريت على ٤٣ مريضًا يعانون من نقص هرمون النمو المنعزل مجهول السبب (٢٤ ذكرًا و ١٩ أنثى) ، تتراوح أعمارهم (٤-١٤ عامًا) بمعدل ± الانحراف المعياري (١٠,٤ ± ٣,٠) أعوام. تضم مجموعة المرضى تسعة وعشرين طفلاً عولجوا بهرمون النمو البشري المؤتلف وأربعة عشر طفلاً دون علاج. و٤٥ فردًا يتمتعون بصحة جيدة على ما يبدو و يتطابقون مع المرضى في العمر والجنس.

استخدمت عينات المصل لقياس المتغيرات البيوكيميائية ، الاسبروسين ، السيراميد ، مالونديالديهيد والجلوكوز.

أوضحت نتائج الدراسة الحالية وجود فرق معنوي (p <٠,٠٥) في مستويات الأسبروسين والسيراميد والجلوكوز بين مرضى نقص هرمون النمو والاصحاء. بينما لا يوجد فرق معنوي (P> ٠,٠٥) في مستويات مالونديالديهيد بين المرضى والاصحاء .

ايضا بينت هذه الدراسة ان هناك علاقة ارتباط موجبة معنوية بين كل من الاسبروسين والسيراميد ، الاسبروسين و الجلوكوز و السيراميد والجلوكوز.

وزارة التعليم العالي

والبحث العلمي

جامعة بابل

كلية الطب

مستوى الاسبروسين والسيراميد المصلي عند الاطفال الذين يعانون من نقص هرمون النموالمنعزل في محافظة بابل

رسالة

مقدمة إلى عمادة كلية الطب في جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير

في العلوم/ كيمياء حياتية سريرية

من قبل

منتظر محمد هادي فاضل

بكالوريوس تقنيات التحليلات المرضية

كلية التقنيات الصحية والطبية/ كوفة

٢٠١٦-٢٠١٧

اشراف

الأستاذ المساعد

د. سيناء بدر محمد

الأستاذ المساعد

د. رحاب فيصل العابدي

٢٠٢٢ ميلادية

١٤٤٤ هجرية