A Study of Plasma Ghrelin Level in Patients with Different Gastric Diseases

Thesis

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دراسة مستوى هورمون الجيريلين فى البلازما فى الحالات المختلفة لأمراض المعدة

رســـالة

توطئة للحصول علي درجة الماجيستير في الباطنة العامة

مقدمة من

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List of appreviations

COX 1	Cyclo-oxygenase 1
COX 2	Cyclo-oxygenase 2
DU	Duodenal ulcer
ECL	Enterochromaffin-like
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
GH	Growth hormone
GHS	Growth hormone secretagogue
GHS-R	Growth hormone secretagogue receptor
GU	Gastric Ulcer
HCL	Hydrochloric acid
IF	Intrinsic factor
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IL	Interleukin
LHA	Lateral hypothalamic area
MHC	Major histocomptability complex
mRNA	Messenger ribonucleic acid
NSAID	Non steroidal anti-inflammatory drugs
NUD	Non-ulcer dyspepsia
PPI	Proton pump inhibitor
PPIs	Proton pump inhibitors
PUD	Peptic ulcer disease
PVN	Paraventricular nucleus
TGF	Transforming growth factor
TXA2	Thromboxane A2
VEGF	Vascular endothelial growth factor
VMN	Ventromedial nucleus
ZES	Zolinger Ellison syndrome

CONTENTS

CHAPTER 1

No I	sex	age	Wt	Ht	BMI	W	Hip	W/H	WBC	Hb	Plt	ALT	AST	BUN	Cr	Ghrelin
1	♀	30	79	152	34	106	119	0.9	4.2	11.2	285	22	24	10	0.9	39
2	♀	42	67	156	28	91	108	0.8	5.2	12	186	21	23	11	1	41
3	♀	55	47	149	21	77	95	0.8	7.3	10	601	19	22	12	0.8	47
4	♀	29	47	145	23	77	90	0.9	6	10.2	241	29	24	17	0.8	30
5	♂	40	79	175	26	88	107	0.8	6.3	11.6	214	25	21	18	0.6	35
6	♂	22	40	158	16	77	92	0.8	5.5	13.6	228	11	29	10	1	37
7	♂	52	50	165	18	75	90	0.8	6.7	10	91	13	11	8	0.7	40
8	♀	48	68	153	29	98	113	0.9	7	10	210	20	24	19	1	36
9	♂	49	50	165	18	75	85	0.9	5	9	320	36	44	24	0.6	29
10	♀	38	60	155	25	90	104	0.9	8	11	190	38	58	14	0.5	33

Clinical and laboratory data of group I (Chronic gastritis group)
Number of patients: 10 patients

No II	sex	age	Wt	Ht	BMI	W	Hip	W/H	WBC	Hb	Plt	ALT	AST	BUN	Cr	G
11	♂	25	55	170	19	80	96	0.8	7	13	240	29	31	9	0.8	44
12	♀	50	64	152	28	90	105	0.9	5	10	211	14	24	11	0.7	47
13	♀	52	84	153	35	100	115	0.9	7	10	147	49	40	10	0.9	40
14	♀	38	81	165	30	90	100	0.9	6	10	262	23	24	18	0.8	39
15	♂	32	96	172	33	104	110	0.9	9.8	13	209	24	28	10	0.8	59
16	♀	40	75	149	33	105	110	1	8	10	231	19	19	14	1	45
17	♀	46	85	150	38	100	121	0.8	10	10	240	20	25	12	1	36
18	♂	32	62	175	31	86	95	0.9	8	14	250	23	40	15	0.9	48
19	♂	48	94	155	39	120	113	1.1	6	14	240	41	59	14	0.8	33
20	♂	28	73	166	26	97	104	0.9	8	10	310	20	30	10	0.7	35
21	♀	45	80	160	31	105	121	0.9	10	11	300	25	21	12	0.8	40
22	♀	42	81	160	31	104	120	0.9	9	9	300	24	20	13	0.8	46
23	♂	35	73	165	26	96	104	0.9	10	11	270	20	31	9	0.8	53
24	♂	30	94	155	39	120	113	1.1	6	13	300	18	60	9	0.8	29
25	♀	29	84	150	38	100	121	0.8	10	10	240	20	25	12	1	33

Clinical and laboratory data of group I (Gastric ulcer group)
Number of patients: 15 patients

No III	sex	age	Wt	Ht	BMI	W	Hip	W/H	WBC	Hb	Plt	ALT	AST	BUN	Cr	G
26	♂	20	70	175	26	82	112	0.7	6	13	120	20	21	11	0.8	110
27	♀	46	60	160	23	70	90	0.8	5	10	152	30	41	15	0.9	98
28	♀	28	45	150	20	70	95	0.7	4	11	211	30	42	13	0.9	130
29	♂	27	80	185	23	102	125	0.8	5	16	301	40	42	10	0.8	90
30	♂	26	63	182	21	80	99	0.8	7	14	120	30	28	10	1.2	119
31	♂	50	70	175	26	82	111	0.7	7	13	312	40	31	12	0.7	104
32	♀	42	65	165	25	93	113	0.8	4	10	287	30	21	11	0.6	89
33	♂	32	68	190	19	77	95	0.8	5	13	190	20	18	10	0.9	95
34	♂	35	60	165	22	82	92	0.9	6	12	212	30	28	11	0.5	85
35	♂	42	75	170	25	108	115	0.9	8	13	312	40	22	10	1.5	115
36	♂	35	57	170	20	80	95	0.8	6	10	270	30	21	11	0.8	113
37	♀	36	64	165	24	75	90	0.8	7	13	210	20	18	10	0.9	100
38	♂	30	67	188	19	76	95	0.8	6	14	280	30	20	11	1.3	92
39	♂	41	70	174	26	81	112	0.7	7	12	312	20	11	10	1.4	80
40	♂	45	80	185	23	102	125	0.8	8	10	219	21	10	11	1.3	82

Clinical and laboratory data of group I (Acute gastritis group)
Number of patients: 15patients

No IV	sex	age	Wt	Ht	BMI	W	Hip	W/H	WBC	Hb	Plt	ALT	AST	BUN	Cr	G
41	♀	25	71	165	26	95	110	0.9	6	10	297	31	34	10	0.6	60
42	♂	36	60	177	20	80	95	0.8	7.6	15	205	23	34	10	0.5	55
43	♀	50	50	158	20	82	92	0.9	10	7.8	523	35	21	16	1.1	65
44	♀	48	50	159	20	81	92	0.9	10	8	500	30	20	18	1	63
45	♂	49	70	160	27	90	105	0.9	8	10	420	11	20	20	1.2	58
46	♀	50	70	175	26	82	111	0.7	7	13	312	40	31	12	0.7	70
47	♂	42	65	165	25	93	113	0.8	4	10	287	30	21	11	0.6	63
48	♀	46	85	150	38	100	121	0.8	10	10	240	20	25	12	1	57
49	♀	32	62	175	31	86	95	0.9	8	14	250	23	40	15	0.9	53
50	♂	40	79	175	26	88	107	0.8	6.3	11.6	214	25	21	18	0.6	62

Clinical and laboratory data of group I (Control group)
Number of Subjects: 10 persons

INTRODUCTION

Ghrelin, first described by Kojima et al in 1999, is a 28-amino acid n-octanoylated peptide recently isolated from the rat stomach, placenta, kidney, pituitary and hypothalamus. **(Galas et al., 2002)**

Ghrelin, predominantly derived from the stomach, may target neuroendocrine networks within the central nervous system to regulate energy homeostasis. The novel hormone ghrelin is a potent orexigen that may counterbalance leptin **(Banks et al., 2002).**

Ghrelin is produced in a variety of human tissues, but messenger ribonucleic acid (mRNA) is most highly expressed in the epithelial cells lining the fundus of the stomach. **(Ariyasu et al., 2001)** Thus plasma ghrelin level decreased in chronic atrophic gastritis **(Hajim et al., 2005)**. Also plasma ghrelin level decreases by as much as 65% after gastrectomy, and this is consistent with the finding of decreased plasma level after gastric bypass surgery. **(Cummings et al., 2001)** As ghrelin has potential effects on gastric motility and acid secretion, **(St Pierre et al., 2003)** it is conceivable that ghrelin plays a pathophysiological role in various disease conditions of upper digestive tract.

Aim of work

To evaluate the status of serum ghrelin in different gastric diseases in some Egyptian patients.

Chapter 1:

GHRELIN

Ghrelin was first described in 1999 by Kojima et al. it was identified as the long-awaited endogenous ligand to the growth hormone (GH) secretagogue receptor (GHS-R) and a peripheral metabolic signal informing the brain about stomach nutrient load. Ghrelin quickly became the focus of intense investigation in many laboratories and was the subject of more than 600 publications by early 2004. **(Michael and Kevin, 2004)**

Structure of Ghrelin:

Ghrelin is synthesized as a preprohormone, and then proteolytically processed to yield a 28-amino acid peptide. An interesting and unique modification is imposed on the hormone during synthesis in the form of an n-octanoic acid bound to one of its amino acids, this modification is necessary for biologic activity **(Galas et al., 2002).**

Ghrelin is the only known natural peptide in mammalian biology in which acylation of one amino residue is required for at least the majority of its biological activities. Under the influence of a still unknown acyl-transferase, a hydroxyl group of serine at position 3 of the ghrelin molecule is octanoylated. This posttranslational modification of ghrelin is essential for binding and activation of the GHS-R 1a, for the GH-releasing capacity of ghrelin, and most likely also for its action on endocrine axis, energy balance, and glucose homeostasis. **(Aart et al., 2004).**

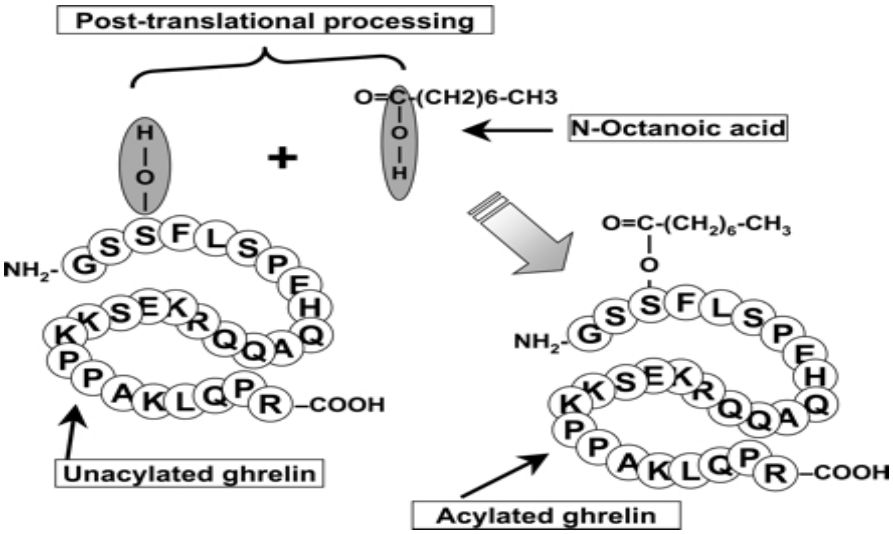

Fig (1): Post-translational processing of ghrelin molecule. (Aart et al., 2004).

Several naturally occurring variants of ghrelin have been reported based on the acylation at the serine-3 position, including nonacylated, octanoylated (C8:0), decanoylated (C10:0), and possibly decenoylated (C10:1) ghrelin. Any other synthetic variant of ghrelin with a chemical modification of either the acyl group or the N-terminal amino residue sequence did not activate or bind the receptor GHS-R 1a. However, ghrelin did still bind and activate the GHS-R 1a in vitro after modification or even significant deletion of C-terminal amino residues. It however remains unclear whether the same modalities are relevant in vivo. Although the major active form of human ghrelin is a 28-amino acid peptide with an octanoylation at the serine-3 position, the vast majority (80–90%) of circulating ghrelin has been found to be nonacylated. This predominant form of serum ghrelin seems to be devoid of any effects on endocrine axes or energy balance, as previously expected based on its inability to bind and activate GHS-R 1a, which is still the only identified ghrelin receptor. However, nonacylated ghrelin does have cardiovascular and antiproliferative effects and it

seems tempting to speculate that these activities are mediated by yet to be identified receptor families or subtypes. In the absence of further information on the tissue specificity, reversibility, balance, and enzyme kinetics of the (des-) octanoylation process, the information one can possibly gain from plasma ghrelin quantification is very limited but should include the analysis of both total and acylated ghrelin **(Aart et al., 2004).**

Ghrelin receptor and its mechanism of action:

Ghrelin displays strong GH-releasing activity, which is mediated by the activation of the so-called GH secretagogue (GHS) receptor type 1a (GHS-R1a). Before the discovery of ghrelin, this orphan receptor had been shown to be specific for a family of synthetic, peptidyl and nonpeptidyl GHS. GHS-Rs are concentrated in the hypothalamus-pituitary unit but are also distributed in other central and peripheral tissues **(Aart et al., 2004).**

The natural ligand for the GHS-R was announced in 1999 as ghrelin, and ghrelin was named for its ability to provoke growth hormone secretion (the suffix ghre means "grow") **(Yoshihara et al., 2002).**

The discovery of this receptor followed by the discovery of synthetic GHS, which specifically binds it. This makes the discovery of ghrelin an example of reverse pharmacology, which in this case means that it started with the synthesis of analogs and it ended with the discovery of a natural ligand via the discovery of a natural receptor. Synthetic GHS are a family of ligands, including peptidyl and nonpeptidyl molecules. The first synthesized molecules were non-natural peptides [GH-releasing peptides GHRPs)] that were designed by Bowers and Momany in the late 1970s. They were

metenkephalin derivatives devoid of any opioid activity. **(Aart et al., 2004).**

GH secretagogues act through a specific G protein-coupled receptor. Two types of GHS-R cDNAs have been identified and designated receptors 1a and 1b. cDNA 1a encodes a receptor, named GHS-R 1a, of 366 amino acids with seven transmembrane 41 kDa. The 1b cDNA encodes a shorter form, named the GHS-R 1b, which consists of 289 amino acids with only five-transmembrane regions. **(Camina et al., 2003)**

The human GHS-R 1a shares 96 and 93% identity with the rat and pig GHS-R 1a, respectively, which strongly suggest that the GHS-R 1a is highly conserved across the species and probably does have an essential biological function.

The binding of ghrelin and synthetic to the GHS-R 1a activates the hospholipase C signaling pathway, leading to increased inositol phosphate turnover and protein kinase C activation, followed by the release of Ca^{+2} from intracellular stores. GHS-R activation also leads to an inhibition of K^{+} channels, allowing the entry of Ca^{+2} through voltage-gated L-type, but not T-type channels **(Aart et al., 2004)**

Activation of intracellular calcium mobilization is one of the earliest known cellular signals elicited by ghrelin in HEK-293 cells expressing the GHS-R1a, ghrelin induces a biphasic cytocalcium elevation characterized by a spike phase of the response, which is due to calcium influx across the plasma membrane triggered by aperture of capacitative calcium channels (store-operated calcium channels). Upon repeated administration, ghrelin showed a marked suppression of ghrelin-mediated elevations of intracellular calcium. This homologous desensitization represents an important physiological mechanism that modulates receptor