

## ORIGINAL ARTICLE

## EFFECT OF GHRELIN ON ERYTHROPOIETIC INDICATORS IN MYELOSUPPRESSED RATS

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**Background:** Ghrelin is a 28-amino acid acylated peptide released from the enteroendocrine cells of stomach. Aim of the study was to find out the possible beneficial role of ghrelin in erythropoiesis of myelosuppressed rats and its possible mediator. **Methods:** This randomized control trial was carried out at the Department of Physiology Foundation University Medical College in collaboration with the National Institute of Health, Islamabad, from February 2013 to June 2014. A total of 130 Sprague-Dawley rats were randomly divided into four groups. Group I was treated with ghrelin only (1 nmol/100µl), group II received intraperitoneal carboplatin and 5-fluorouracil (5-FU) only while group III received chemotherapy with rat ghrelin. Group IV received growth hormone releasing hormone antagonist in addition to chemotherapy and ghrelin. Erythrocyte count, haemoglobin, reticulocytes count and serum growth hormone levels were measured on day 7 and 14. **Results:** The erythrocyte count, haemoglobin and reticulocytes count showed a drop on day 14 in group II, III and IV. This fall was significantly less in group III as compared to group II and IV. The serum growth hormone levels in group III were significantly higher as compared to group IV ( $p<0.05$ ). **Conclusions:** In myelosuppressed rats treated with rat ghrelin, the reduction in erythrocyte count, haemoglobin and reticulocyte count was less as compared to myelosuppressed rats suggesting valuable role of ghrelin in stimulating erythropoiesis. While decreased erythrocyte count and growth hormone level in group treated with chemotherapy, ghrelin and GHRH antagonist suggest growth hormone as possible mediator of ghrelin in erythropoiesis.

**Keywords:** Ghrelin, Growth hormone, Erythropoiesis, Reticulocyte, Myelosuppression

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## INTRODUCTION

Ghrelin, a 28-amino acid peptide is endogenous ligand for growth hormone secretagogue receptor (GHS-R).<sup>1</sup> It is produced mainly by the enteroendocrine cells of stomach and is considered as a potent circulating orexigenic hormone regulating food intake, energy expenditure, adiposity and growth hormone (GH) secretion.<sup>2</sup> The wide distribution of ghrelin and its receptors is attributed to its diverse biological functions. It has been proposed that ghrelin receptors are distributed on immune cells and it causes release of growth hormone from these immune cells.<sup>3</sup>

The growth hormone can act in autocrine or paracrine manner in the bone marrow.<sup>4</sup> Growth hormone response to ghrelin can be suppressed by inhibiting endogenous growth hormone releasing hormone system.<sup>5</sup> It has been shown that nearly 80% of the growth hormone secretagogue mediated growth hormone release is suppressed by administration of a growth hormone releasing hormone antagonist.<sup>6</sup> The beneficial effects of growth hormone in stimulating erythropoiesis has been observed in number of studies.<sup>7,8</sup> Growth hormone has been shown to stimulate haematopoietic cell recovery and lower the side effect caused by myelosuppression.<sup>7</sup> Recombinant human growth hormone increases colony formation by human myeloid and erythroid progenitors *in vitro* and can

synergize with other cytokine such as granulocyte monocyte colony stimulating factor in haematopoiesis.<sup>9</sup>

There is conflicting literature available related to the role of ghrelin in erythropoiesis. The beneficial effects of ghrelin in stimulating erythropoiesis have been observed in a study done in Guilan University of Iran. The study reported an increase in hematocrit percentage and erythrocyte count in ghrelin treated rats.<sup>10</sup> The positive effect of ghrelin in stimulating erythropoiesis can also prove beneficial for combating side effects of hypoxia.<sup>11</sup> This is further supported by the observed low levels of ghrelin in patients of iron deficiency anaemia. The low levels of ghrelin in anaemic patients result in anorexia with consequent delay in growth and development.<sup>12</sup> On the other hand, study conducted by Narin showed no beneficial effect of ghrelin on red blood cell and haemoglobin concentration.<sup>13</sup> Similarly intracerebrovascular administration of rat ghrelin showed no significant effect on erythropoietic activity of broiler chicken.<sup>14</sup> The present study was designed to find out the probable role of ghrelin in erythropoiesis of myelosuppressed rats and whether the effect is mediated by GH release.

## MATERIAL AND METHODS

This randomized control trial was conducted at Department of Physiology, Foundation University Medical College, Islamabad, in collaboration with

National Institute of Health, Islamabad. Permission from Ethical Committee of Foundation University Medical College was obtained prior to commencement of study.

Animal house facility of National Institute of Health, Islamabad was utilised. A total of 130 healthy male *Sprague Dawley* rats, 3–4 months old and weighing 250–300 g were selected. They were divided randomly into 4 groups with 30 rats each. Remaining 10 rats served as control. Free access to food and water was provided and room was well ventilated with controlled temperature range of 20–22 °C. A 12-hours light and dark cycle was maintained.

Group I (Ghrelin treated group, n=30) was treated daily with subcutaneous ghrelin (Bioworld USA-Cat No: 21970208) in a dose of 1  $\eta$ mol/100  $\mu$ l N/S for 14 days.<sup>10</sup> Group II (Chemotherapy drug-treated group, n=30) was administered carboplatin intraperitoneally to each rat in a dose of 70 mg/Kg on the first day followed by intraperitoneal administration of 5-FU in a dose of 50 mg/Kg/day on the 2<sup>nd</sup> and 3<sup>rd</sup> days to induce myelosuppression.<sup>7</sup> Group III (Chemotherapy and ghrelin treated group, n=30) was treated with both chemotherapy drugs for 3 days and ghrelin for 14 days. Group IV (Chemotherapy, ghrelin and growth hormone releasing hormone antagonist treated group, n=30) in addition to chemotherapy and ghrelin, each rat was administered GHRH antagonist (Sigma Aldrich-USA Cat No: SAB1300904) intravenously in a dose of 400  $\mu$ g/Kg daily for 14 days to counter growth hormone releasing effect of ghrelin if any.<sup>5</sup>

Proper anaesthesia was given with ether and terminal intracardiac blood sampling of 10 rats was done on day 0 and sampling of group I–IV was done on day 7 and 14. Ten rats were sacrificed in each group at each time point. Blood (2.5 ml) was transferred into the vacutainers containing ethylenediaminetetraacetic acid (EDTA) while rest of samples (2.5 ml) were centrifuged to separate serum. Red blood cell count, haemoglobin, and reticulocytes were estimated by using automated haematology cell analyser Sysmex-xt 2000i. Enzyme linked immunosorbent assay was used to measure serum growth hormone (ELISA Kit, Millipore Inc. USA Cat No: EZRMGH-45K was used).

SPSS-17 was used for statistical analysis. Descriptive statistics were expressed along with Means $\pm$ SD. The statistical significance of the difference of various quantitative changes was evaluated by ANOVA followed by Tukey HSD (honestly significant difference) *post hoc test* for multiple comparisons. The difference was regarded as significant at  $p < 0.05$ .

## RESULTS

The haemoglobin, red blood cell counts, reticulocyte count, and growth hormone levels showed an increase in group I, and drop in group II and group III compared to control group (Table-1 and 2).

The drop in red blood cell count and reticulocyte count on day 14 in group III was less as compared to group II ( $p < 0.05$ ) and group IV ( $p < 0.05$ ) showing beneficial role of ghrelin in stimulating erythropoiesis. The serum growth hormone levels on the other hand were significantly less in group II and group IV as compared to group III on both days ( $p < 0.05$ ) (Table-2 and 3).

**Table-1: Haematological parameters and serum growth hormone levels of group I vs group III on day 7 and 14**

Parameter	Day	Control Day zero	Group I	Group III	p
Haemoglobin (g/l)	7	120.6 $\pm$ 9.15	133.5 $\pm$ 10.89	120.0 $\pm$ 13.46	0.10
	14		130.61 $\pm$ 5.11	89.8 $\pm$ 8.6	<0.001*
RBC count ( $\times 10^{12}$ )	7	7.03 $\pm$ 0.87	7.42 $\pm$ 1.03	7.61 $\pm$ 0.63	1.00
	14		8.19 $\pm$ 0.45	6.35 $\pm$ 0.71	<0.001*
Reticulocyte (%)	7	1.55 $\pm$ 0.61	2.28 $\pm$ 0.61	1.68 $\pm$ 0.65	0.218
	14		2.39 $\pm$ 0.52	2.37 $\pm$ 0.70	1.00
Growth hormone ( $\eta$ g/ml)	7	8.57 $\pm$ 4.96	11.28 $\pm$ 2.98	10.24 $\pm$ 1.73	0.99
	14		12.63 $\pm$ 4.08	13.09 $\pm$ 0.41	0.98

\*significant

**Table-2: Haematological parameters and serum growth hormone levels of group II vs group III on day 7 and 14**

Parameter	Day	Control Day zero	Group II	Group III	p
Haemoglobin (g/l)	7	120.6 $\pm$ 9.15	114.9 $\pm$ 14.9	120.0 $\pm$ 13.46	0.974
	14		74.4 $\pm$ 8.9	89.8 $\pm$ 8.6	0.038*
RBC count ( $\times 10^{12}$ )	7	7.03 $\pm$ 0.87	6.86 $\pm$ 0.72	7.61 $\pm$ 0.63	0.466
	14		5.16 $\pm$ 1.03	6.35 $\pm$ 0.71	0.031*
Reticulocyte (%)	7	1.55 $\pm$ 0.61	0.71 $\pm$ 0.32	1.68 $\pm$ 0.65	0.003*
	14		1.08 $\pm$ 0.29	2.37 $\pm$ 0.70	<0.001*
Growth hormone ( $\eta$ g/ml)	7	8.57 $\pm$ 4.96	3.24 $\pm$ 0.73	10.24 $\pm$ 1.73	<0.001*
	14		5.12 $\pm$ 0.67	13.09 $\pm$ 0.41	<0.001*

\*significant

**Table-3: Haematological parameters and serum growth hormone levels of group III vs. group IV on day 7 and 14**

Parameter	Day	Control Day zero	Group III	Group IV	p
Haemoglobin (g/l)	7	120.6 $\pm$ 9.15	120.0 $\pm$ 13.46	114.9 $\pm$ 11.34	0.974
	14		89.8 $\pm$ 8.6	75.3 $\pm$ 8.3	0.063
RBC ( $\times 10^{12}$ )	7	7.03 $\pm$ 0.87	7.61 $\pm$ 0.63	6.73 $\pm$ 0.917	0.256
	14		6.35 $\pm$ 0.71	4.72 $\pm$ 0.52	0.000*
Reticulocyte (%)	7	1.55 $\pm$ 0.61	1.68 $\pm$ 0.65	0.85 $\pm$ 0.33	0.018*
	14		2.37 $\pm$ 0.70	0.88 $\pm$ 0.44	<0.001*
Growth hormone ( $\eta$ g/ml)	7	8.57 $\pm$ 4.96	10.24 $\pm$ 1.73	4.53 $\pm$ 0.31	<0.001*
	14		13.09 $\pm$ 0.41	5.00 $\pm$ 1.88	<0.001*

\*significant

## DISCUSSION

Erythrocyte count and haemoglobin level showed a delayed response to chemotherapy because of their longer life span. Ghrelin has been shown to stimulate erythropoiesis. The reduction in erythrocyte count and haemoglobin level was less in ghrelin treated

myelosuppressed rats. It has been observed previously that ghrelin resulted in an increase in hematocrit percentage and red blood cell count. Similarly, subcutaneous ghrelin administration to hypoxic animals induced polycythemia in rats which was a beneficial compensation for hypoxia.<sup>11</sup> The results of these studies are consistent with our findings. The study conducted by Taati *et al*<sup>10</sup> also showed that ghrelin increased red blood cell count and hematocrit, but it had no effect on haemoglobin concentration. The study conducted by Akarsu *et al*<sup>12</sup> stated that there was a significant relation between haemoglobin concentration and peripheral ghrelin level. It was shown that patients with iron deficiency anaemia had low peripheral ghrelin levels.<sup>12</sup> Other studies have also indicated that low blood ghrelin levels could be the cause of anaemia. The results are consistent with findings of current study. On the other hand, studies conducted by Narin *et al*<sup>13</sup> and Aghdam *et al*<sup>14</sup> did not show beneficial effect of ghrelin on stimulation of erythropoiesis. These differences can be attributed to the difference in animal species, method and dose of drug administration, and time interval between treatment and sampling.

It has been suggested by some studies that this effect of ghrelin on erythropoiesis may be due to direct effects of ghrelin on cell division and replication in bone marrow.<sup>15</sup> However other studies have shown that most important function of ghrelin is growth hormone secretion.<sup>16</sup> Petri *et al*<sup>17</sup> have also shown that that growth hormone has a stimulatory effect on erythropoietin in mammals thus enhancing erythropoiesis. The involvement of growth hormone as possible mediator of ghrelin in haematopoiesis is supported by results of present study. There was no significant change in erythropoietic activity of GHRH antagonist treated group as compared to the chemotherapy group. The growth hormone levels also failed to increase in this group suggesting that growth hormone secretagogue activity of ghrelin is essential for ghrelin to stimulate erythropoiesis.

## CONCLUSION

There is a beneficial role of ghrelin in stimulating erythropoiesis of myelosuppressed rats. Growth hormone may be acting as possible mediator of ghrelin in erythropoiesis.

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