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Pre/post-surgical investigation of some angiogenic factors related with cancer and obesity

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Abstract

Objective: Obesity is one of the important health problem for developed and developing countries. Due to literature some obesity related factors may trigger the tumor formation. For tumor development, tumoregenic cell and tissue needs to oxygen and nutrients. Once the tumor has developed, it stimulates the angiogenesis by producing chemical signals and grows by supplied oxygen and nutrients with newly formed vessels. Aim of this study is to compare some angiogenic factors before and after surgery which will supply more information about the link between the cancer and obesity.

Material and Methods: Serum samples were obtained before and 48 hours after surgery. Adrenomedullin, vascular endothelial growth factor, hipoxia inducible factor1-α and matrix metallo proteinase-2 levels were investigated in cancerous and noncancer patients. Angiogenic factors were assayed by ELISA method.

Results: Higher levels of angiogenic factor were detected in cancer tissues more than in non-cancer tissues, in pre-obese and obese patients. It is seen that in humans, in post-operated patients angiogenic factors are higher in obese individuals, while non-cancer patients are also higher in obese groups and angiogenic factors which stimulate angiogenesis.

Conclusion: In the treatment of cancer, as defined previously the anti-angiogenic factors should be considered and applied as epigenetic phenomen. Due to our finding cancer related elevated angiogenic and growth factor biochemicals had been also increased in obes population and in cancer patients with surgical intervention. In the light of literature and our findings, instead of drugs, radiation therapy or surgery, which have many side effects in the treatment of cancer, we need to focus to this epigenetic phenomen for cancer patients. In general, we can say that both obesity and surgical applications lead to an increase in angiogenic factor levels, and that the healing process of wounds causes a marked increase in angiogenic factor levels

Keywords: Angiogenic Factor, Surgery, Obesity, Cancer

Introduction

Vessel formation is a process which starting with embryonic stage and is an ongoing process not only in a healthy state but also in disease states such as cancerogenous. Vessel formation, that is, angiogenesis, is caused by the sprouting of new blood vessels from endothelial precursor cells. Signal, may come from embryonic development, wound healing or the tumor cell (1). In adult organisms, angiogenesis is necessary for many processes such as wound healing, growth, sex hormone releasing and formation of vessel tissue during pregnancy (2,3). Also tissue hypoxia causes sprouting angiogenesis and budding of a new capillary sprout laterally from a preexisting vessel (4,5). Briefly vasculogenesis is de novo formation of blood vessels from endothelial cell procursors (6). The World Health Organization defines obesity as an accumulation of excess fat tissue that causes a risk to health. According to the World Health Organization (WHO 1998) (7), individuals with Body Mass Index (BMI) over 30 evaluates as obese. Overweight and obesity are seriously risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings. The National Cancer Institute states that there is a strong relationship between elevated BMI in men and an increased risk of colerectal cancer. Obesity is also associated with increased risks of the

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following cancer types, and possibly others as well; esophagus, pancreas, colon and rectum, breast (after menopause), endometrium, kidney, thyroid, gallbladder. Angiogenesis is inevitable necessary for tumor growth and methasthasis of tumor cells to distant locations. Hypoxia is a critical process for tumor angiogenesis and is carried out primarily by the transcription of hypoxia-sensitive genes and HIF (8). Several mechanisms were considered for vascularization of tumors including endothelial sprouting and bone marrow-derived endothelial cells and so forth. Endothelial sprouting is a process that is controlled by balance between ″pro- and anti-angiogenic″ factors. Endothelial sprouting is a basic mechanism for tumor vascularization. During sprouting, pericytes detach and blood vessels dilate and the process is under control of VEGF and angiopoietins (9). During bone marrow-derived endothelial cell process, circulating cells in the peripheral blood may participate in vessel formation (10). The purpose of our work is to examine the levels of some angiogenic factors in pre- and post-operative individuals with and without obese and cancer and non-cancerous individuals.

The gelatinases (MMP-2 and MMP-9) are subsequently enriched by a region of three-fi bronectin type II repeats within their catalytic domains. Those two enzymes are responsible for the final degradation of fibral collagens after initial cleavage by collagenases (11). Many publications link increased expression of gelatinases as key proteases with malignant tumor ability to metastasis. According to cancer development data, degradation of extracellular matrix is crucial for malignant tumor development and spread and thus has an indispensable role in prognosis and selection of the therapy method. Detection of active MMP-2 (e.g. in circulating blood) could be more sensitive than other, well-established methods (12). Human matrix metalloproteinases (MMPs, matrixins) are a family of over 20 different endopeptidases that are able to degrade various components of the extracellular matrix (ECM). There are several preclinical research publications confirms MMP-2 involvement in to all human cancers. A high level of MMP-2 has been shown to predict adverse outcome in patients with gastric, pancreatic, and prostate cancers (13). Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix (ECM) in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. VEGF is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate such as in hypoxic conditions (14). VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels. The overexpression of VEGF is a contributing factor to the development of disease. For example, solid tumors require an increased blood supply if they are to continue growing beyond a certain size and tumors that express VEGF are able to continue growing because they can develop this enhanced blood supply, a process referred to as angiogenesis. Cancers that express VEGF are therefore able to grow and spread (metastasize) to other organs and regions of the body. Hypoxia-inducible factor-1 (HIF1) is a heterodimeric transcriptional complex and important molecule in the regulation of oxygen levels in mammals, especially in hypoxic tissues (15,16). Increase of HIF-1α transcription is considiring the result of hypoxia-stimulation (17). HIF-1α is a transcription factor that is involved in tumor growth and methasthasis, especially in response to hypoxia. In some studies, results has been shown that HIF-1α protein expression increased in some human cancer types (18). The proliferation of cancer cell reflects the rate of angiogenesis, cancer cell proliferation causes hypoxia in the tissue, and cellular adaptation to hypoxia is the key step in the formation of the tumor. This adaptation is regulated by HIF-1α and plays an important role in oxygen homeostasis (15). Adrenomedullin (AdM) is a multipotentially, regulatory peptide that was first isolated from human pheochromocytoma extracts by Kitamura et al. (1993) (19). AdM is synthesized both by tumour cells and by normal adrenal medulla cells, as well as by many other tissues including macrophages, mast cells, endothelial, and vascular smooth muscle cells. (20). It has also as a local paracrine and autocrine functions with multiple biological activities such as vasodilatation, cell growth, regulation of hormone secretion, natriuresis, and antimicrobial effects (21). Larrayoz et al. (2014) have been stated that adrenomedullin increased tumor cell proliferation, stimulated angiogenesis, and suppressed the immune system. Adrenomedullin is also activated by HIF and it has been considered essential for survival of tumor cells (22).

Material and method

Serum samples were obtained from 25 cancer patients and 22 non-cancer patients before and 48 hours after surgery. Approximately 5 ml of blood was collected in EDTA- or heparin-containing tubes. Samples were centrifuged at 8000 g for 15 min, followed by collection of the plasma. Plasma samples were stored at 80 oC until elisa assays were performed. In our study, serum samples were taken from 9 obese, 7 pre-obese and 9 normal-weight cancer patients and non-cancer patients in 10 obese, 7 pre-obese and 5 normal weights. Adrenomedullin (AdM) vascular endothelial growth factor (VEGF), hipoxia inducible factor-1 alpha (HIF1-α) and matrix metallo proteinase-2 (MMP-2) levels were investigated for all cases. Angiogenic factor levels were assayed colorimetrically by ELISA kits(AdM; CK-30105,

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Hangzhou Eastbiopharm Co., Ltd. VEGF; EK0540 Booster Biological Technology Ltd, MMP-2; ER0051, Fine Test, HIF1-α; ER0191, Fine Test) (23). Analysis of angiogenic factors in serum samples from patients was performed at Inonu University Molecular Biology Laboratory. Differences in results between pre-op/post-op and cancer/noncancer groups were analyzed using an unmatched Student t test. p value < 0.05 was considered statistically significant.

Results

Angiogenic factor levels are shown in Table 1. According to Table 1, it is seen that angiogenic factor levels are about 2-fold increased, especially in cancerous in blood serum samples of cancer patient. Similarly, angiogenic factor levels increase after surgery. Adrenomedullin and HIF1-α levelsin post-op cancer group increased when compared to other groups. Especially in patients with cancer, an increase of about 50% compared to those without cancer was detected. However, MMP-2 and VEGF levels increased by 90-100% in post-op cancer group compared to pre-op cancer group. When the increases observed in angiogenic factor levels in the obese group were compared proportionally, a more significant increase was observed in cancer patients. Especially, VEGF, MMP-2 and HIF1-α levels in cancer patients after surgery increased two-fold compared to normal weight; it was 1.4 times more in obese groups of non-cancer patients (Table 1, Figure 1-4).

The highest ADM level was found to be 332,85 ± 8,52 ng /l as the result of surgical application in cancer patients and obese individuals (P <0,05). ADM levels were found to be higher in non-cancer but obese individuals (Figure 1). VEGF levels have also increased after surgery in cancer and obese patients, as in ADM levels. VEGF level was 238,39 ± 12,23 ng / l after surgery in cancer and obese patients (P <0,05) (Figure 2). MMP-2 levels in cancer and obese patients after surgery increased two-fold compared to pre-operative and normal weight patients. MMP-2 levels were 40.02 ± 2.07 ng / L (P <0.05), 18.05 ± 1.05 ng / l and 19.56 ± 1.22 ng / l, respectively (Figure 3). HIF1-α level was measured as 182,67 ± 4,92 (pg / l) in cancer and obese group after crush application (P <0,05) (Figure 4). Similarly, there was an increase in angiogenic factor levels after the operation. Proportionally, the highest increase was observed in VEGF and HIF levels in cancer patients. These results indicate that the angiogenic factor levels are increased due to the healing process of surgical wound healing after surgery.

Table 1: Angiogenic factor levels

Adrenomedullin Levels (ng/l)				
	Cancer		**Non-Cancer**	
Groups	Pre-op	Post-op	Pre-op	Post-op
Obese	199,91±5,57	332,85±8,52*	187,12±7,42	262,02±11,02*
Pre-obese	142,66±4,76	262,85±6,21	161,90±6,27	245,60±10,56
Normal Weight	118,32±4,32	197,79±9,07	126,65±6,35	191,56±8,15
Vascular Endothelial Growht Factor Levels (ng/l)				
Groups	Pre-op	Post-op	Pre-op	Post-op
Obese	119,04±5,22	238,39±12,23*	82,43±5,26	121,55±7,54*
Pre-obese	93,17±3,88	182,45±10,08	66,39±3,88	98,29±5,55
Normal Weight	66,92±4,32	116,46±8,45	54,95±3,05	87,93±4,27
Matrix Metalloproteinase-II Levels (ng/l)				
Groups	Pre-op	Post-op	Pre-op	Post-op
Obese	18,05±1,05	40,02±2,07*	11,27±0,89	17,42±1,22*
Pre-obese	14,40±2,31	32,61±1,85	9,63±1,21	14,61±0,84
Normal Weight	10,87±1,52	19,56±1,22	8,75±0,76	13,22±0,69
Hipoxic Inducible Factor1-α Levels (pg/l)				
Groups	Pre-op	Post-op	Pre-op	Post-op
Obese	85,39±2,47	182,67±4,92*	83,26±3,68	121,08±5,27*
Pre-obese	72,51±2,09	149,23±5,12	70,50±3,08	112,63±5,18
Normal Weight	57,01±1,64	99,46±5,08	56,13±2,88	90,08±4,85

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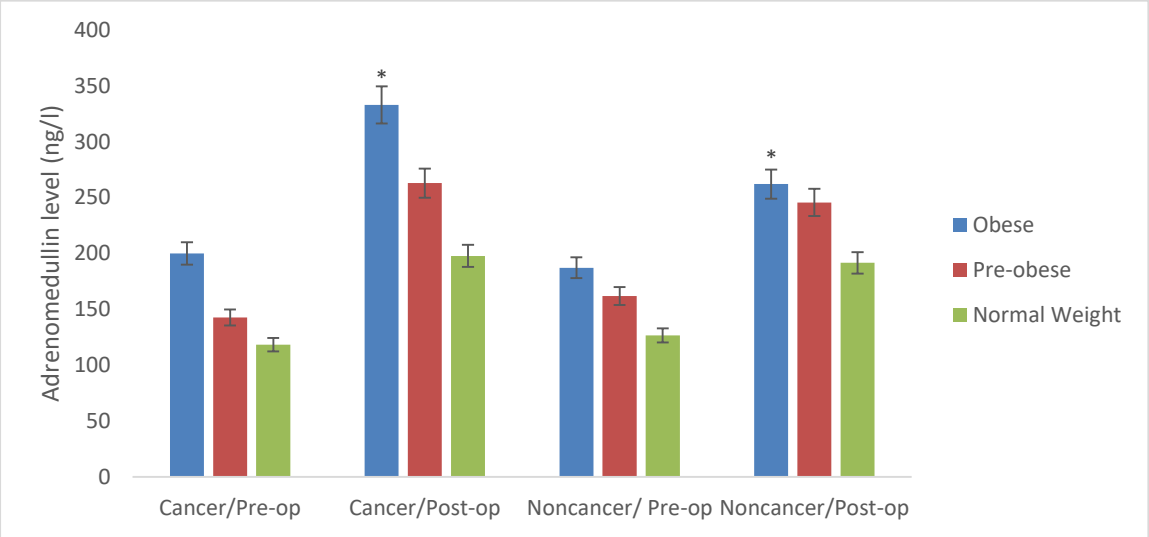

Figure 1: Adrenomedullin levels (*Significant on 0,05 levels).

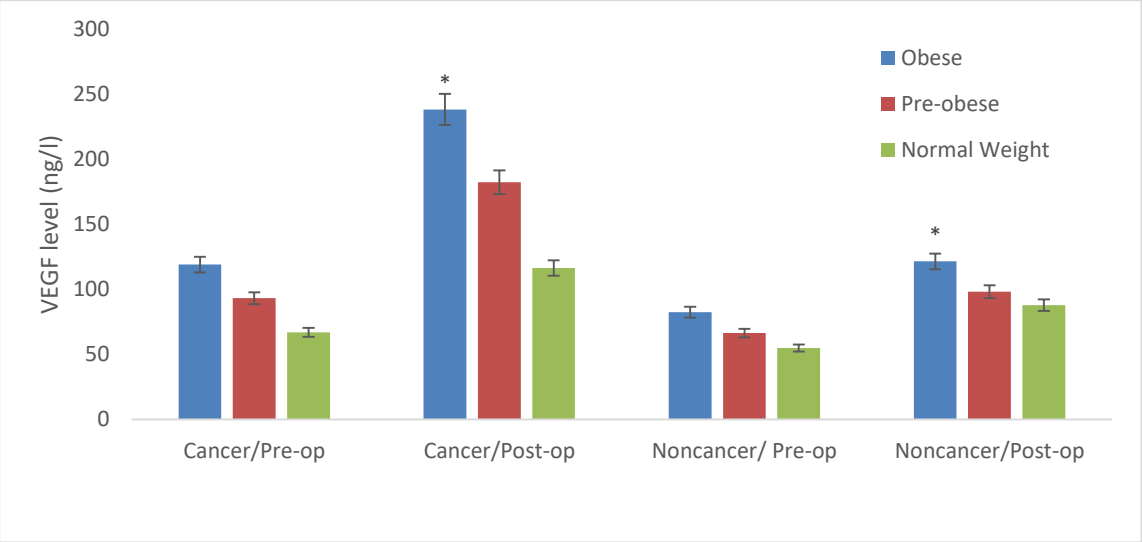

Figure 2: VEGF levels (*Significant on 0,05 levels).

Discussion

Numerous studies have been performed to reveal the relationship between surgical intervention and angiogenic factor release. Belizon et al. (2006) (24), Kong et al. (2010) (25) reported significant increases in postoperative VEGF levels in their study. Jarmila et al. (2004) (26) reported that surgery is a condition that stimulates angiogenesis and causes the release of many angiogenic factors.

For tumor development, it needs oxygen and once the tumor has developed it stimulates the formation of new blood vessels by producing chemical signals and grows by providing more oxygen and nutrients with newly formed vessels. Angiogenesis is a process that involves many steps.

This means that every step in this process is a potential target for new cancer treatments. The hope here is that the tumor can not get enough nutrients and oxygen to reach the end hunger and death. Inhibitors of angiogenesis or anti-angiogenic drugs may play an important role in the treatment of certain types of cancer by inhibiting new blood vessel formation. It should be thought that drugs can show their effect here in the epigenetic process. Angiogenic factors can contribute to tumor formation while providing vascularity for the organism. For example, it is now well known that adrenomedullin is a peptide with multiple effects and it may have a protective function as antioxidant, but it may also cause harmful effects by participating in tumor angiogenesis. The need for

organisms to be regulated in such a way as to bring about positive effects of these chemical molecules will accelerate their efforts to inhibit the formation of new blood vessels, which contribute to the development and spread of cancer. As a result, surgical operations, chemotherapy, and radiotherapy treatments will contribute significantly to molecular-physiological measures.

Hypoxia inducible factor 1-alpha (HIF 1-a) is particularly found in obesity, with a body mass index of 30 and above; Due to the increase of the body mass, the formation of oxygen-free environment will cause this factor to be released and oxygen and nutrients will be provided by the formation of new veins in the tissues. Matrix metallo proteinase enzymes contribute to the formation of new blood vessels by weakening the blood vessel wall, while VEGF also provides new blood vessel formation as a growth factor. Other angiogenic factors such as these also provide vascularity. The important difference is that if the tumor formation starts in some way for some reason, the resulting tumor becomes an uncontrollable growth that negatively affects the life of the living by using this system which is not alien to itself. The mission of researchers should be to prevent the tumor from abusing this system that the organism possesses.

Recent studies suggest anti-anjiogenic factors may be used in the treatment of cancer. Clinical experience suggests that anti-angiogenic therapy is a valid approach to medicine, but that it needs to work harder to make it realistic (27-29).

While the matrix metalloproteinase enzyme causes the destruction of adrenomedullin, hypoxia causes an increase in adrenomedullin (22). Hypoxia may develop due to obesity (30), which is expected to increase in adrenomedullin level; It is expected that the increased MMP-II enzyme due to tumor formation will also destroy adrenomedullin. This reflects a paradoxical situation. In our study, also more adrenomedullin and VEGF were detected in cancerous tissues. As we mentioned earlier, angiogenesis is a process that emerges with the unification of many steps and this process can explain the paradoxical situation.

Conclusion

The angiogenesis process is a convergence of many steps. This means that each step in this process is a potential target for new cancer treatments. The hope here is that the tumor will be disappear depending on the lack of nutrients and oxygen. Rationalized antiangiogenic process should be considered additional vehicle in the treatment of cancer. In the light of literature and our findings, instead of drugs, radiation therapy or surgery, which have many side effects in the treatment of cancer, we need to focus to this epigenetic phenomen for cancer patients.. In our study, we compared preoperative and postoperative levels of some angiogenic factors in non-cancer and cancerous and non-obese and obese individuals We are postulating that the angiogenesis process should be evaulated as an independent parameter. Because the organism will effort to maintain its instinctual life in every adverse situation and will continue the angiogenesis process for the life of the tissues, regardless of the disease or health. More importantly, it is to be thought that the angiogenesis process can be used more effectively, in cancer cases. Antianogenogenic factors may also affect normal tissues with the tumor, so it is important to consider studies that would inhibit blood transfusion to other tissues outside the tumor tissue.

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Ethical issues: All Authors declare that Originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits.

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