Evaluation of IGF1 Serum Levels in Malignant Melanoma and Healthy Subjects

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Abstract. *Background/Aim: There were two aims in the present study. The first was to evaluate the usefulness of insulin-like growth factor 1 (IGF1) for melanoma detection. The second was to correlate changes of serum levels of IGF1 with the Breslow score and sentinel node metastasis positivity. Patients and Methods: We examined a group of 216 cases, 77 patients with melanomas and 139 healthy probands. We determined the serum IGF1 levels of each patient using an IRMA radioisotope IGF1 assay kit. Serum samples were collected prior to surgery or any other form of treatment. All melanoma diagnoses were histologically verified. Results and Discussion: Based on the statistical evaluation between the melanoma group and group of healthy individuals, we observed statistically significant differences in IGF1 serum levels. The median IGF1 levels in the melanoma group was 154.1 ng/ml compared to 111.2 ng/ml in the group of healthy individuals (p=0.0036). The changes of the IGF1 levels related to the Breslow score categories were statistically significant (p=0.0027). Lastly, we compared the results between the positive and negative metastatic affection of the sentinel nodes. The median IGF1 levels in the negative group was 173.5 ng/ml compared to 205.8 ng/ml in the positive group. This difference was statistically significant (p=0.0407). Conclusion: Serum levels of IGF1 were significantly higher in patients diagnosed with melanoma compared to the healthy control group. The changes of the IGF1 levels related to the Breslow score categories were statistically significant. Serum levels of IGF1 were significantly higher in the group with the positive metastatic affection of the sentinel nodes than in negative patients.*

Melanoma is a malignant tumor originating from the melanin-producing cells – melanocytes (1). The incidence of melanomas has been increasing over the past decades. According to the World Health Organization (WHO) statistics 132,000 melanoma skin cancers occur currently each year globally. Skin cancer is being diagnosed in every third cancer. As the ozone level has been depleting, an increase of melanoma incidence has been estimated for the future (2, 3). The insulin-like growth factor 1 (IGF1) plays an important role in the growth, differentiation and development of the cells (4). IGF1 is also involved in the pathogenesis of various types of human neoplasias (5).

The aim of this work was to evaluate the usefulness of IGF1 in melanoma detection and to correlate the changes of serum levels of IGF1 with the Breslow score and sentinel node metastasis positivity.

Patients and Methods

Patients. Serum levels of IGF1 in a group of 216 cases were measured from January 2010 till June 2012. This group consisted of 77 patients with melanomas. The second one, a control group of the healthy probands, consisted of 139 persons. We compared the serum levels of IGF1 between these two groups of individuals. For a detailed evaluation of the melanoma group, we stratified this group into the four sub-groups based on the Breslow score. In the last step we created two subgroups of melanoma patients according the metastasis positivity in the sentinel nodes. The first sub-group consisted of 11 patients with the metastasis positivity and the second one consisted of 33 persons without metastasis findings. All melanoma diagnoses were histologically verified.

Serum samples. Samples of venous blood were collected using the VACUETTE blood collection system (Greiner Bio-one Company, Kremsmünster, Austria). Blood was centrifuged for 10 min at 1,700

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×g. Serum samples were immediately frozen to –80°C. Samples were thawed only once, just prior to analyses. Serum samples were collected at the time of diagnosis, prior to surgery or any other form of treatment.

Methods. IGF1 serum levels were measured using the immunoradiometric assay (IRMA) radioisotope IGF1 assay kit (Immunotech S.A., Marseille, France).

Statistical analyis. The SAS 9.2 (Statistical Analysis Software release 9.2; SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. A summary of statistical findings for age and serum levels of IGF1 is presented (Tables I, II). The Wilcoxon test was used to compare distributions of values between the melanoma and the control group. The Kruskal-Wallis test was used for the evaluation of changes of the IGF1 levels through Breslow score categories (Table III). The Wilcoxon test was used to compare distributions of IGF1 values in the sentinel nodes groups with and without metastasis affection (Table IV). We evaluated the age differences between the patients' groups very precisely. The age adjustment was used in the cases of a statistical difference between the compared groups.

Results

The age characteristics of both groups of patients are shown in Table I. Statistical evaluation between the melanoma group and the group of healthy persons is shown in the Table II. We observed statistically significant differences in IGF1 serum levels. The median IGF1 levels in the melanoma group was 154.1 ng/ml compared to 111.2 ng/ml in the group of healthy persons with $p=0.0036$. The relationship between Breslow score and IGF1 serum levels is shown in Table III: We observed an increase of IGF1 levels from sub-group 1 to sub-group 3. However, the levels in the fourth sub-group decreased to the levels of sub-group 1. The changes of the IGF1 levels through the Breslow score categories were statistically significant ($p=0.0027$). Table IV shows the results of comparison between the positive and negative metastatic affection of the sentinel nodes. The median IGF1 levels in the negative group was 173.5 ng/ml and the corresponding median in the positive group was 205.8 ng/ml. The difference was statistically significant ($p=0.0407$).

Discussion

The role of growth factors in the development of the malignant melanoma has been recently discussed. Several clinical studies with reference to serum levels and tissue receptor expression for the individual growth factors have been already published. We have focused on the comparison of IGF1 serum levels between patients with melanoma and healthy controls.

The age of the control group of healthy individuals was similar to the age of the group of patients with melanoma. The age parameter appeared to be the most problematic factor for

statistical evaluation. IGF1 serum levels are significantly decreasing with age (6). When stratifying into the sub-groups based on the other criteria, the age distribution of the small sub-groups was not equal. Therefore, we have also tested the statistical significance of the age difference between the sub-groups. When the age difference was statistically significant ($p<0.05$), age adjustment has been performed in order to rule-out any incorrect conclusions. Based on our personal experience from our previous clinical trials, the age adjustment has been a crucial factor for IGF1 serum levels changes interpretation (7).

Our group of healthy cases consisted of patients who were examined in the surgical ambulance of our Faculty Hospital. We had therefore all the current and history medical data. We applied a similar selection criteria as in the studies of IGF1 reference values in the population (8, 9). We excluded patients with diabetes, high blood glucose, high insulin levels, patients after surgery, polymorbid patients and persons with a history of cancer diseases. Even though a rigorous selection of the healthy persons has been implemented, the group of healthy probands exhibited a very large variation of IGF1 serum levels. Because we did not find any disease that might, according to current knowledge, influence IGF1 levels, we decided to keep all probands in the group of healthy subjects. Nobody was excluded from this group. For a better presentation of the data distribution in the healthy and melanoma groups the mean, median, both quartiles and minimum and maximum levels are presented in Table I. The distribution of IGF1 in the group of healthy subjects demonstrates a very large and unexplained, for the time being, individual variability in IGF1 levels (10).

When comparing data of serum levels between the patients with melanoma and healthy individuals, we found that IGF1 serum levels were significantly higher in the melanoma group. To our knowledge there are only two studies evaluating serum levels of IGF1 in melanoma and healthy controls with opposite results compared to our study results (11, 12). Frenkel *et al*. proposed IGF1 to be a predictive biomarker for metastatic uveal melanoma but also with opposite results since levels of IGF1 were lower in metastatic melanoma compared to healthy subjects (13). In our study, the age of the two groups was not significantly different ($p=0.8876$); therefore, no age adjustment was used.

Focusing on the melanoma patients group, we stratified this group further into four sub-groups based on the Breslow score, which is the most important prognostic factor in melanoma, if sentinel lymph node is not examined. As primary tumor thickness increases, there is a significant decrease in survival (14, 15). IGF1 serum levels in the individual sub-groups were evaluated and correlated with the degree of invasiveness. The Kruskal-Wallis test for evaluation of IGF1 changes through all the sub-groups was used. The sub-groups formed based on the Breslow score

Table I. *Age characteristics of the patient groups*.

Status	Count (N)	Age (years)				p-Value (Wilcoxon test)
		Mean	Median	Minimum	Maximum	
Melanoma	77	56.5	57.4	11	87	0.8876
Healthy persons	139	55.9	56.7	16	83	

Table II. *Melanomas vs. healthy individuals*.

Analyte (units)	Status	Mean	Median	Lower quartile	Upper quartile	Min. Max.	p-Value (Wilcoxon test)
IGF1 (ng/ml)	Melanoma	157.1	154.1	116.4	203.8	61.93 324.9	0.0036
	Healthy	119.4	111.2	98.16	170.4	48.92 340.5	

Table III. *IGF1 serum levels according Breslow score*.

Analyte (units)	Breslow score (mm)	Count (N)	Mean	Median	Minimum	Maximum	p-Value (Kruskal-Wallis test)
IGF1 (ng/ml)	>1	20	155.2	153.9	52.21	255.2	0.0027*
	1-2	27	172.3	166.8	78.18	238.6	
	2-4	15	186.9	169.6	81.89	324.9	
	<4	15	151.5	154.4	114.2	219.9	

*p-Value of age adjusted IGF1.

Table IV. *IGF1 serum levels and sentinel nodes*.

Analyte (units)	Status	Count (N)	Mean	Median	Minimum	Maximum	p-Value (Wilcoxon test)
IGF1 (ng/ml)	Negative	33	164.5	173.5	62.75	255.2	0.0407*
	Positive	11	201.2	205.8	121.9	324.9	

*p-Value of age adjusted IGF1.

were significantly different according to the age parameters; therefore, IGF1 values were age-adjusted. IGF1 serum levels correlated with increasing Breslow score only till the third subgroup. When the Breslow score was higher than 4 millimeters, IGF1 serum levels were decreasing. This finding agrees with the literature data (11,12). The present clinical study supports the theory that IGF1 plays a certain role, particularly in the period of cancer initiation. It supports the tumor growth at the early stages and it has also anti-apoptotic properties, probable via the activation of Akt and MAP kinase pathways (16). Various factors play the role in subsequent stages of cancer. Hyperactivation and deregulation of Akt and MAP kinase pathway increase activity of the signal transductors. Recent studies support the theory that several transductors are common for several pathways and mediate the signal transduction between the several type of the cell growth pathways (17). Based on this knowledge we assume that the value of the serum levels of IGF1 is not the main promoter of the next development of the cancer cells in advanced cancer stages.

Finally, we have studied two sub-groups of patients based on the metastatic positivity of sentinel lymph nodes. The status of the sentinel lymph node is the most important prognostic factor for the recurrence and survival prediction (18). Comparing these sub-groups with negative and positive metastatic process in sentinel lymph nodes, we found that IGF1 serum level was statistically significantly higher in patients with positive metastatic affection (p=0.0407). To our knowledge, there is no literature evidence concerning this evaluation.

Conclusion

IGF1 serum levels were significantly higher in patients diagnosed with melanoma compared to the healthy control group. These changes of the IGF1 levels in relationship to the Breslow score categories were statistically significant. Serum levels of IGF1 were significantly higher in the sub-group with the positive metastatic affection of the sentinel nodes than in negative patients.

Acknowledgements

References

1   Bennett DC: How to make a melanoma: what do we know of the primary clonal events? Pigment Cell Melanoma Res *21(1)*: 27-38, 2008.

2   Ferlay J, Parkin DM and Steliarova-Foucher E: Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer *46(4)*: 765-781, 2010.

3   Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D and Bray F: Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer *49(6)*: 1374-1403, 2013.

4   Larson Z: Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol Pathol *54*: 311-316, 2001.

5   Pollak M: Insulin and insulin-like growth factor signaling in neoplasia. Nat Rev Cancer *8(12)*: 915-928, 2008.

6   Brabant G and Wallaschofski H: Normal levels of serum IGF-I: determinants and validity of current reference ranges. Pituitary *10(2)*: 129-133, 2007.

7   Kucera R, Cerna M, Narsanska A, Svobodova S, Strakova M, Vrzalova J, Fuchsova R, Treskova I, Kydlicek T, Treska V, Pecen L, Topolcan O and Pazdiora P: Growth factors and breast tumors, comparison of selected growth factors with traditional tumor markers. Anticancer Res *31(12)*: 4653-4656, 2011.

8   Alberti C, Chevenne D, Mercat I, Josserand E, Armoogum-Boizeau P, Tichet J and Leger J: Serum concentrations of insulin-like growth factor (IGF-1) and IGF binding protein-3 (IGFBP-3), IGF-1/IGFBP-3 ratio, and markers of bone turnover: reference values for French children and adolescents and z-score comparability with other references. Clin Chem *57(10)*: 1424-1435, 2011.

9   Rosario PW: Normal values of serum IGF-1 in adults: results from a Brazilian population. Arq Bras Endocrinol Metabol *54(5)*: 477-481, 2010.

10 Morimoto LM, Newcomb PA, White E, Bigler J and Potter JD: Variation in plasma insulin-like growth factor-1 and insulin-like growth factor binding protein-3: genetic factors. Cancer Epidemiol Biomarkers Prev *14(6)*: 1394-1401, 2005.

11   Park SL, Setiawan VW, Kanetsky PA, Zhang ZF, Wilkens LR, Kolonel LN and Le Marchand L: Serum insulin-like growth factor-I and insulin-like growth factor binding protein-3 levels with risk of malignant melanoma. Cancer Causes Control *22(9)*: 1267-1275, 2011.

12 Santonocito C, Paradisi A, Capizzi R, Concolino P, Lavieri MM, Lanza Silveri S, De Luca D, Catricala C, Di Carlo A, Zuppi C, Ameglio F and Capoluongo E: Insulin-like growth factor I (CA) repeats are associated with higher melanoma's Breslow index but not associated with the presence of the melanoma. A pilot study. Clin Chim Acta *390(1-2)*: 104-109, 2008.

13 Frenkel S, Zloto O, Peer J and Barak V: Insulin-like growth factor-1 as a predictive biomarker for metastatic uveal melanoma in humans. Invest Ophthalmol Vis Sci *54(1)*: 490-493, 2013.

14 Breslow A: Prognostic factors in the treatment of cutaneous melanoma. J Cutan Pathol *6(3)*: 208-12, 1979.

15 Balch CM, Buzaid AC, Soong SJ, Atkins MB, Cascinelli N, Coit DG, Fleming ID, Gershenwald JE, Houghton A Jr., Kirkwood JM, McMasters KM, Mihm MF, Morton DL, Reintgen DS, Ross MI, Sober A, Thompson JA and Thompson JF: Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol *19(16)*: 3635-3648, 2001.

16 Peterson JE, Kulik G, Jelinek T, Reuter CW, Shannon JA and Weber MJ: Src phosphorylates the insulin-like growth factor type I receptor on the autophosphorylation sites. Requirement for transformation by src. J Biol Chem *271(49)*: 31562-31571, 1996.

17 Malaguarnera R and Belfiore A: The emerging role of insulin and insulin-like growth factor signaling in cancer stem cells. Front Endocrinol 5(10): doi: 10.3389/fendo.2014.00010, 2014.

18 Abbasi NR, Shaw HM, Rigel DS, Friedman RJ, McCarthy WH, Osman I, Kopf AW and Polsky D: Early diagnosis of cutaneous melanoma: revisiting the ABCD criteria. JAMA *292(22)*: 2771-2776, 2004.