RESEARCH ARTICLE

Clinical Significance of Serum Ykl-40 (Chitinase-3-Like-1 Protein) as a Biomarker in Melanoma: an Analysis of 112 Turkish Patients

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Abstract

Background: Angiogenesis plays an essential role in tumor growth and serum levels of YKL-40 , a strong angiogenic factor that promotes tumor vessel development, has been found to be elevated in various cancers. We here investigated correlation between melanoma parameters and serum YKL-40 levels, to assess potential diagnostic, prognostic and predictive values. **Material and Methods:** Data for 112 pathologically confirmed cutaneous melanomas of any stage were examined retrospectively. ELISA assays were used to measure serum YKL-40 in plasma samples. **Results:** The baseline serum YKL-40 levels were significantly higher in patients than healthy controls (174.88 vs 120.10 ng/mL, $p<0.001$). However, values did not correlate with clinicopathological parameters, ($p>0.05$), and furthermore there was no apparent prognostic influence on melanoma survival (HR: 1.568; 95% CI, 0.580-3.051; $p=0.838$). **Conclusion:** Serum YKL-40 can be useful for diagnosis of melanoma, but reliability in assessing prognosis is questionable. We believe that efforts should be made to understand the interaction between YKL-40 and the tumor environment, and establish whether it might be the target for treatment of malignancies.

Keywords: Melanoma- biomarkers- YKL-40- CHI3L1- prognostic- diagnostic

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Introduction

The American Joint Committee of Cancer (AJCC) has classified melanoma into prognostic groups based on clinicopathological factors and LDH (lactate dehydrogenase) has been accepted as one of the independent prognostic serum factors in this classification (Balch et al., 2009). Nevertheless, LDH is rather non-spesific and it tends to elevate in many circumstances apart from malignancies, such as hemolysis. Apart from LDH, several other prognostic serum biomarkers have been investigated in melanoma, including S-100B; MIA (melanoma inhibiting activity protein); TA90IC (tumor associated antigen 90 immune complex); and YKL-40 (also called chitinase-3-like-1 protein [CHI3L1]) (Schmidt et al., 2006; Faries et al., 2007; Díaz-Lagares et al., 2011; Lugowska et al., 2015).

YKL-40/ CHI3L1 (also called breast regression protein-39 [BRP-39] in the mouse, human cartilage glycoprotein-39 [HC-gp39], 38-kDa heparin-binding glycoprotein [gp38k]) is a lectin that belongs to mammalian chitinase like family and that binds collagen, heparin and chitin, however it does not degrade chitin (Schmidt et al., 2006; Salamon et al., 2014; Ma et al., 2015). YKL-40 is secreted by number of nonmalignant cells, such as neutrophils, epithelial cells, smooth muscle cells, macrophages and chondrocytes and it is stimulated by various mediators, such as IFN-γ, IL6, IL13, IL1β (Low et al., 2015). It stimulates the growth of connective tissue cells and, even though not secreted by fibroblasts it influences the growth of fibroblasts by acting synergistically with insulin-like growth factor 1 (IGF-1) (Recklies et al., 2002). Mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI-3K) signaling cascades in fibroblasts are activated by YKL-40, and in turn both the extracellular signal regulated kinase (ERK) 1/2 MAPK and protein kinase B (AKT)-mediated signaling cascades are phosphorylated and thus the mitogenesis is controlled (Recklies et al., 2002). The PI-3K pathway, especially AKT phosphorylation, plays a pivotal role in cell survival, thus it explains the significance of YKL-40 in tissue remodeling and apoptosis. The migration and adhesion of vascular smooth muscle cells are also enhanced by YKL-40.

We aimed, in this study, to analyse the level of YKL-40 in serum from melanoma patients in order to evaluate its prognostic, predictive and diagnostic values in melanoma.

Materials and Methods

This study included 112 consecutive melanoma patients with histologically confirmed diseases who

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had been treated and followed up between January 2013 and January 2015. The patients had not received chemotherapy and/or radiotherapy over the last 6 months. The stage of the disease was determined according to the AJCC (American Joint Committee on Cancer) and UICC (International Union against Cancer) staging systems. Prior to onset of the treatment, the patients had been processed through a detailed assessment including clinical history, physical examination and a series of blood tests, such as lactate dehydrogenase and complete blood count. Those with Eastern Cooperative Oncology Group (ECOG) performance status 2 or less and appropriate blood chemistry tests had received chemotherapy on an outpatient basis consisting interferon-α, temozolamide, dacarbazine and cisplatine. Depending on the stage of their diseases the patients also underwent radiotherapy. The immunotherapy agents, such as pembrolizumab and nivolumab, and the targeted therapy agents, such as vemurafenib/cobimetinib and dabrafenib/trametinib were used for systemic therapy for metastatic or unresectable disease. Follow-up included clinical, laboratory, and radiological assessments every 8 weeks during chemotherapy periods and every 12 weeks when no chemotherapy was given. Response was determined according to the revised RECIST criteria version 1.1. Forty three age- and sex-adjusted healthy controls were also included in the analysis to compare the serum YKL-40 levels. The informed consents were obtained from all patients and the study was reviewed and approved by our Regional Ethics Committee.

Serum samples were obtained on first admission prior to any treatment was given and following centrifugation they were stored at -20°C until analysis. The YKL-40 ELISA (SUNRED Biotechnology Company, Shangai, China) measured the level of human YKL-40 in the samples using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Serum samples and standards were added to the wells which had been pre-coated with human YKL-40 monoclonal antibody. Streptavidin-horseradish peroxidase (HRP) and biotinylated-Fab monoclonal capture antibody conjugates were applied to form immune complexes and then were left to incubate at 37 °C temperature for 1 hour. Unbound streptavidin-HRP was washed away, and then a colorless chromogen solution was added and incubated at 37 °C for 10 min (protected from light). The colorless solution was turned blue and the intensity of this color change was proportional to the amount of YKL-40 in the sample. The reaction was terminated by an acidic stop-solution and it turned yellow. The end product were measured by an automated ELISA reader (ChroMate® 4300 microplate awareness technology) at 450 nm. The results were expressed as ng/ml.

The statistical calculations were executed by SPSS software (version 21.0, SPSS Inc., Chicago, Illinois, USA). Continuous variables were divided using median values as cut-off points. Mann Whitney U test was used to analyze differences between groups with nonparametric data distribution. Survival was calculated from the date of first admission to hospital to death resulting from any cause or to the last contact with the patient or any family member. The survival time was analyzed by the Kaplan-Meier method and the differences in survivals were assessed by the log-rank statistics. A p value ≤0.05 was considered significant.

Results

The median age at the diagnosis of 112 patients was 52 years (range, 16-85). Male sex was predominant among the patients (62%). The majority of the patients had truncal lesions (55%) and metastatic disease (61%) with predominantly M1c disease (72%). The baseline serum YKL-40 levels of the patients were significantly higher compared with the healthy controls (174.88 vs 120.10 ng/mL, p<0.001) (Table 1). None of the known clinical parameters, such as age; site of lesion; lymph node involvement; stage; lactate dehydrogenase level; sex; histology; Breslow thiskness; Clark invasion level; presence of ulceration or regression, and response to therapy was found to be correlated with serum YKL-40 levels (p>0.05) (Table 2).

The median survival of all patients was 20.8 months (95% CI, 10.7-30.9). The 1- and 2-year overall survival rates were 67.3% and 44.4%, respectively. The patients with truncal lesions (p=0.027), node involvements (p=0.08), multiple node involvements (p=0.047), metastasis (p<0.001), advanced metastasis (p<0.001), anemia (p<0.001), elevated erythrocyte sedimantation rate (ESR) (p=0.003) and failure in response to chemotherapy (p=0.006) showed poor survival (Table 2). On the other hand, serum YKL-40 level did not have a prognostic influence in melanoma survival (HR: 1.568; 95% CI, 0.580-3.051; p=0.838) (Table 2).

Discussion

We measured serum YKL-40 (CHI3L1) concentration in 112 melanoma patients and found that the baseline serum YKL-40 levels of the patients were significantly higher compared with the healthy controls (174.88 vs 120.10 ng/mL, p<0.001). YKL-40 concentration was also found to be correlated with none of the clinicopathological parameters (p>0.05). As expected, truncal lesions (p=0.027), node involvements (p=0.08), multiple node involvements (p=0.047), metastasis (p<0.001), advanced metastasis (p<0.001), anemia (p<0.001), elevated erythrocyte sedimantation rate (ESR) (p=0.003) and failure in response to chemotherapy (p=0.006) were correlated with poor survival, hovewer, serum YKL-40 level did not have a prognostic influence in melanoma survival (HR: 1.568; 95% CI, 0.580-3.051; p=0.838).

YKL-40 was analyzed in 234 stage I (n=162) and II (n=72) melanoma patients within a 66 month-long

Table 1. The Values of Serum Assay YKL-40 Levels in Melanoma Patients and Healthy Controls

Assay	Patients	(n=112)	Controls	(n=43)	
	Median	Range	Median	Range	p
YKL-40 (ng/ml)	174.88	98.10-438.24	120.1	79.17-432.60	< 0.001

Table 2. Distribution and Survival Comparisons of Serum YKL-40 Levels on Various Patient/Clinical Parameters

Parameter	YKL-40 Distribution p	Survival p
Age, years <50/≥50years	0.51	0.77
Sex male/female	0.95	0.76
Site of lesion axial/extremity	0.41	0.027
Histology nodular/nonnodular	0.71	0.41
Breslow thickness ≤4mm)/>4mm	0.71	0.74
Clark invasion level I-III/IV-V	0.25	0.88
Ulceration yes/no	0.31	0.33
Mitosis 0-2/≥3	0.32	0.11
Regression yes/no	0.71	0.62
TIL yes/no	0.48	0.19
Node involvement yes/no	0.27	0.08
Type of node involvement single/multiple	0.28	0.047
Metastasis yes/no	0.7	<0.001
M1 status ab/c	0.7	<0.001
Serum LDH level high/normal	0.89	0.89
Anemia yes/no	0.77	<0.001
ESR** high/normal	0.76	0.003
Response to chemotherapy yes/no	0.49	0.006
YKL-40 low<median>high	-	0.838

**erythrocyte sedimentation rate

median follow-up period and found to be an independent prognostic factor for recurrence and death (Schmidt et al., 2006). In this study, 13% of the patients versus healthy controls were found to have increased serum YKL-40 concentration prior surgery and these patients had worse prognosis compared with the healthy controls. Similarly, in early stage and locally advanced melanoma (pathological stages I-III in 148 patients) it was found that 59% of patients with ulceration (a very important prognostic indicator) had elevated levels of serum YKL-40 compared to 26% of patients without ulceration (p=0.012) and there was a correlation between YKL-40 and matrix metalloproteinase 9 (MMP-9) and vascular endothelial growth factor (VEGF) (Lugowska et al., 2015).

MMPs degrade type IV collagen causing disruption of extracellular matrix and the basement membrane thus the migration of tumor cells ensue and factors mediating neoangiogenesis, such as VEGF, are released. Shao described that YKL-40 stimulates vascularization and migration of endothelial cells and up-regulates VEGF receptor 2 that in turn induces FAK-MAPK thus mediates neoangiogenesis along with other factors, such as VEGF (Shao, 2013). He also explained that coupling of membrane-bound receptor syndecan-1 with integrins was activated by YKL-40. Not only did he recognize YKL-40's crucial influence in angiogenesis but he also drew attention to its role in tumor associated macrophage (TAM)-mediated tumor development. The author suggested that because YKL-40 (after produced by TAMs and other YKL-40-producing cells, such as neutrophils, in tumor environment) played a critical role in macrophage differentiation and maturation it was worthy of questioning whether the dissemination ability of tumor cells that was enhanced by inflammatory cytokines, MMPs, and growth factors secreted and regulated by TAMs is regulated by TAM-associated-YKL-40.

An elevated serum YKL-40 was found to be independently correlated with poor survival in metastatic melanoma (Schmidt et al., 2006). Furthermore, it was found that this combination was independent of the AJCC M stage classification. However, in another study YKL-40 was found neither diagnostic nor prognostic and nor was it as effective as the other four biomarkers in separating the distant metastasis from other type of metastasis (Diaz-Lagares et al., 2011). Supporting this result, two other studies that assessed various biomarkers in advanced melanoma reported that YKL-40 had no correlation with either survival or prognosis (Egberts et al., 2012; Weide et al., 2015). However, of these two studies, Egberts et al, found that YKL-40 had a significant correlation with the stage of the disease.

Similarly, higher serum level of YKL-40 and additionally the association of YKL-40 level with poor survival were also reported in colorectal cancer (CRC), prostate cancer and glioblastoma/high grade glioma patients in various studies (Junker et al., 2005; Pelloski et al., 2005; Brasso et al., 2006; Abd El-Fattah et al., 2016). Elevated YKL-40 expression was found in human glioblastoma cells after they were exposed to hypoxia or radiation and astrocytes with high YKL-40 expression were more resilient to radiation and their potential for invasion was increased in vitro; these findings suggested that YKL-40 interceded as a cellular survival factor. Furthermore, elevated level of YKL-40 was found to be associated with high grade gliomas, and failure of radiotherapy and poor survival. It was stated that YKL-40 staining was more useful than glial fibrillary acidic protein in distinguishing glioblastoma from anaplastic oligodendroglioma, and YKL-40 and glial fibrillary acidic protein immunohistochemistry combined provided a more reliable and accurate diagnosis in anaplastic

oligodendrogliomas.

A neutralizing monoclonal anti-YKL-40 antibody (mAY) was created and found to prevent vascularization in glioblastome through dysfunction of intercellular N-cadherin and it was suggested that the treatment with mAY and ionizing irradiation (IR) synergistically hindered vascularization and spread of the tumor (Shao et al., 2014). They also speculated that more aggressive glioblastomes might be treated with a combination of mAY, IR and bevacizumab (an anti-vascular endothelial growth factor (VEGF) antibody) and for bevacizumab-resilient cancers that express YKL-40 bevacizumab might be replaced by a mAY.

Indeed, mAY was shown to abolish all the cascades that were mediated by YKL-40 and were involved in angiogenesis, such as the coordination of membrane bound receptor syndecan-1 and integrin αvβ3, the activation of the FAK-Erk1 and Erk2 signaling cascades, the increase in the expression of Flk-1/KDR (a VEGF receptor 2 that intercedes VEGF angiogenesis) and the activation of the tyrosine phosphorylated form of Flk-1/KDR (Faibish et al., 2011). Elevated YKL-40 expression resulting from γ-irradiation eventually abrogated apoptosis and enhanced angiogenesis, so mAY conversely sensitized tumor cells to γ-irradiation via a decrease of PI3K-independent AKT phosphorylation and mAY consistently was found to abolish endothelial cell angiogenesis; these findings agreed with the opinion that creating a therapeutic pathway by nullifying YKL-40 activity with monoclonal antibodies might be the next step in cancers that express high YKL-40 (Shao, 2013).

Despite the efforts there are still many questions to be answered, e.g. Is targeting YKL-40 in fact a possible therapeutic solution for cancer, or which cancers might benefit from such a treatment and which cancers, if any, must not be treated with anti-YKL-40, or should anti-YKL-40 be used in combination with novel agents or must it be used alone. The small patient number was one of the limitations of our study. This was, we believe, why we did not find a significant relationship between YKL-40 level and any of the clinicopathologic parameters, so in conclusion, we found that YKL-40 level was diagnostic, but, however it had neither prognostic nor predictive value in melanoma. Because this was a retrospective analysis and some of the data were missing it might have played a role in failure of establishing a representative distribution, therefore future studies assessing YKL-40 has to be designed accordingly. We believe that investigation of YKL-40 in cancer is worth the time and effort and that future studies should also focus on therapeutic approaches targeting YKL-40.

Declarations

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Role of the funding source

There is no funding source.

Ethical approval

This article does not contain any studies and experiments with human participants or animal performed by any of the authors. This study was approved by the Regional Ethics Committee.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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