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Serum Amyloid A and Lipoprotein Associated Phospholipase A_2 Levels in Patients with Malign Melanoma: Correlations with Clinical Assessment and Stage

Malign Melanomlu Hastalarda Serum Amiloid A ve Lipoprotein İlişkili Fosfolipaz A_2 Düzeyleri: Klinik Değerlendirme ve Evre ile Korelasyon

Abstract

Objective: The lack of validated, sensitive, and specific biomarkers for early diagnosis and follow-up of patients with malign melanoma (MM) is a major problem today. In this study, we aimed to investigate the correlations of two inflammatory biomarkers-serum amyloid A (SAA) and lipoprotein associated phospholipase A_2 (Lp-PLA_2)-in clinical follow-up and staging of MM.
Methods: Lactate dehydrogenase (LDH) and C-reactive protein (CRP) (Routine), SAA and S100B (ELISA), and Lp-PLA_2 (PLAC® Test) activity levels were examined in histologically and clinically confirmed MM patients (n=131) and in healthy controls (n=27).
Results: Sedimentation rate and LDH, CRP, S100B, SAA, Lp-PLA_2 activities were found to be significantly higher in MM patients compared to control group ($p<0.05$). SAA showed the strongest correlation with disease stage (Spearman's correlation coefficient=0.622, p=0.000). Receiver operating characteristic analysis revealed that SAA exhibited the largest area under the curve=0.984, p=0.000, highest sensitivity (95%) and specificity (93%). Pearson's test indicated a weak positive correlation between SAA levels and Lp-PLA_2 activity (r=0.311, p=0.000).
Conclusion: This is the first study to evaluate the activity of inflammatory biomarker Lp-PLA_2 in melanoma patients. Both SAA and Lp-PLA_2 were in highest levels in stage 4 patients, and they are thought to be candidate biomarkers to be used in detecting tumor progression. According to our results, SAA is the biomarker which correlates best with disease stage in MM.
Keywords: Melanoma, prognosis, serum amyloid A, lipoprotein associated phospholipase A_2, inflammation, biomarker

Öz

Amaç: Malign melanomlu (MM) hastaların erken tanı ve takibinde kullanılabilecek onaylanmış, sensitif ve spesifik biyobelirteçlerin eksikliği günümüzde önemli sorun teşkil etmektedir. Bu çalışmada yangı ilişkili iki biyobelirteç olan serum amiloid A (SAA) ve lipoprotein ilişkili fosfolipaz A_2'nin (Lp-PLA_2) melanomun klinik izlemi ve evrelemesindeki korelasyonunu araştırmayı amaçladık.
Yöntemler: Histolojik ve klinik olarak doğrulanan MM hastaları (n=131) ve sağlıklı gönüllülerden (n=27) alınan kan örneklerinde laktat dehidrogenaz (LDH) ve C-reaktif protein (CRP) (Routine), SAA ve S100B (ELISA), Lp-PLA_2 aktivite düzeyleri (PLAC® Test) çalışılmıştır.
Bulgular: MM hastalarında kontrol grubuna kıyasla sedimantasyon düzeyi, LDH, CRP, S100B, SAA ve Lp-PLA_2 aktivitesi düzeyleri anlamlı olarak daha yüksek bulunmuştur ($p<0,05$). Hastalık evresiyle en güçlü korelasyon gösteren belirteç SAA olarak saptanmıştır (Spearman's korelasyon analizinde rho katsayısı 0,622, p=0,000). İşlem karakteristik değerlendirmelerine göre en geniş eğri altında kalan alanı gösteren=0,984, p=0,000, en yüksek sensitivite (%95) ve spesifite (%93) SAA'da saptanmıştır. Pearson testi sonuçlarına göre SAA düzeyleriyle Lp-PLA_2 aktivitesi arasında zayıf güçte pozitif korelasyon saptanmıştır (r=0,311, p=0,000).

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Sonuç: Bu çalışma literatürde ilk defa melanom hastalarında enflamatuvar bir belirteç olan Lp-PLA_2 aktivitesinin değerlendirildiği çalışmadır. Hem Lp-PLA_2 hem SAA düzeylerinin evre 4 hastalarda en yüksek düzeylere ulaştığı, tümör progresyonunu araştırmada aday biyobelirteçler olarak kullanılabileceği düşünülmektedir. Sonuçlarımıza göre SAA MM'de hastalık evresiyle en iyi korelasyon gösteren belirteçtir.
Anahtar kelimeler: Melanom, prognoz, serum amiloid A, lipoprotein ilişkili fosfolipaz A_2, yangı, biyobelirteç

Introduction

Melanoma accounts for 2% of all cancers and its overall incidence is increasing dramatically worldwide (1). This highly aggressive cancer associated with high mortality can be treated most effectively if the primary tumor is detected early (2). Following excision of the primary tumor, careful follow-up for early detection of local relapse, regional or distant metastases or secondary tumor occurrence is critical to disease prognosis (3).

Currently available methods for early melanoma detection, prognosis assessment and disease monitoring focus on clinical and histological metrics of the primary tumor at diagnosis and on the use of serologic markers and advanced radiographic imaging as follow-up. Imaging-based detection or monitoring methods are useful, but their use is often limited due to economic and health concerns. Serological biomarkers, on the other hand, are minimally invasive, convenient, cost-effective and may provide new insights into the disease biology. Serological biomarkers previously investigated include lactate dehydrogenase (LDH), C-reactive protein (CRP), S100 proteins, melanoma inhibiting activity, angiogenesis factors including vascular endothelial growth factor, cell adhesion molecules including soluble intracellular adhesion molecule, cytokines and cytokine receptors including interleukin (IL)-6, IL-10 and serum amyloid A (SAA). However, none of these biomarkers possess ideal predictive criteria and there is currently no consensus on optimal follow-up strategy (4,5).

Early dissemination of metastases and the poor prognosis following metastasis highlight the need for continued biomarker research for melanoma. Achieving better understanding of the pathology, determining tumor burden and stage progression represent aims that assessment of reliable biomarkers could aid (6-8).

This study focused on two novel inflammatory markers in melanoma patients based on the well-characterized association of chronic inflammation with tumorigenesis and disease progression (9).

The first is SAA, one of the acute phasemarkers produced in the liver (10). The protein is increased even from early stages in ovarian cancers and in others, such as esophageal, lung and endometrial cancers, it is proposed to have a prognostic value (11). Similarly, SAA is significantly increased in all melanoma stages (1 to 4), the most significant increase being in the advanced disease. In early stage patients with significantly increased SAA levels, this increase is also correlated with disease progression (12).

A second inflammation-related biomarker we investigated is lipoprotein associated phospholipase A_2 (Lp-PLA_2), a secretory isoform of the PLA_2 family. This enzyme specifically hydrolyzes phosphatidylcholines in low-density lipoprotein (LDL) particles within the arterial intima-media to form lysophosphatidic acid (LPA), oxidated free fatty acids (FFA) and arachidonic acids, precursors of eicosanoids which are potent proinflammatory agents (13).

Extensive research conducted on the biology and role of the enzyme in the last decade have shown that Lp-PLA_2 is an independent risk factor of coronary events and it has a role in stroke, diabetes, apoptosis (14-16). LPA's, the products of the PLA_2 family, have been identified as a major tumor-promoting factor in ovarian cancer ascites and recently some isoforms of PLA_2 are proposed to have an oncogenic role and therapies targeting secreted PLA_2 ($sPLA_2$) inhibition are in search (17-19).

In acute phase response, elevated acute phase form of SAA (A-SAA) is shown to induce PLA_2 enzyme activity. A-SAA associated high-density lipoprotein (HDL) is damaged by $sPLA_2$ 2-3 times more easily compared to normal HDL. Thus, increased PLA_2 hydrolysis of HDL in acute phase is proposed to be A-SAA mediated (10).

The relationship between SAA and PLA_2 in inflammation and the known role of proinflammatory state in tumor formation and progression was the primary motivation for this study. Our hypothesis was that if the SAA levels and Lp-PLA_2 activities are significantly higher in melanoma patients compared to controls and/or if they are correlated with clinical assessment and staging of malign melanoma, then they might serve as possible diagnostic tools for detection of melanoma progression. This, in turn, might bring value to improve early detection of stage change and/or patient monitoring. To the best of our knowledge (PubMed search with keywords "Lp-PLA_2", and "melanoma") no studies have explored the role of Lp-PLA_2 in melanoma and whether the levels of SAA in melanoma patients is associated with Lp-PLA_2 activity.

Methods

This study included 131 histologically and clinically confirmed melanoma patients according to American Joint Committee on Cancer (AJCC) staging who were followed-up at Ege University Faculty of Medicine, Department of Dermatology. Patients at different disease stages, but who had not received systemic treatment for melanoma, anti-obesity medications or therapies for estrogen replacement, thyroid maintenance, lowering lipid level or treating chronic inflammation during the previous three months were recruited to the study. Twenty-seven healthy volunteers with the same age span who did not have a history of systemic disease or infection or any pathological findings on physical examination were included in the control

group. All subjects underwent a standardized interview and were questioned about medical history specifically including physician-diagnosed hypertension, cardiovascular events, hyperlipidemia, diabetes, etc. Current medications were also recorded. None of the patients in the melanoma or control groups had cardiovascular disease history or abnormal lipid profile as assessed by total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels.

The protocol for the study was approved by the Ege University Faculty of Medicine Ethics Committee on Clinical Research (approval number: 13-6/54) and written informed consent was obtained from all subjects.

Blood samples were collected in Vacutainer tubes (Becton Dickinson, USA) both with and without EDTA between 8.00-10.00 a.m., centrifuged within 1 h after collection at 2000 g for 10 min and stored at 80 °C until analysis. We evaluated A-SAA levels and Lp-PLA_2 activities in stage 1, 2, 3 and 4 melanoma patients along with other known serologic inflammatory biomarkers (LDH, CRP and S100B) used in the follow-up. All laboratory analyses were carried out blinded with respect to patient or control status.

LDH and CRP levels were analyzed using a Beckman Coulter Unicel DxC 800 Synchron auto analyser. SAA and S100B levels were analyzed by sandwich based ELISA based commercial kits (CusoBio Inc., Wuhan, China). LP-PLA_2 activity was determined using the PLAC® Test enzyme assay for Lp-PLA_2 activity (DiaDexus Inc., San Francisco, USA) and the rate of formation of the colored reaction product (4-nitrophenol) was spectrophotometrically determined using a Unicel DxC 800 Synchron (Beckman Coulter, USA). Lp-PLA_2 activity was calculated from the rate of absorbance change.

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, USA). Student's t-test was used to test for significant difference between the means for control group and patient group data. Variance analysis was used to determine significance of difference between means for control and patient groups with respect to melanoma clinical stage. The post-hoc Tukey test was performed for biomarker values whereby significant difference was detected by variance analysis. Spearman's rank correlation was used to assess relationships between the variables. A p value <0.05 was considered as significant. The sensitivity and specificity of each marker were assessed by the areas under the respective receiver operating characteristic-area under the curve (ROC-AUC) to evaluate the ability of each marker to discriminate melanoma patients from healthy controls.

Results

The malign melanoma group consisted of 131 patients (67 female, 64 male), the control group included 27 cases (15 female, 12 male). Characteristics and biomarker values for all patients are summarized in Table 1. There was no significant difference with respect to gender, age and body mass index, between the patient and control groups. T-test analysis showed significantly higher sedimentation rate, LDH, CRP, S100B, SAA levels and Lp-PLA_2 activity in the malign melanoma patient group compared to the control group (p=0.000, p=0.014, p=0.000, p=0.000, p=0.000,

Table 1. Malign melanoma and control group characteristics and studied biomarker values

	Malign melanoma group (n=131)		Control group (n=27)		p values
	Mean ± SD	Min-max	Mean ± SD	Min-max	
Age (in years)	47.21±12.33	20.00-65.00	44±10.54	26.00-64.00	0.210
Body mass index	26.74±4.62	17.58-41.80	25.36±3.86	19.38-35.99	0.156
LDL-cholesterol (mg/dL)	124.04±33.93	67.0-214.0	119.00±28.43	81.00-196.00	0.213
HDL-cholesterol (mg/dL)	48.76±23.80	20.0-117.0	53.50±16.82	28.00-95.00	0.246
Total-cholesterol (mg/dL)	206.43±37.48	142.0-303.0	205.00±57.50	136.00-312.00	0.862
Sedimentation rate (mm/h)	17.11±14.46	2-92	8.19±4.45	2-16	0.000
LDH (IU/L)	198.70±90.78	110.00-963.00	154.70±32.34	113.00-219.00	0.014
CRP (mg/dL)	0.59±1.18	0.10-9.70	0.18±0.09	0.00-0.30	0.000
S100B (µgr/L)	0.22±0.29	0.01-1.48	0.106±0.061	0.020-0.270	0.000
SAA (µgr/mL)	13.95±13.25	1.60-122.40	2.37±1.43	1.10-7.40	0.000
Lp-PLA_2 (nmoL/min/mL)	179.14±44.49	58.90-315.40	148.65±36.64	98.60-218.00	0.001

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, LDH: Lactate dehydrogenase, CRP: C-reactive protein, SAA: Serum amyloid A, Lp-PLA_2: Lipoprotein associated phospholipase A_2, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 2. Mean, standard deviation, minimum/maximum values for investigated biomarkers in controls and malign melanoma patients at various clinical stages

		n	Mean	Standard deviation	Minimum	Maximum
LDH (IU/L) ANOVA, p=0.001	**Controls**	27	154.70	32.34	113.0	219.0
	1	33	168.55	26.77	110.0	211.0
	2	32	185.66	47.60	116.0	294.0
	3	31	201.10	57.84	125.0	411.0
	4	29	242.03	165.56	117.0	963.0
CRP (mg/dL) ANOVA, p=0.007	**Controls**	26	0.18	0.09	0.00	0.30
	1	33	0.52	1.26	0.10	7.50
	2	32	0.48	0.39	0.10	1.80
	3	31	0.31	0.23	0.10	1.30
	4	29	1.18	1.97	0.10	9.70
S100B (µgr /L) ANOVA, p=0.000	**Controls**	27	0.11	0.06	0.02	0.27
	1	33	0.11	0.03	0.01	0.18
	2	32	0.14	0.07	0.05	0.30
	3	31	0.40	0.45	0.06	1.48
	4	29	0.26	0.31	0.06	1.43
SAA (µgr/mL) ANOVA, p=0.000	**Controls**	27	2.37	1.43	1.10	7.40
	1	33	9.87	3.91	1.60	18.60
	2	32	13.33	11.41	4.50	67.80
	3	31	12.88	6.72	3.89	36.40
	4	29	20.71	23.01	5.00	122.40
Lp-PLA_2 (nmoL/min/mL) ANOVA, p=0.000	**Controls**	27	148.65	36.64	98.60	218.00
	1	33	155.94	40.22	58.90	238.10
	2	32	187.38	37.25	119.60	315.40
	3	31	179.05	45.35	89.00	245.30
	4	29	197.50	48.86	121.30	298.00

LDH: Lactate dehydrogenase, CRP: C-reactive protein, ANOVA: Analysis of Variance, SAA: Serum amyloid A, Lp-PLA_2: Lipoprotein associated phospholipase A_2

p=0.001, respectively). The melanoma group patients were classified according to the AJCC guidelines on staging and classification; stage 1 (n=33), stage 2 (n=32), stage 3 (n=31), stage 4 (n=29). Additionally, there were three mucosal, two uveal and one melanoma *in situ* patients in this group.

Analysis of investigated biomarkers for healthy controls and melanoma patients in various stages (Table 2) indicated that there were significant differences for LDH between controls and stages 2 and 3 (p=0.044, p=0.004, respectively), CRP between controls and stage 2 (p=0.001), S100B between controls and stage 3 (p=0.010), stages 1 and 3 (p=0.009), stages 2 and 3 (p=0.027), SAA between controls and stages 1, 2, 3 and 4 (p=0.000, p=0.000, p=0.000, p=0.002 respectively), Lp-PLA_2 between controls and stage 2 (p=0.05), controls and stage 4 (p=0.000), stages 1 and 2 (p=0.024), stages 1 and 4 (p=0.001).

Correlation between the disease stages and LDH, CRP, S100B, SAA levels and Lp-PLA_2 activity evaluated using Spearman's rho correlation coefficient (r_s) revealed a positive relation for all parameters (r_s=0.360, p=0.000; 0.387, p=0.000; 0.408, p=0.000; 0.622, p=0.000, 0.361, p=0.000, respectively), indicating the strongest correlation for SAA.

Pearson's correlation test showed a weak positive correlation between SAA levels and Lp-PLA_2 activity (r=0.311, p=0.000). Additionally, SAA and LDH (r=0.272, p=0.001), Lp-PLA_2 and S100 (r=0.227, p=0.004) or LDH (r=0.216, p=0.007) showed weak positive correlations.

ROC analysis was used to determine the threshold or "cut-off" values which allow positive or negative discrimination of patient from control group with respect to individual biomarkers. ROC curves for the biomarkers evaluated in the present study are shown in Figure 1. Among the biomarkers

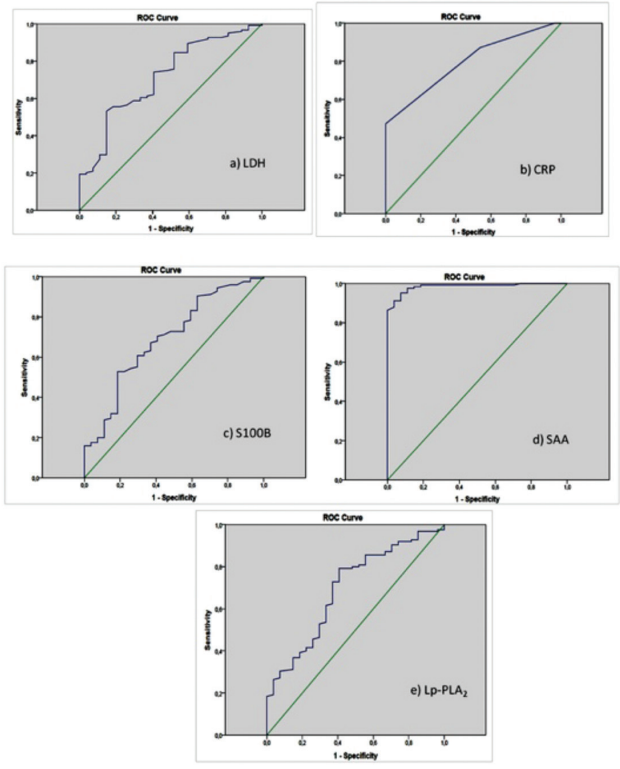

Figure 1. Receiver operating characteristic curves for lactate dehydrogenase (a), C-reactive protein (b), S100B (c), serum amyloid A (d) and lipoprotein associated phospholipase A_2 (e). Among the biomarkers tested in our study, serum amyloid A (area under the curve=0.984, p=0.000) has the largest area under the curve with the highest sensitivity (95%) and specificity (93%) at the cut-off value of 4.75 µgr/mL. Area under the curve values for the other biomarkers were lactate dehydrogenase (0.715, p=0.000), C-reactive protein (0.706, p=0.000), S100B (0.692, p=0.002) and lipoprotein associated phospholipase A_2 (0.695, p=0.001)

ROC: Receiver operating characteristic, LDH: Lactate dehydrogenase, SAA: Serum amyloid A, Lp-PLA_2: Lipoprotein associated phospholipase A_2, CRP: C-reactive protein

tested in our study, SAA (AUC=0.984, p=0.000) has the largest AUC with the highest sensitivity (95%) and specificity (93%) at the cut-off value of 4.75 µgr/mL. AUC values for the other biomarkers in this study were LDH (0.715, p=0.000), CRP (0.706, p=0.000), S100B (0.692, p=0.002) and Lp-PLA_2 (0.695, p=0.001). Choosing a cut-off point of 155 nmoL/min/mL for Lp-PLA_2 yielded a sensitivity of 72.8% and a specificity of 63%; for S100B (cut-off 0.10 µgr/L) 70.4% sensitivity, 59.3% specificity, for LDH (cut-off 155 IU/L) 59.3% sensitivity, 74.4% specificity; for CRP (cut-off 0.25 mg/dL) 73% sensitivity, 67% specificity.

Discussion

This study addressed the need for contributing to the research of reliable biomarkers for early detection at diagnosis or follow-up of malign melanoma patients. Both is crucial as early excision of the tumor at diagnosis and/or using appropriate therapies as disease stage changes are needed for the control of this highly aggressive tumor. We focused on the relationship between inflammation and tumor development and evaluated five inflammatory markers (LDH, CRP, S100B, SAA and Lp-PLA_2) in controls and malign melanoma patients in different disease stages to correlate the biomarker changes with the clinical evolution of the disease. Significantly elevated levels/activities of all biomarkers in patients compared to controls indicated the role of inflammation in malign melanoma development. ROC analysis of biomarkers in discriminating patients from controls showed that SAA is the best available marker with AUC=0.984. Our results also indicated that SAA had the highest sensitivity (95%) and specificity (93%) in diagnosing patients. This study was the first to assess Lp-PLA_2 activity, a secreted form of phospholipase enzymes which are attributed to have a role in tumor development and progression via their inflammatory role, in melanoma patients. Significantly elevated activities in malign melanoma patients compared to controls, the increase in activity in advanced stages of disease (with a significant difference in early stage changes) and the positive correlation between the enzyme and SAA promises initial hope for conducting further studies on this new biomarker.

Malign melanoma is a highly aggressive tumor with early dissemination of metastases. Its incidence is increasing worldwide and unfortunately advanced stages are among the most aggressive and treatment-resistant human cancers. Therefore, early detection and assessment of stage progression is currently the main aim of biomarker research in this field. Various serological biomarkers were previously characterized with respect to melanoma related patient measures. For example, LDH and S100B are useful for assessing prognosis of patients in advanced stages, however they are of little use in early stages (8). LDH level is currently the only serological biomarker recommended by the AJCC staging system (4) for melanoma along with histological and cytometric measures that include Breslow tumor thickness, ulceration, number of mitosis and nodal involvement. LDH becomes increased during tumorigenesis due to both the well known Warburg effect in cancer cells and release of the cytoplasmic enzyme through necrotic cell damage (20). Elevated LDH levels strongly correlate with tumor burden, disease progression and decreased survival; therefore the enzyme is accepted as a strong independent adverse prognostic factor in metastatic melanoma (21-24). However, LDH level does not perform well in discriminating melanoma patients entering stage 4 from earlier stages. Moreover, LDH is prone to false positive results as it is released during other non-melanoma conditions as hemolysis, hepatitis, myocardial infarction, muscular disorders and infections (25). Similar to previous studies, this study showed significantly increased LDH activities in the malign melanoma patient group compared to the control group. Our results are consistent with those of Deichmann et al. (25) who reported that LDH is not of value in discriminating patients entering stage 4.

CRP, synthesized by hepatocytes in response to IL-6 secreted from melanoma cells, is associated with progression of distant metastases during therapy and shortened survival in metastatic melanoma patients (25,26). Another study (27) proposed CRP as an independent prognostic indicator of both early and advanced stages. However, as a non-specific acute phase protein, the marker was reported to have low specificity, limiting its clinical usefulness. In the present study, CRP levels were significantly increased in malign melanoma patient group compared to the control group and a significant difference was found between control and stage 2 patients.

S100 proteins are members of a large family of proteins with complex and multifactorial biology that includes escaping from immune system control and contributing to active tumorigenic processes such as cell proliferation, metastasis and angiogenesis (28). S100B levels reflect tumor mass and are also reported to be very sensitive, potentially superior for detecting the development of metastasis in melanoma (29) but suffer from low sensitivity in practise (30). Furthermore, S100B is not useful for screening or early melanoma detection as its level is only increased with advanced clinical stage and only very few patients exhibit elevated levels in early-stage disease (24,30). We found S100B levels were significantly higher in the malign melanoma group compared to the control group. Notably, stage 3 patients showed significantly higher levels compared to stage 1 and stage 2 patients.

SAA protein is a sensitive indicator of inflammation produced in the liver and an apo-lipoprotein primarily associated with plasma HDL. In chronic inflammation associated with tumor formation, SAA levels continue to increase substantially (10,31,32). Increased levels of SAA were reported in association with hepatic, gastric, lung, ovary, renal, breast, uterine, colon, pancreatic and prostate cancers (32).

Inflammatory responses contribute to induction and progression of tumor formation, malign transformation, invasion, and metastasis besides their negative effects in immune surveillance and treatment response (33). Thus, cancer-related inflammation is recently the target of innovative diagnostic/therapeutic strategies and it is attributed to be the 7th sign of cancer besides the well known 6 hallmarks (sustained growth signal, escape from growth receptors, resistance to cell death, replicative immortality, induction of angiogenesis and invasion/metastasis (34,35).

A previous study by Findeisen et al. (12) investigated known prognostic serum biomarkers CRP, LDH, S100B and SAA in melanoma patients. Increased serum values of LDH, CRP, SAA and S100B were found to be associated with low survival rates in stage 4 melanoma patients. This study concluded that SAA is a strong prognostic factor in all melanoma stages, while SAA and CRP in combination had greater predictive power for prognosis of early stage patients.

Similarly, our melanoma patient group had significantly greater SAA levels compared to that for the control group (p=0.000). Significant differences in SAA levels were revealed between the control group and groups at each stage (stage 1, p=0.000; stage 2, p=0.000; stage 3, p=0.000; stage 4, p=0.002). We found a positive correlation between disease clinical stages and SAA levels (r_s=0.622, p=0.000), representing the best combination of sensitivity and specificity (95%, 93%, respectively).

PLA_2's are a superfamily of esterases that hydrolyze the sn-2 ester bond in glycerophospholipids, releasing FFA and LPA. During the acute inflammatory phase, PLA_2's also hydrolyse HDL, which now contains A-SAA instead of apolipoprotein A1. This A-SAA stimulated activity increases production of FFA, eicosanoids and LPA's (10). Generation of proinflammatory lipid mediators (such as thromboxanes, prostaglandins and leukotrienes) from the released FFA's explains how PLA_2's mediate inflammatory responses and play a role in cellular injury (36). On the other hand, LPA's, the other product of PLA_2 hydrolysis, are identified as a major tumor-promoting factor in ovarian cancer ascites (17,18). Another research have shown that LPA is a potent chemoatractant for melanoma cells in general, and that outward-oriented gradients of LPA are self-generated by melanoma cells. They determined that LPA chemotaxis provides a strong drive for melanoma cells to invade outwards; cells create their own gradients by acting as a sink, breaking down locally present LPA, and thus forming a gradient that is low in the tumour and high in the surrounding areas (37).

To date, more than 30 PLA_2 isoforms and related enzymes have been identified with various tissue distributions, biological functions and substrate specificities. Among these, $sPLA_2$ and Lp-PLA_2 are secreted enzymes and especially $sPLA_2$'s are the focus of cancer studies including breast, colon, pancreas and prostate cancers. New studies (19,38) also showed some evidence other PLA_2's involvement in promoting the development of various cancers, mainly ovarian cancer. It has been proposed that some isoforms of $sPLA_2$ have oncogenic roles and therapies targeting $sPLA_2$ inhibition are currently under investigation (19,38).

Lp-PLA_2 [also called platelet activating factor acetylhydrolase (PAF-AH)] is a secreted circulatory enzyme of the PLA_2 family which is primarily produced by inflammatory cells (monocytes, macrophages, T-lymphocytes and mast cells) (39). It plays a role in the inflammation of blood vessels and its activity is bound to atherogenic small-dense LDL particles (40). Also, the importance of substrates and products of PAF-AH on key cellular functions has been evaluated in cell-based analyses which revealed that these metabolites can have pro- and antitumorigenic functions. Studies in genetically engineered mice lacking PAF-AH expression and genetic manipulation of PAF-AH levels in cancer cells demonstrated diverse functions of the protein in models of melanoma, prostate cancer, colon cancer and others (41).

Thus, although recent studies have shown that Lp-PLA_2 is an independent risk marker for cardiovascular disease, including coronary heart disease, and ischemic stroke, the mechanism of its regulation has not been fully elucidated yet. Modest increases in Lp-PLA_2 activity have also been observed in a wide range of inflammatory pathologies such as pregnancy-induced and essential hypertension, ischemic stroke,

diabetes mellitus, glomerulonephritis, rheumatoid and non-rheumatoid arthritis, and chronic cholestasis (42,43).

Interestingly, a recent study (39) investigated the influence of SAA on the expression of Lp-PLA_2 and found that recombinant SAA up-regulated Lp-PLA_2 expression in a dose and time-dependent manner in both THP-1 cells and apolipoprotein E (-/-) mice via a formyl peptide receptor like-1/mitogen-activated protein kinases/peroxisome proliferator-activated receptor-γ signaling pathway.

We designed the current study to further investigate the role of inflammation in tumorigenesis. In doing so, we discovered a possible relationship between SAA and PLA_2's. Although other PLA_2's have been investigated in various cancer types, this is the first study that evaluates Lp-PLA_2 in malign melanoma patients. Our study showed a significant, positive correlation between Lp-PLA_2 activity and SAA in different stages of malign melanoma. We chose to determine Lp-PLA_2 activity instead of Lp-PLA_2 mass measurements because the enzyme mass represents Lp-PLA_2's mass in intact lipoproteins, whereas the activity shows the phospholipase activity. Lp-PLA_2 activity test requires very small amounts samples (1-10 µL of plasma), is easy to perform and feasible to develop into an automated test.

Study Limitations

This study also has potential limitations. First, using a case-control design does not allow assessment of any causal role of Lp-PLA_2 in the initiation or the progression of malignant melanoma. However, the primary aim of our pilot study was to investigate the potential association between melanoma clinical stages and Lp-PLA_2 among other well-known inflammatory biomarkers. Secondly, PLA_2 enzymes may be elevated in patients with other inflammatory diseases and/or cancers. This can lead to false positive conclusions and is a potential caveat for the clinical application of PLA_2 activity as an efficacious predictive biomarker. However, differential diagnostic tests could be used to confirmation or rule out other known causes of elevated PLA_2 activity.

Conclusion

The results of this study support the notion that Lp-PLA_2 and SAA, alone or together, may be good predictive biomarkers for assessment of malignant melanoma staging. In our study, both SAA and Lp-PLA_2 were elevated in melanoma patient sera initially from stage 1 and reached the highest levels in stage 4, suggesting a positive association with tumor progression. Serial Lp-PLA_2 measurements could be used to reduce the number and overall cost of other diagnostic procedures such as imaging techniques. This in turn, will have high value in restaging the patient and/or starting a change in therapeutic strategy when necessary. Further prospective cohort studies with larger patient populations including complete follow-up data and serial blood measurements are required to establish the significance of the enzyme's activity as either diagnostic or prognostic marker for malignant melanoma. Nevertheless, future studies on the significance of elevated Lp-PLA_2 activity in disease detection and progression will be highly interesting.

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Ethics

Ethics Committee Approval: The protocol for the study was approved by the Ege University Faculty of Medicine Ethics Committee on Clinical Research (approval number: 13-6/54).

Informed Consent: Written informed consent was obtained from all subjects.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.K., F.S., G.K.Y., F.Ö., I.K., Design: Y.K., F.S., Y.A., G.K.Y., F.Ö., Data Collection or Processing: F.S., Y.A., G.K.Y., E.A., E.S., Analysis or Interpretation: F.S., Y.A., E.A., E.S., Y.K., I.K., Literature Search: Y.K., F.S., Writing: Y.K., F.S., I.K.

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