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Evaluation of sialic acid levels in patients with hydatidiform mole: a preliminary study

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Summary

Purpose of investigation: This study aims to investigate whether hydatidiform mole (HM) disease with malignant potential is significantly associated with increased sialic acid (SA) levels. *Materials and Methods:* A total of 114 women were enrolled in this study. Patients were divided into three groups including HM (Group 1, n=34), control group including non-pregnant healthy patients (Group 2, n=42), and another control group including healthy pregnant patients within 12 weeks of gestation (Group 3, n=38). Serum-free SA levels were measured. *Results:* There was a statistically significant difference in serum-free SA levels among the groups ($p \leq 0.001$). Patients with HM had significantly higher levels compared to the control groups. *Conclusion:* The present study results showed that there was a significant correlation between HM and serum SA level.

Key Words: Hydatidiform mole; Sialic acid; Pregnancy.

Introduction

A hydatidiform mole (HM) is a gestational trophoblastic tumor (GTN) originating from the placental site with a potential for local invasion and distant spread. The HM is classified as complete (CHM) and partial (PHM) subtypes according to the histopathological criteria [1]. Sialic acid (SA) is a generic term for derivatives of neuraminic acid [2]. SA plays an important role in ensuring the proper and healthy functioning of biological systems. In humans, the alteration of SA levels is known to be associated with various disorders and conditions such as cardiovascular diseases, inflammatory diseases, endocrine diseases, and neurologic diseases. Free SA is very rarely observed in organisms [3]. Sialic acid levels are increased in certain types of cancer [4]. Increases in the levels of total serum SA (TSA) and lipid-bound SA (LSA) have been observed in various pathologies such as advanced ovarian cancer [5], cervical cancer [6], breast cancer [7] and endometrial cancer [8]. To the best of the present authors' knowledge, SA activities have not been reported previously in patients with HM in the English literature. In this preliminary study, they aimed to investigate whether HM disease with malignant potential is significantly associated with increased SA levels.

Materials and Methods

Between April 2009 and November 2009, a total of 114 women who were admitted to Gaziantep University, Faculty of Medicine, Obstetrics and Gynecology Outpatient Clinic were prospectively analyzed. Patients were divided into three groups including HM (Group 1, n=34), control group including non-pregnant healthy patients (Group 2, n=42), and another control group including healthy pregnant patients with a single viable fetus within the first trimester of pregnancy (Group 3, n=38). Informed consents were obtained from the patients. The study protocol was approved by the Ethics Committee for Clinical Research of Gaziantep University, Faculty of Medicine.

The diagnosis of HM (complete or partial HM) was based on histopathological examination of the molar tissue samples, which were taken by suction curettage under mask anesthesia. Blood samples (six ml of whole blood) were drawn from the cubital vein into regular tubes. The samples were centrifuged at 1,600 r.p.m. for five minutes and stored at -80 °C until used.

Serum SA operating procedure

Serum-free SA levels were measured using the Sialic Acid Quantitation Kit, a commercial enzymatic, photometric method in accordance with manufacturer instructions.

Test Principle:

First, N-Acetylneuraminic acid (NANA) aldolase catalyzes the reversible reaction (Reaction 1):

1) NANA ↔ N-Acetylmannosamine + pyruvic acid

The pyruvic acid can be reduced to lactic acid, β-NADH, and lactic dehydrogenase (Reaction 2)

2) Pyruvic acid + β-NADH ↔ Lactic acid + β-NAD

Under the proper conditions, the first forward reaction predominates, and when coupled with β-NADH, the reaction is com-

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Table 1. — *SA levels among the groups*.

	Group 1 mean ± SD *(min-max)*	Group 2 mean ±SD *(min-max)*	Group 3 mean± SD *(min-max)*	*p*
SA*	0.96 ± 0.12 *(0.73-1.25)*	0.56 ± 0.33 *(0.12-1.09)*	0.65 ± 0.28 *(0.16-1.17)*	<0.001

*SA Unit: µmol/L

Table 2. — *SA levels of complete and partial mole pregnancies*.

	Group 1 - C mean ± SS *(min-max)*	Group 1 - P mean ± SS *(min-max)*	Group 2 mean ± SS *(min-max)*	Group 3 mean ± SS *(min-max)*	*p*
SA*	0.98 ± 0.11 *(0.86-1.24)*	0.91 ± 0.13 *(0.73-1.25)*	0.56 ± 0.33 *(0.12-1.09)*	0.65 ± 0.28 *(0.16-1.17)*	0.301

*SA unit: µmol/L

pleted. β-NADH oxidation at 340 nm and NANA samples can be accurately measured spectrophotometrically.

Statistical analysis

Statistical analysis was performed using SPSS v17.0 software. Categorical variables were expressed in number and percentage, while continuous variables were expressed in mean and standard deviation (SD) (in minimum and maximum, if necessary). A one-way analysis of variance (ANOVA) was used for the comparison of continuous variables when the assumptions were met, whereas the Kruskal-Wallis test was carried out when the assumptions were not met. The *t*-test or Mann-Whitney U test performed used to compare differences between two independent groups with Bonferroni correction. The Spearman's correlation coefficient was used to assess the strength of the correlation among these continuous variables producing abnormal distribution. A p value of < 0.05 was considered statistically significant.

Results

There was no difference in mean age between the subjects ($p = 0.256$).

Serum SA levels were significantly higher in the HM group (Group 1) than compared to the controls (Group 2 and Group 3; $p = 0.001$) (Table 1). In the HM group, 23 subjects had CHM, while 11 had PHM. There was no difference in SA levels between the CHM and PHM subgroups (Table 2).

Discussion

A HM has a potential for local invasion (15%) and distant metastasis (4%) [9]. Normal trophoblasts exhibit a broad range of synthetic activities, including the synthesis of steroid hormones and glycoproteins. The most effective approach for identifying GTN is assessing the level of hCG. The serum and urine hCG levels are closely parallel to the number of viable tumor cells. As the level of hCG is associated with the activity of viable tumor cells, monitoring increases or decreases in hCG levels is more important than employing radiological or other diagnostic methods. The monitoring of the disease or the planning of treatment does not require histological diagnosis; as such, decisions regarding the monitoring/treatment approach are based on hCG values [10]. SA can be found freely or bound to proteins and lipids (gangliosides) at the terminal positions of oligosaccharides [11]. SAs are generally located in the inner and outer surfaces of lysosomal membranes as well as at the terminal positions of the main and side chains of oligosaccharides. SA, therefore, is the primary molecule, which is encountered by biochemical compounds, which interacts with cells and other cells. This feature of SA and its being negatively charged at physiological pH are directly associated with the functions of SA in the organism [12]. Polysialic acid (PolySia) is an anionic large homopolymer composed of α-2,8-linked SA residues that are normally found on cell surfaces. PolySia, which has been subject to considerable study in the nervous system, is involved in the modulation of cell development (by increasing cell migration), and in the regulation cell differentiation. PolySia is also observed in the immune cells of children and adults, and serves as an indicator for numerous cancer pathologies [13]. Hromatka *et al.* [13] identified PolySia in the trophoblast of human placenta. Cytotrophoblasts and syncytiotrophoblasts express PolySia during the initial trimester of pregnancy, although PolySia expression gradually decreases as the pregnancy progresses. In a model of chorionic villous growth, it has been observed that PolySia causes the migration of cytotrophoblasts. Furthermore, in an *in vitro* model, it was determined that removing PolySia from the cytotrophoblasts' environment had the effect of decreasing their ability to penetrate and invade basement membranes. In addition, biopsies from patients with gestational trophoblastic pathologies (such as malignant choriocarcinomas and benign molar pregnancies) exhibited overexpression of PolySia. These findings suggest that PolySia assumes an active and functional role in the normal development of the human placenta, and that PolySia is also involved in the invasion of trophoblast tumors. In this context, the aim of the present study was to demonstrate clinically the strong relationship between SA and trophoblast tumors.

The SA level was increased in parallel to increased tumor burden and degree of metastatic diseases [4]. Some studies demonstrated that SA levels might be increased in cancer patients without clinical symptoms [14]. In addition, many studies showed that measurement of SA level might be used in cancer patients in the assessment of progression and regression of the disease, when combined with other biomarkers, particularly. The function of SA as a tumor biomarker is associated with abnormal glycolization of cancer cell membranes through the activation of new glycosyltransferases, a specific characteristic of tumor cells. The role of SA in the distant metastasis is associated with the increase in the capacity of endothelial binding [15]. There are also some studies in the literature conducted on this subject.

Shimizu *et al.* [16] observed that serum SA levels were increased in gynecologic tumors including myomas, benign

ovarian tumors, cervical cancer, endometrial cancer, and ovarian cancer using an enzymatic method. Cancer patients with poor prognosis exhibited considerably higher SA levels than cancer patients with good prognosis, regardless of the type of treatment being employed. In addition, higher SA levels were indicative of the cancer's clinical course. For patients receiving combination therapies and who require monitoring for extended periods of time, achieving effective follow-up by using tumor markers alone may prove extremely difficult. However, SA will serve as a useful marker even for the follow-up of such patients, since it is a non-specific marker for cancers of different histologies. Similarly, Yue *et al.* [17] reported that serum LSA was more cost-efficient and easy-to-use than Ca125 and might be used in patients with ovarian cancer. In another study investigating a biochemical index for diagnosis and treatment of cervical cancer, Patel *et al.* [18] measured serum TSA, LSA, and lactate dehydrogenase using high-specific spectrophotometric methods. Compared to the control group, the level of all markers were significantly higher ($p \leq 0.001$) in the cervical cancer group. No significant changes were observed in the markers for early (1-2) and advanced (3-4) stages. Among patients who were non-responsive to radiotherapy, the TSA and LSA values were significantly higher compared to patients responsive to radiotherapy ($p < 0.05$ and $p < 0.01$, respectively). The authors reported that TSA was the most sensitive biomarker (90.74%) and might be helpful to identify patients with cervical cancer and follow their treatment responses, when combined with other biomarkers. On the other hand, Vivas *et al.* [5] concluded that TSA and LSA levels were not a contributing factor to early diagnosis of cervical cancer or clinical staging of the tumor. In the present study, the authors demonstrated significant differences in serum SA levels among the HM group, healthy pregnant controls, and non-pregnant controls. Serum SA levels were significantly higher in the HM group, compared to control groups. However, they observed no significant differences in SA levels between the CHM and PHM subgroups.

Conclusion

In conclusion, SA level measurement alone appears to have a limited value in the preliminary diagnosis of a malignant disease. On the other hand, SA level may be helpful in the assessment of the progression and regression of a disease during therapy, when combined with other markers such as HCG, particularly. To become clinically useful, however, assay methods need to be refined. To confirm these views, the present authors have planned a second prospective study that will demonstrate the importance of SA levels in potentially invasive and metastatic molar patients, and which will demonstrate the response to administered chemotherapeutic medications based on variations in SA levels.

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