

HPLC WITH UV DETECTION OF PSEUDOURIDINE AS TUMOR MARKER FOR BREAST TUMOR IN VIVO⁺

Amal Rasheed Ferhan^{*}

Dr. Rajha Al- Naiemi^{**}

Saosun Abd Al-Shaheed^{***}

Abstract

This work was done to evaluate the role pseudouridine status which is modified nucleoside in the serum of breast tumor patients.

Serum Pseudouridine was measured by HPLC (high performance liquid chromatography with UV detection), and to study its effect on serum of 12 normal healthy volunteers compared to values obtained from 30 subjects with breast tumor groups (malignant and benign) , all subjects aged between (30-60) years as compared with control.

The statistical analysis for the data obtained in the present study showed that the concentration level of pseudouridine was significantly elevated in the patients with malignant tumors as compared with controls but serum showed slightly increased but not significant in benign breast tumor patients as compared with controls.

The result observed shows that the concentration levels of this type of modified nucleosides could be a useful parameter for the early diagnosis of patients with breast tumor , so this method suggested that this compound is a sensitive marker for breast tumor patients.

المستخلص

في هذه الدراسة تم التقصي عن احد النيوكليوسيدات المحورة الموسومة (Pseudouridine) في مصل الدم للنساء المصابات باورام الثدي (الحميد والخبيث) .

واعتمدت طريقة القياس على الطور العكوس للكروماتوغرافيا السائل ذي الاداء العالي باستخدام مجس الاشعة فوق البنفسجية (UV) الذي وصف التحليل الكمي لهذا النيوكليوسيد ودراسة تأثيره في مصل الدم لـ (١٢) متبرع من النساء والتي قورنت مع القيم التي تم الحصول عليها من (٣٠) مريضة مصابة باورام الثدي ممن تتراوح اعمارهم بين (٣٠-٦٠) سنة والذين قسموا الى مجموعتين الأولى : المصابات باورام حميدة وعددهم (١٤) حالة والثانية : باورام خبيثة وعددهم (١٦) حالة .

من النتائج الاحصائية التي تم الحصول عليها ، اضطرت الدراسة بان مستوى (Pseudouridine) قد اعطى زيادة معنوية لمرضى سرطان الثدي الخبيث مقارنة مع الاشخاص الاصحاء (controls) في حين ان مستوى (Pseudouridine) قد اضهر تذبذباً لمرضى اورام الثدي الحميدة لكل من الناحية الاحصائية لم تعطي فروقاً معنوية مقارنة مع مجموعة الاشخاص الاصحاء .

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^{*} Assis. Prof . the Medical Technical Institute of Al-Mansour, Baghdad, Iraq /f_fanar2004@Yahoo.com

^{**} Assis. Prof Veterinary Medicine- R.Naiemi@Yahoo.com

^{***} Assis Lecturer -.Technical College – Baghdad – Iraq

نستنتج مما سبق ان دراسة هذا النوع من المركبات ذو فائدة لتشخيص الاورام السرطانية المبكرة للمرضى كذلك للمرض كذلك اعتبارها كمؤشرات حساسة .

Introduction :

Nucleosides can be defined as compounds in which purin or pyrimidine is linked glycosidically to a sugar [1] . The main sources of nucleosides are nucleic acid .Most modified nucleosides detected in nucleic acids are relatively simple structural modification of nucleosides which are found in t-RNA [2] , about 50 modified nucleosides have been isolated from t-RNA of various organisms and the structure of 37 of these nucleosides have been determined [3] , Methylation is the most common from of t-RNA modification . Fig (1) give structure of base methylated nucleosides found in t-RNA.

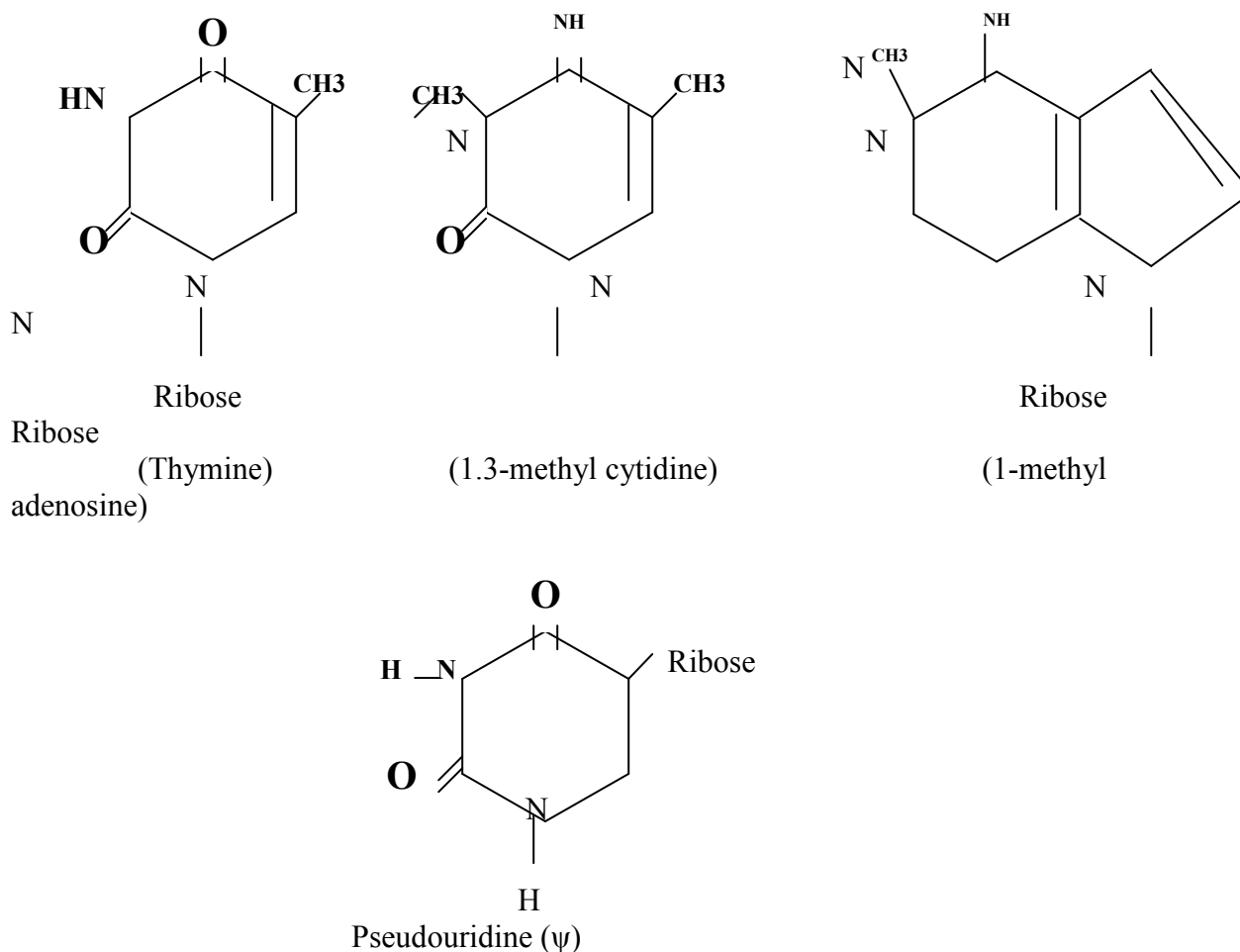


Fig (1) : structures of methylated nucleosides found in t-RNAs.

Pseudouridine (ψ), the most common modified nucleosides [4.5].

it does not fit the usual definition of modified nucleosides because neither the base nor the ribose is substituted , An acidic hydrogen in (ψ) would provide a specific reactive site in t-RNA polymer not provided by uridine . These modifications would there by cause a considerable change in the chemical properties of t-RNA at their point of location [6].

The alterations in t-RNA which have been studied mostly in relation to carcinogenesis [7.8] . Many studies have reported the relation ship between malignant

tumors and various modified nucleosides and its relation to cancer metabolism . [9.10].

One of these tumors is breast tumor (malignant and benign) the breast is constantly responding to changes in hormonal nutritional genetics psychological and environmental stimuli that cause continual cellular change [11] .

Breast cancer is the second most common cancer to they cancer as a cause of death from a cancer among women in the world .

During the literature survey they have been found that there are several types of biomarker that may vary in concentration and nature between normal cells and tumor cells [12].

These types include enzymes activity , alteration in the concentration of some hormone levels and their receptors and some of minerals [3]. The rather unexpected discovery was made of a group of enzymes with methylate nucleic acids at the polynucleotide level present in t-RNA might be responsible for carcinogens [7].

Modified nucleosides have been thought to be derived predominantly from degradation of TRNA and have been shown to be excreted in urine of patients with various malignant diseases [14.15].

Similar findings were reported by Liebich et al (1996) . who investigated serum in patients with liver , larynx and breast cancer , it has been found that serum was significantly elevated in those patients as compared with controls [16] . Also Douglass , C, et al (1980) who represented that urine levels were significantly elevated in breast cancer [17] . Another study found that the concentration of serum levels were significantly higher in the patients with lung cancer than that in patients with pulmonary infections diseases as compared with controls [18].

Material & Method :

A total of 30 women with diagnosed breast tumor aged (30-60) years have been examined who had been admitted in medical city (early detection of breast tumor) , and they were sub divided into two groups 16 patients with study and informed consent was obtained from all subject the patient groups were included in this study as a control groups .

The results were presented as a mean standard deviation and range , all statistical . analyses were done using SPSS package and were performed using student t- test and taking $p < 0.05$ as the lowest limit of significance .

A venous blood (3ml) was collected from controls and patients after clotting the samples were centrifuged at (600Xg) for 10 min . 0.2ml serum was diluted with 0.2 ml of (0.2M Na⁻phosphate buffer PH 8.4 and centrifuged of (1000Xg) for 10 min to remove clotting . 20 ul from the filtrate was injected in HPLC instrument named .

The condition were used in this work for the estimation of Pseudouridine in breast tumor patients were :

Column type C18 (ODS)

Concentration and type of mobile phase (0.03) M ammonium dihydrogen phosphate buffer PH =5.3 with 2.5% methanol .

Flow Rate of mobile phase (1 ml /min)

UV detector the wave length was 23 nm

Column temperature (was 25C)

Results and Discussion

Breast tumor was described, as either benign or malignant, breast cancer disease is a common tumor in females world – wide [13], knowledge about modified nucleosides metabolism and their disturbances in tumor tissue or in organism carrying a malignancy, might help to elucidate the relation of the modified nucleosides to tumor and to decide whether these modified nucleosides could be used as additional biochemical marker for the diagnosis and prognosis of tumor development and after treatment with chemotherapy and radiotherapy. Tamura et al (1986), showed that 59% of patients with small cell lung cancer (SCLC) and 27% of those with non (SCLC) had a urinary pseudouridine (ψ) level above the mean value plus 2 for healthy controls [18], and the same group in (1987) presented another study and reported the concentration of (ψ) in 24 patients with (SCLC), 13 patients with non – SCLC with advanced stages, they found that the mean serum (ψ) concentration was significantly higher in patients with SCLC than that in patients with pulmonary infectious disease.

Nakona et al (1990), presented a study included the excretion of modified nucleosides by determination of urinary (ψ)

They found elevated (ψ) in –patients with gastrointestinal cancer as compared with normal urine [19].

These results suggest that urinary (ψ) might be clinically useful as complementary marker to the monitoring of the disease status of patients with Leukemia and Lymphoma [20]. Finally the sera of breast cancer patients showed that the level of (ψ) in serum was significantly elevated in these patients. The mean level $\pm$ SD of (ψ) in serum of malignant breast tumor patients were (8.12 ± 2.376 , $P < 0.002$) as compared with control (mean $\pm$ SD = 2.52 ± 0.085) while mean $\pm$ SD of (ψ) in serum of benign breast tumor patients were (2.75 ± 0.532 , $p > 0.05$) as compared with controls as shown in table (1). The results showed significant increase in serum (ψ) in malignant breast tumor patients as compared with both (benign breast tumor patients & healthy controls).

However no significant difference was found between benign groups & healthy controls. The elevation of concentration in serum (ψ) is due to increase cellular activity in malignant tissue and this has been interpreted as being due to a greater turnover rate of t-RNA in tumor tissue than in normal tissue [21].

The metabolic turnover of t-RNA is facilitated and modified nucleosides are produced and excreted in large amount [10], the methylated bases may be more specific in this regard since increase t-RNA methylase activity in a common concomitant of neoplastic growth was detected.

In earlier studies, the urine of 62 patients with diseases other than cancer was analyzed and considered modified nucleoside as biological markers for other disease. The disease syndromes were (Hepatitis, Obstructive lung disease, Cirrhosis, Acute pancreatitis, Psoriasis), they found the levels of several methylated nucleosides such as (ψ) most frequently elevated when compared to normal urine [9].

Schoch et al (1990) recently reported that selected modified urinary nucleosides could be used as indicators for whole – body turnover of r-RNA, r-RNA and m-RNA. Moreover, these modified nucleosides also used to calculate the biological half – lives of t-RNA and r-RNA, they found increased r-RNA turnover as a result of increased protein synthesis in cancer patients. The rapid turnover of both t-RNA and r-RNA ascribed to AIDS patients is thought to occur from the break down of whole – body cells, while the half – lives of both RNA's determined from the urine of cancer and AIDS patients were remarkably reduced [22].

Table (1) : Serum Pseudouridine Level (Mean $\pm$ SD) of patients with Benign and Malignant Breast Tumors and healthy controls.

Groups	Pseudouridine Level (Mean $\pm$ SD) (μ mol/L)	Probability (P value)
Control	2.52 $\pm$ 0.0850	
Benign	2.75 $\pm$ 0.532	0.073*
Malignant	8.12 $\pm$ 2.376	0.002 **

(*) For comparison between Benign breast tumor patients and controls.

(**) For comparison between Malignant breast tumor patients and controls.

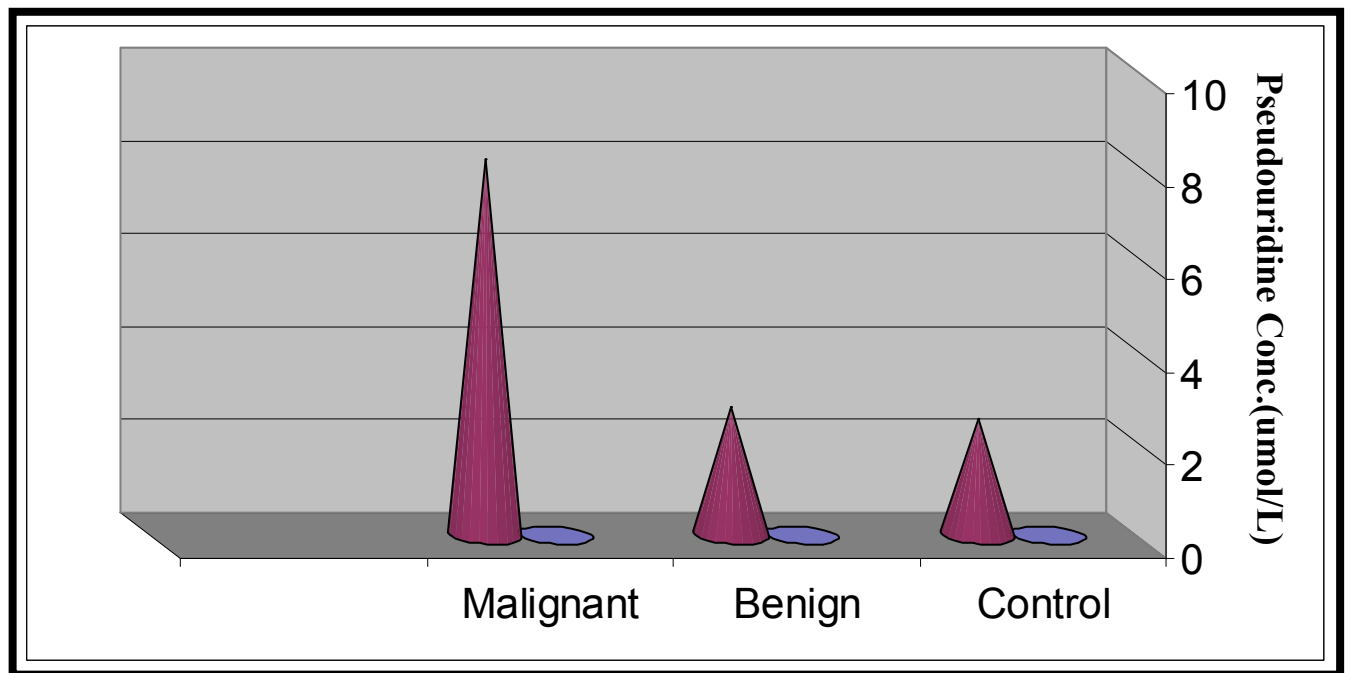


Figure (1) : The mean of Pseudouridine level in the sera of patients with malignant and benign breast tumors as compared with healthy controls

References

1. Rawn, J.D Biochemistry "Intern ed., printed in the united states of America,(1989).
2. Zachan, H.G. *Angew chem. Intern. Ed.* 8, 711, (1969).
3. Stacheline, M. *Experientia* 27,1, (1971).
4. Chon, W.E. *J.Biol. chem.* 235, 1488, (1960).
5. Yamaizumi, Z. Nishimura, S., Limburg, K. and Closkey, J. A. *J.Am. chem. Soc.* 101, 2224, (1979).
6. Miller, C.O. *scienc* 157, 1055. (1967).
7. Kerr, S.J. *Biohem. Biophys. Acta* 407, 392. (1978).
8. Borek, E. *Tumor boil.* 5,1. (1984).
9. Waalkes, T.P., Abeloff, M.D. and Ettinger, D.S. *Eur. J. Cacer clin-Oncol.* 18, 1269, (1982).
10. Mandel. L.R. Simivasan, P.R. and Borek, E. *Nature London* 209, 586, (1996).
11. Porth , C.M., *pathophysiology 4th. Ed. Philadelphia*, J.B. lippincott, (1994).
12. Garofale. J.A, A Shir, K.H. and Menemdez, *Cancer*, (1980).
13. Anthorng, S.E.Eugena, Biskurt, J.I. and Jean , D.W. *Harrisons Principles of Internet medicine*, 4th ed. New york (1998).
14. Terwin, R.W. Glaser , R.Kelly, D.R and Speicher, C.E. *Cancer (phila)* 49, 2513, (1982).
15. Fischbein, A. Sharma, O.K. and Borek, E. *Cancer Res.* 43, 2971, (1983).
16. Libich, H.M., Stefano, C. and Schmid, H.R. *J. of Chromatogram A.* 28, 763 (1-2) , 193, (1996).
17. Donglass, C., Waalkes, T.P. and Gehrke, C.W- *Journal of surgical oncology* 14, 267, (1980).
18. Tamura , S., Fujii , j., Nakano , T., Hud , T. and Higashio , K. *Cancer Rec .* 47,6138,(1987)
19. Nakanao , k and Yasaka, T. *j of Chromatogram* ,515,537,(1990) .
20. Kunihiiko, H.; Konno , T. Ishiwata , S. and Misugahi , M, *Clinica . Chemica . Acta* 206,181 ,(1992).
21. Baliga ,B.S., Gehrke , C. W., Kuo , K.C. and Belman , S. *Cancer Res .* 37,3362, (1977).
22. Schoch , G., Sander , G. and Topp , H. *Modified Nucleosides and Nucleosides in Urine and Serum as selective markers for the whole –body turnover of t-RNA , r-RNA and m-RNA .* In .part C. Amsterdam : Elsevier ,PP.C 389 ,(1990).