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The clinical relevance of the correlation of NM 23-H1 expression with PAP, PSA and Androgen Receptors in patients with Prostate Cancer and BPH

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ABSTRACT

We investigate the correlation of the tissue expressions of NM23-H1, androgen receptor, serum PSA and PAP in patients with prostate cancer and benign prostatic hyperplasia to search for the possible relevance for the prognosis. Seventy-five patients were included to study, of these, 55 were diagnosed with prostate cancer and 20 with benign prostatic hyperplasia. Twenty-eight percent of stage I, 54% of stage II, 33 % of stage III and IV cases show the NM23-H1 expressions. The metastatic and nodal diseases (55%) mostly showed the strong intensity of NM23-H1. The correlations between stages and NM23-H1 expression were not statistically significant. A variable but steady NM23 staining from all different stages was noted. However, no correlations among NM23-H1, AR, PSA, PAP and tumor grading in terms of disease prognosis were seen, except for the moderately differentiated tumors with moderate PSA expression levels which have the statistically significant correlation with increased expression of the NM23-H1. [Turk J Cancer 2009;39(4):136-145]

KEY WORDS: Prostate cancer, benign prostatic hyperplasia, NM23-H1, prostate specific antigen, prostate specific acid phosphatase, androgen receptor

INTRODUCTION

Prostate cancer (PCa) is one of the main causes of morbidity with relatively low mortality and is one of the leading causes of cancer-specific death among men (1). Although the mean age for diagnosis is placed between 60-70, more than 60% of cases diagnosed with PCa were at mean age of 75 years or older and there are still cases reported under the age of 45 (2,3). Having the available diagnostic tools and prostate specific antigen (PSA) screening provides the early diagnosis for the patients, but the factors correlating the poor prognosis of some of the early prostate cancer cases are still not clear. On the other hand, for older men it becomes controversial to initiate a contemporary treatment of prostate cancer, if diagnosis does not provide enough information about the aggressiveness of the disease and if the treatment modality is more aggressive than the disease itself which, in turn, increases the risk of side effects (4-7). The options for a proper treatment modality are necessarily to be improved with some other parameters in addition to the stage, grade, DNA polyploidy and PSA.

Serum PSA detection is used for disease monitoring among men diagnosed with PCa (8-11). Later, the tissue specificity for the prostate epithelium defined, and the high level of the serum PSA or remote expression of

PSA in another tissue were found compatible with the diagnosis of the prostatic origin (12). In combination with histopathological evidences, serum and tissue PAP levels, Gleason score and clinical stage of the disease, PSA levels can be predictive for the outcome of disease as well as the treatment, but still with some limitations (13,14). Serum PSA level increases with age, and therefore the PCa cases of young males remain unnoticed until the disease progress and becomes clinically detectable, whereas the older males with PCa develop less aggressive disease. Recently, the increased serum PSA level and the low mortality rate for elderly were associated and this may explain the slow growth in prostate cancer with aging (15). However, a recent review on "the use of classical and novel biomarkers" revealed the heterogeneity of the studies resulting in the inconclusiveness to use a single classical marker or the novel biochemical and histological markers on disease prognosis. Some of these new markers, including modified Gleason grade, STAT5, PAP, p53, Bcl-2, Androgen receptor (AR) with CAG (Cysteine-Adenine-Guanine) repeats, were inconsistent with disease prognosis (16). Another classical tissue marker of PCa is AR which is also not accepted as a single marker (16-19). Although the basal secretion of PSA level is unclear for disease prediction, for some cases, it is found reliable to combine the tissue AR, PAP and PSA levels for diagnosis (17, 19-21). AR is the marker observed in primary PCa varying through the disease progression in both hormone-sensitive and hormone refractory PCa (10,13, 16-18).

Considering the fact that, the disease aggressiveness is directly related with its metastatic potential it is important to determine the levels of tumor suppressor gene expression in PCa. In this respect, a tumor suppressor gene product, NM23-H1, received a great attention and, in turn, studies focused to dissect the mechanism underlying its anti metastatic function (22-25). However, the heterogeneity of PCa and the clinical studies were resulted in inconsistency to explain the role of NM23-H1 in disease prognosis. The expression level in healthy prostate tissue was reported with the same intensity in PCa, where healthy prostate gland showed more basal activity than the secretory. Since an increasing expression of NM23-H1 was seen with dormant cells and the decreasing levels in metastasized cancer cells, NM23-H1 appears to be a possible prognostic marker combined with other classical markers for disease aggressiveness (22-27). Considering again that younger males are susceptible to develop more aggressive and highly metastatic prostate cancer, and elderly may be at risk for undergoing any up-to-date treatment modalities, new approaches to combine the novel and conventional parameters for the diagnosis and the prognosis of disease should be studied to show whether the patient may benefit from therapy in a short term, or the disease has a potential to become aggressive during the life time. On the other hand, based on recent studies with the classical markers, such as serum PSA and PAP levels, as well as tissue AR expression in combination with novel markers, such as nm23-H1, one can easily conclude that the more extensive clinical studies are necessary to reach more reliable results (2, 4-39).

In this study, we investigated the expression levels of NM23-H1, androgen receptor, PAP and PSA in both prostate cancer and benign prostate hyperplasia (BPH) to understand and correlate their roles in disease progress, metastatic propensity and prognosis (9,37,40,41).

METHODS

In this study, 75 patients were included, where 55 were diagnosed with PCa and 20 with BPH, admitted to University of Ankara, Department of Urology and Pathology. Of these, six patients with PCa died during the study, and 49 patients with PCa were followed with the mean follow up of 19 months (3-48 months). Both BPH and PCA patients underwent the serum PSA and PAP analysis at the time of staging. During the follow-up of the PCa patients, the pathological specimens were obtained by Prostate Needle Biopsy (PNB) in 32 patients, by Transurethral Resection of Prostate (TUR-P) in 8, and by Radical Prostatectomy (RP) in 15 patients. The specimens for BPH cases were obtained for 14 patients by TUR-P, and by Transvesical Prostatectomy of Prostate (TV-P) for 6 patients. The control follow-up was performed via rectal examination, bone scintigraphy, transrectal and abdominal ultrasonography, chest X-ray, serum prostate specific acid phosphatase (PAP) and prostate specific antigen (PSA) levels, where the patients were not subjected to any chemotherapy at the time of the sampling. The staging was assessed according to TNM classification (36), and for the histological grading the Gleason System (42) was used after Hematoxylin and Eosin (H&E) stain-

ing. Immunohistochemistry was used for demonstrating the expression of NM23-H1, androgen receptor, PAP and PSA antigens in both PCa and BPH tissues, where the staining was performed on paraffin-embedded tissue specimens using the Streptavidin-biotin peroxidase (ABC Kit Novocastra, Newcastle, UK) for antigen localization with (polyclonal) antibodies against PAP and PSA (Immunon, Pittsburgh, PA,USA), Androgen Receptor and NM23-H1 (Novocastra, Newcastle, UK) (43-45). The samples, as five-micrometer-thick tissue sections, were deparaffinized in Xylene (3x5min), rehydrated through a graded alcohol and washed in distilled water. Then the heat-induced epitope retrieval was performed followed by cooling and washing steps in phosphate buffered saline (PBS). Non-specific endogenous peroxidase reactivity was blocked using periodic acid (3%). Tissue specimens were treated with primary antibodies at room temperature for two hours and then incubated with a secondary biotinylated antibody. The visualization was carried out with aminoethylene carbazole (AEC) as a chromogen. The preparations were counterstained with Mayer's Hematoxylin. Positive controls were prepared from BPH tissue for PAP and PSA, from PCa for AR and from breast cancer for NM23-H1. These samples were also used as the negative controls in the absence of relevant primary antibodies. The samples for NM23-H1, PSA and PAP displayed a cytoplasmic and granular red brown staining pattern. For AR, the nuclear red-brown granular pattern was observed. The intensity and density of their expressions were evaluated separately. Here, the intensity patterns were scaled as 0 for negative, 1 for weak, 2 for fair, and 3 for strong. The density patterns were determined as the percent of the cells stained with respect to the total cells analyzed and reported as 0 for 0-25%, 1 for 25-50%, 2 for 50-75%, and 3 for 75-100%.

Statistical evaluation

Statistically, Kruskal-Wallis variants analysis, Mann-Whitney U test, Chi-Square test, Kendall's tau-c test, Spearman correlation test were applied to compare the expressions of NM23-H1, AR, PSA and PAP, their correlations with each other, and with the tumor grading, staging, serum PAP and PSA levels. For the statistical significance, alpha level was assigned as p=0.01 for Mann-Whitney U test and Kruskal-Wallis analysis, and as p=0.05 for other analyses.

RESULTS

The mean follow up for the prostate cancer patients was 19 months (3-49 months) and the staging of PCa patients were as follows, 7 patients were in T1 (12.7%), 11 in T2 (20%), 16 in T3 (29.1%), and 21 in NM stage (38.2%). The mean serum PSA and PAP levels were 32.9 ng/ml and 25.3 IU/ml, respectively for PCa. The serum levels for BPH were measured as 6.5 ng/ml for PSA and 4.31 IU/ml for PAP. The PSA levels and tumor staging were correlated and the mean PSA was found as 8.2 ng/ml in T1 stage, 13.5 ng/ml in T2, 26.9 ng/ml in T3 and 78.2 in NM stage (Figure 1). High serum PSA levels for patients with metastasis were also verified statistically (Mann-Whitney-U test, $p<0.01$). PCa samples after H&E staining showed the majority Gleason grade as 6 (47.3%) and the cases mostly were in the grading range of 6 to 8 (94.6%). Immune histochemistry staining for PAP (Figure 2) and PSA (Figure 3) showed the similar red-brown cytoplasmic and granular staining pattern.

The strong expression pattern was observed for 70.7% for PAP and 46.6 % for PSA and their expression levels were correlated with the disease stages (Figure 4). Here, 13% of stage II and 24% of Stage III cases exhibited weaker PSA stain compared to PAP, whereas one patient with stage T1 was positive for PAP but negative for PSA. Except five PSA negative NM cases, all cases with me-

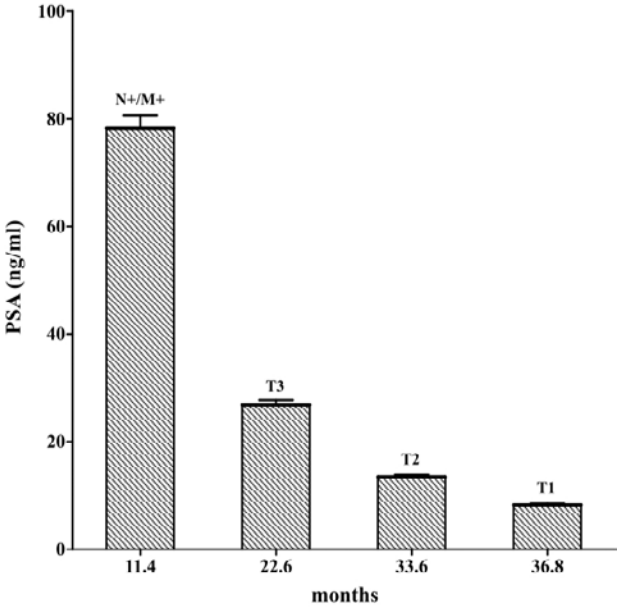

Fig 1. The serum PSA expression levels correlated with the corresponding tumor stages with respect to the mean follow-up in months (Mann-Whitney U, P<001)

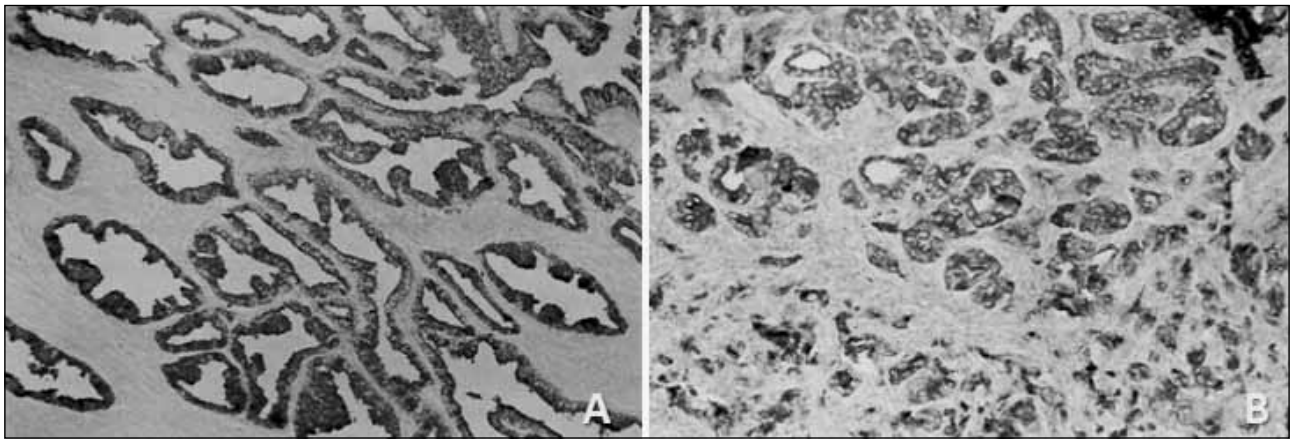

Fig 2 (A&B). (A): IHC staining of BPH and (B): IHC staining of PCa samples against PAP with strong staining pattern shown here for benign glands of BPH (x25) and for atypical acinar glands and infiltrative tumor cells of high grade PCa (x50)

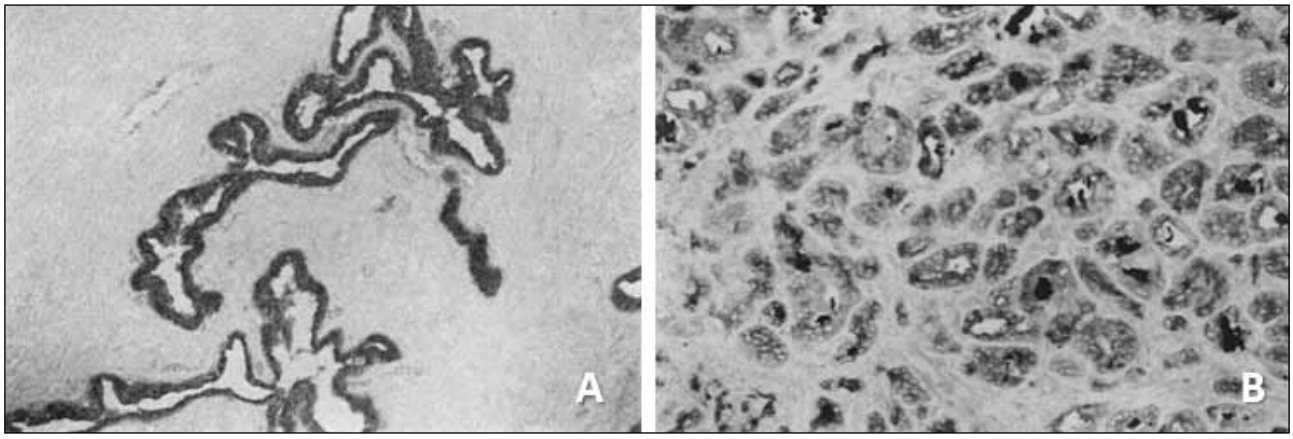

Fig 3 (A&B). (A): IHC staining of BPH and (B): IHC staining of PCa samples against PSA shown here for BPH with strong staining pattern and for PCa with Gleason grade 3 with moderate to strong stain patterns (x25)

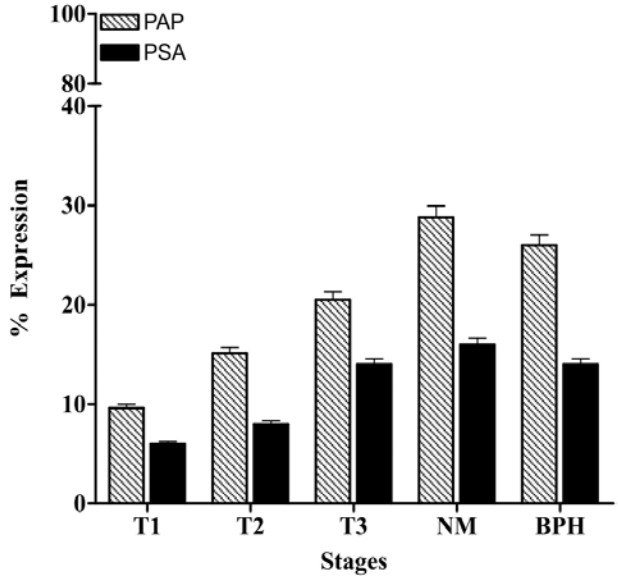

Fig 4. IHC staining against PAP and PSA is presented as percent tissue expression and correlated with the disease stages

tastasis revealed positive PAP and PSA staining (Figures 2 and 3). The mean serum PSA level was determined as 39.2 ng/ml in cases with the Gleason grade 0-7, and was 42.6 ng/ml in cases with the Gleason grade 8-9. No statistical difference between high or low Gleason grade with serum PSA level was determined (t test: p= 0.054).

NM 23-H1 immunohistochemistry showed extensive density and intensity staining with red-brown and granular staining pattern located in cytoplasm (Figure 5). The strong intensity pattern was observed for the 28% of Stage I, 54% of stage II, 33% of stage III, and 55% of NM tumors (Figure 6-A). The staining density pattern for NM23-H1 was found dense (75-100%) for 78.2% of patients and weak to moderately dense (25-75%) for 16.4% of the patients. Five point four percent exhibited no stain pattern (Figure 6-B). However, no statistical association was determined between the decrease in NM23-H1 inten-

sity or density and advanced tumor staging by chi-square analysis ($p>0.01$).

NM23-H1 and AR was not associated with density or intensity distribution (Kruskal-Wallis Analysis, $p>0.05$). AR, was characterized with nuclear red-brown and granular staining pattern (Figure 7). The strong intensity pattern was observed for 23.1% of patients, and for 68.1% the intensity was scaled in moderate to weak (Figure 6-A). In terms of stain density for AR, 60.8% of patients displayed dense (75-100%) and 24% displayed moderately dense (25-75%) staining pattern (Figure 6-B). Evaluation of AR staining intensity with respect to stage revealed the strong intensity pattern for the 6% of T1 stage, 20% of T2, 21% of T3, 10% of NM and 40% of BPH patients, and the strong association (Table 3) between the elevating AR expression with BPH and early PCa was determined by Chi-Square analysis ($p<0.01$). With Kruskal-Wallis analysis, no significant association between NM23-H1 and AR was determined in terms of their expression intensity ($p>0.05$).

The association of NM23-H1 expression with PAP and PSA, 22 patients (40% of cases) showed both strong

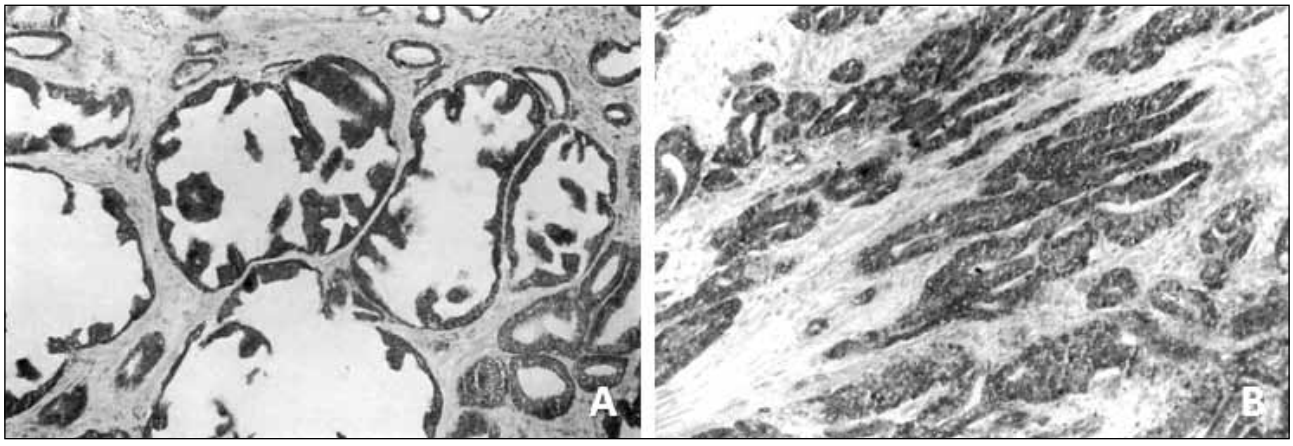

Fig 5 (A&B). (A): IHC staining of BPH and (B): IHC staining of PCa samples against NM23-H1 with strong nuclear staining pattern (x25)

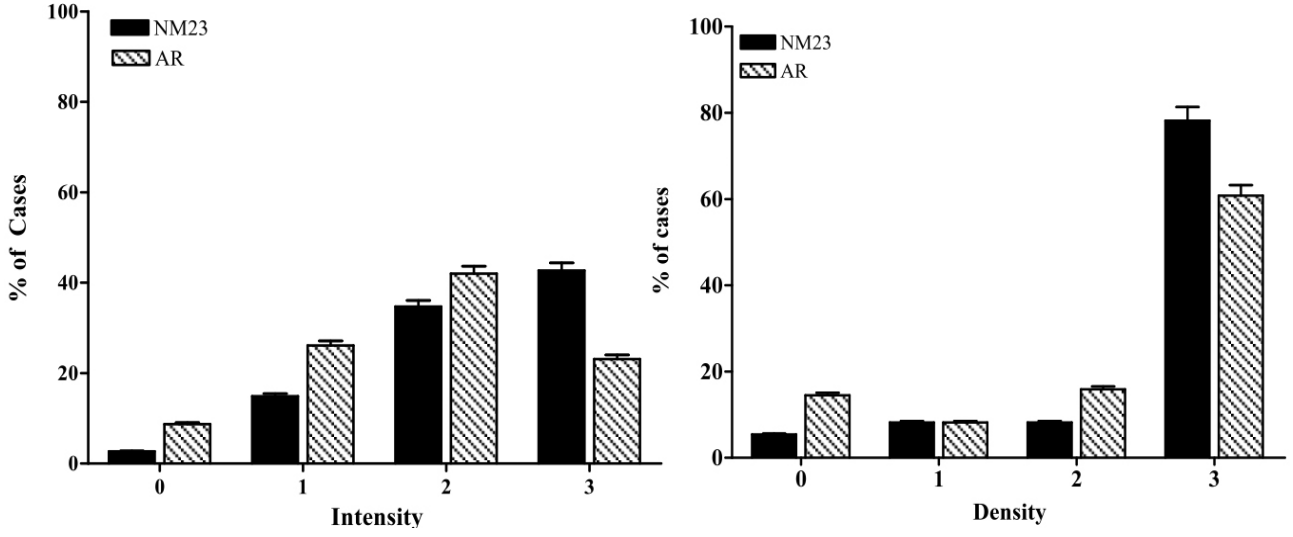

Fig 6 (A&B). (A): NM23-H1 and AR expression distributions based on the degree of the staining intensity. (B): NM23-H1 and AR expression distributions based on the degree of the staining density. NM23-H1 and AR was not correlated in terms of density or intensity distribution (Kruskal-Wallis Analysis, $p>0.05$). The intensity pattern scaling is 0: negative; 1: weak; 2: fair; and 3: strong. The density pattern scaling is 0: negative (0-25%); 1: weak 25-50%); 2: moderate (50-75%), and 3: strong (75-100%)

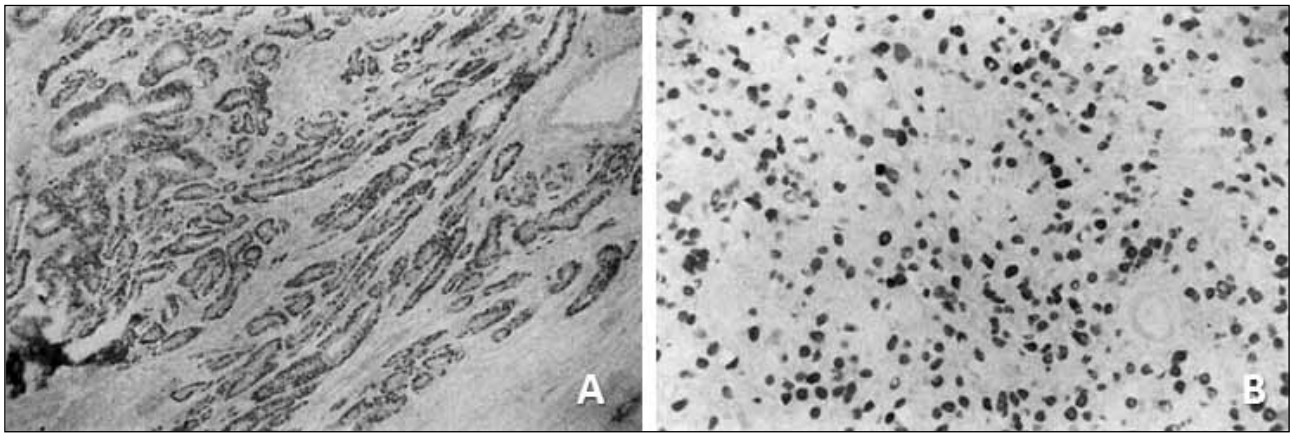

Fig 7 (A&B). IHC staining of (A): moderate and (B): high grade PCa samples against AR with strong nuclear staining pattern (x25)

Table 1
The comparison of NM23-H1 (NM23) and PAP in terms of percent distribution of expression intensity among the samples evaluated

	PAP				Total (%)
NM23	-	+	++	+++	
-	-	-	1	1	2.8
+	-	2	2	9	18.1
++	1	-	4	20	34.7
++++	2	2	6	22	44.4
Total (%)	**3.4**	**6.3**	**18.1**	**72.2**	

Table 2
The comparison of NM23-H1 (NM23) and PSA in terms of percent distribution of expression intensity among the samples evaluated

	PSA				Total (%)
NM23	-	+	++	+++	
-	-	-	-	-	-
+	-	2	1	3	17.5
++	2	3	4	12	36.8
++++	4	3	8	11	45.6
Total (%)	**14.0**	**12.3**	**26.3**	**47.4**	

NM23 and PAP expression and only 11 patients (20% of cases) showed both strong NM23 and PSA expressions (Tables 1 and 2). Mean Serum PSA and PAP values were also compared with the tissue expression levels of NM23-H1, PAP and PSA (Tables 1 and 2). Serum PSA was in the range of 36-38 ng/ml for patients showing both weak and strong tissue expression levels of NM23-H1 ($p>0.01$). For those patients, serum PAP levels were in the range of 29-39 ng/ml. The similar results correlating the serum PSA and PAP with the tissue expression of AR was also observed ($p>0.05$). Kruskal-Wallis one-way analysis showed no statistical association between serum PAP and PSA levels with the NM23-H1 and AR tissue expressions (Table 4). On the other hand, tissues from prostatic cancers showing moderate PSA staining demonstrated slightly more intense NM23-H1 staining and this correlation was statistically significant but weak with Kendall tau-c rank correlation test which showed the coefficient value of 0.018 (Table 5). When PAP and PSA expressions in tissues were compared with their serum levels, patients with weak PSA expressions showed higher serum PSA (37.3 ng/ml) and PAP (40.6 ng/ml) levels whereas, those with strong expressions showed the lower mean serum PSA (22 ng/ml) and PAP (22.3 ng/ml) levels. This reverse correlation for PAP and PSA was statistically significant when applying Kruskal-Wallis analysis ($p<00.1$).

DISCUSSION

Prostate cancers may have distant metastasis even they are localized or in early stages and any criteria providing the information regarding the invasive and metastasis po-

Table 3
The association of NM23-H1 and AR expression with tumor stages

	NM-23		AR		Chi-Square
	n	%	n	%	
Stage I	7	9.6%	6	8.7%	P<0.01
Stage II	11	15.1%	10	14.5%	P>0.05
Stage III-IV	15	20.5%	14	20.3%	P>0.05
N+M+	20	27.4%	19	27.5%	P<0.01
BPH	**20**	**27.4%**	**20**	**29%**	**p>0.05**

n: number of patients, %: percent of NM23-H1 or AR positive patients in corresponding stage

Table 4
The association of serum PSA and PAP levels with tissue expressions of NM23-H1, AR, PSA, PAP

		Serum Levels (ng/ml)			
Tissue expressions		**PSA**		**PAP**	
NM23-H1	0-1	36.8	p>0.05*	39.1	p>0.05*
	2	31.7		29.6	
	3	38.7		39.2	
AR	0	23.0	p>0.05*	19.4	p>0.05*
	1	41.0		32.4	
	2	31.5		24.0	
	3	21.3		13.7	
PSA	0	37.3	p<0.01*	40.6	p<0.01*
	1	31.7		33.0	
	2	32.8		31.3	
	3	22.3		22.0	
PAP	0-1	41.0	p<0.01*	27.7	p<0.01*
	2	36.5		24.4	
	3	35.1		25.7	

* Kruskal-Wallis analysis

tential for the early stages accelerate the aggressive follow up to improve the expected survive rates. PSA is known to be highly specific to prostatic tissue and it appears to have an increasing intensity for well differentiated tumors and Gleason grading doesn't provide the prognostic information by itself for the most of the cancers. (e.g. 80% of cases with cancer show moderate differentiation) (46-49).

Table 5
The comparison of intensity of NM23-H1 tissue expression with the intensity of PSA expression (Kendall's Tau-c rank correlation Analysis, coefficient value is 0.018)

	PSA		AR		Total (%)
NM23	-	+	++	+++	
-	0	0	0	0	0
+	0	2	1	3	17.5
++	2	3	4	12	36.8
+++	4	3	8	11	45.6
Total (%)	**14.0**	**12.3**	**26.3**	**47.4**	

In this study, all patients with metastasis showing a positive staining for PAP whereas, 5 out of those were negative for PSA staining. Patients with a weak PSA expressions showed high serum PAP and PSA levels and the cases with the strong PSA expression shared the lower serum PSA and PAP. This reverse correlation is statistically significant. We noted the different expressions of PAP and PSA from cell to cell even in adjacent cells which cannot be totally explained by the antibody dilutional variations in the same slides. We consider that each individual cells might have a different somatic and metabolic differentiation and maturation as well as the genetic alteration of the enzyme cascade.

The correlation of NM23-H1 and AR expression with the serum PAP and PSA, was not statistically significant. No correlation between Gleason Grading and serum PAP and PSA level were seen. PSA appears to be more specific than PAP. The patients with moderate PSA intensity showed a slightly more intense staining for NM23-H1 with no statistical correlation. No statistically significant relation between cancer stages and NM23-H1 expression was observed either. Only in the patients with moderately differentiated tumors, somewhat a weak correlation between the strong NM23-H1 staining and the moderate PSA expression was noted. The strong AR expression in patients with BPH and early PCa was observed, and for those with the weak or negative AR expressions were correlated with the worse prognosis.

According to previous studies, NM23-H1 level and tissue AR levels may be combined with the disease stage for patient specific prognosis (50-52). These results found supporting the previous studies where the increase in NM23-H1 expression was found in dormant cells and the decrease was observed in metastasized cancer cells, revealing that it appears to be a possible prognostic marker combined with other classical markers for disease aggressiveness (22-27).

Our results based on the staining intensity and density show no statistical significant correlation between cancer stages and nm23-H1 expression. The moderately differentiated tumors with a moderate PSA expression have a statistically significant but a weak correlation with the increased NM23-H1 expression. The combination of the classical markers and histopathological analysis of NM23-H1, AR, PSA, PAP and tumor grading did not reveal a clear correlation for the disease prognosis, however, we observed a variable but steady NM23 staining in all different stages, we consider that tissue expression of NM23-H1 is less likely to be a reliable prognostic parameter for the patients. However, only the moderately differentiated tumors with moderate PSA expression levels did have a weak correlation with an increased expression of the NM23-H1, revealing that for the metastatic potential, NM23-H1 can be combined with classical tumor markers for the tumors where the moderate tissue PSA expressions are seen. This correlation cannot be generalized since NM23-H1 expression features could not be associated significantly with the other differentiation levels or the stages. The further studies are necessary to detail the mechanism of NM23 gene-enzyme pathway for predicting the prognosis for prostate cancer and the reasons of the variations should be clarified for the other crucial pathways of NM23 gene family which may also function as a regulatory gene as well as having the suppressor activity.

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