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ORIGINAL ARTICLE

Evaluating the Effect of Chemotherapy *Notch1* Gene Expression in Patients with Prostate Cancer

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Abstract

Prostate cancer (PCa) affects middle-aged men between the ages of 45 and 60 and is the highest cause of cancer-associated mortalities. Circulating tumor cells (CTC) are found in peripheral blood and show poor prognosis compared to patients not having CTCs in their blood. *Notch1* is actually a critical gene in the *Notch* signaling pathway associated with cell differentiation, proliferation, and apoptosis. This *Notch1* gene has already demonstrated dual roles as both an oncogene and tumor suppressor. This study was conducted between November 2023 and May 2024 at the National Education Hospital of Oncology/Najaf and some privates' clinics of oncologists in Najaf province. This study is carried out on 50 cases of prostate cancer patients and 20 apparently healthy. Chemotherapy significantly downregulated the expression of *Notch1* ($p = 0.0001$) in PCa patients. *Notch1* in untreated patients had higher expression (median = 2.68) compared to treated patients (median = 0.60). This variability suggests that untreated patients exhibit more extreme *Notch1* expression levels. *Notch1* has a lower expression with Zometa & Taxoter treatments compared to more extensive drug regimens ($p < 0.001$). In conclusion the study found that chemotherapy is significantly downregulate *Notch1* expressions in treated patients, particularly with the combination of Zometa and Taxoter, which showed a synergistic effect.

Keywords: *Notch-1*, Chemotherapy, Circulating tumor cells, Gene expression

Introduction

Prostate cancer (PCa) affects middle-aged men between the ages of 45 and 60 and is the highest cause of cancer-associated mortalities (Sekhoacha et al., 2022). Many men with PCa are diagnosed by prostate biopsy and analysis, prostate-specific antigen (PSA) testing, digital rectal examination, magnetic resonance imaging (MRI), or health screening. The risk factors related to PCa include family risk, ethnicity, age, obesity, and other environmental factors. PCa is a heterogeneous disease both on the basis of epidemiology and genetics. The interplay among genetics, environmental influences, and social influences causes race-specific prostate cancer survival rate estimates to decrease, and thus, results in differences observed in the epidemiology of PCa in different countries (Sekhoacha et al., 2022).

In a patient with PCa, circulating tumor cells (CTC) are found in peripheral blood and show poor prognosis compared to patients not having CTCs in their blood. The assessment of the CTCs is linked to some other variables which may influence the outcome, such as age, performance status, serum PSA levels, and other factors. The value of CTC as a prognostic factor is usually measured with the threshold approved by the US FDA of 5 or less CTC per 7.5 ml of peripheral blood. This mentioned value is commonly considered to be an adverse prognostic indicator for PCa (Pantel et al., 2019).

According to RefSeq, This gene encodes a member of the *Notch* family of proteins. Members of this Type I transmembrane protein family share structural characteristics including an extracellular domain consisting of multiple epidermal growth factor-like (EGF) repeats, and an intracellular domain consisting of multiple different domain types. Notch signaling is an evolutionarily conserved intercellular signaling pathway that regulates interactions between physically adjacent cells through binding of *Notch* family receptors to their cognate ligands.

The ATP-dependent transport of solutes across membranes against a concentration gradient is primarily mediated by members of a superfamily of

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proteins known as the ATP-binding cassette transporters. The evolutionary importance of these polytopic membrane proteins is evident from their presence in all eukaryotic species as well as in bacteria and archaea (Higgins, 1992). In humans, the 48 ABC transporters are classified into seven subfamilies (A through G) according to their relative degrees of sequence homology. Subfamily *ABCC* is composed of 12 proteins, at least nine of which collectively mediate the ATP-dependent transmembrane efflux of multiple anticancer drugs and other xenobiotics, their metabolites, and an array of bioactive OAs, including multiple key signaling molecules (Slot et al., 2011).

Octamer-binding transcription factor 4, which is encoded by the *Pou5f1* gene and is a member of the POU-domain transcription factor family, is connected to maintain pluripotency of embryonic stem cells (ESCs) and CSCs (Bigdelou et al., 2020).

Zoledronic acid has been available in the market since the United States Food and Drug Administration (FDA) approval in 2002 for solid tumors. Prostate cancer is one of the established tumor types for which treatment with at least one hormonal therapy had to be met (Ibrahim et al., 2003). The competitor drug in this market has been the RANK-ligand inhibitor denosumab. Denosumab, a monoclonal antibody that binds RANKL was found in a double-blind randomized phase 3 trial to be non-inferior, and with further statistical analyses, more effective than zoledronic acid in reducing the incidence of skeletal related events (SREs) in patients with metastatic castrate resistant prostate cancer (mCRPC) (Fizazi et al., 2011).

Docetaxel chemotherapy was the first treatment to show an improvement in OS in mCRPC following two landmark phase III trials. These trials showed improvement in OS, symptoms, 2 prostate-specific antigen (PSA), and quality of life of mCRPC patients treated with docetaxel and prednisolone versus mitoxantrone and prednisolone (Tannock, 2008); (Berthold et al., 2008). This survival benefit was seen across all age groups, and, following these studies, an established regimen of three weekly intravenous docetaxel for 10 cycles is given as first-line chemotherapy (Berthold et al., 2008).

Materials and methods

Materials

Table 1.

Equipments

Table 2.

Table 1. Materials.

Materials	Category number	Company
1- GoTaq® q-PCR master mix	A6001	Promega-USA
2- Primers	–	Macrogen-Korea
3- Easy-spin™ (DNA free) total-RNA extraction Kit	17,221	Intron-Korea
4- AddScript cDNA Synthesis Kit	22,701	Addbio-Korea

Table 2. Instruments.

	Instruments	Company	Country
1	Microfuge IB Centrifuge	Beckman Coulter	Germany
2	Dry microtubes incubator	ae	UK
3	Mx3005P Stratagene Real-Time system	Agilent	USA
4	Kern PFB balance	Kern & Sohn	Germany
5	Pipettes	DARWELL	China
6	Micro-Pipettes	DARWELL	China
7	EDTA-tube	Shanghai Goldenwell	China
8	Vortex mixer	Shanghai Haiker	China
9	Thermal cycler	Bio-Rad	USA
10	Eppendrof tube	HangZhuo A Gene	China
11	Syringe 5 ml	Becton Dickinson	China

Sample collection

This study was performed after taking the ethical approvals for conducting the research from the Najaf Health Department and the approval of the Scientific Committee for Research in the center of the Najaf Health Department with full compliance with the instructions for biosafety and ethical controls and obtaining the approval of the participants before proceeding to search and maintain their privacy and not disclose the data. The samples were collected from the National Oncology Teaching Hospital in 2024 for several months. This case control study was conducted on 50 whole blood samples of PCa patients, 20 whole bloods of apparently healthy samples. The blood samples are placed in EDTA tube then empty in Eppendorf tubes in deep freezing (−81 °C) until the test take place. The patient's data were collected including group grade, cell grade, Gleason's score, TNM stage, chemotherapy and type of treatment, PSA value and blood parameters. Patient's PCa-related information were also collected, including age, the duration of treatment, the marital status, time of diagnosis as well as the number of doses taken by the patient. The aggressiveness of the disease considered as a priority trait for the samples that are included in this experiment with the consideration of samples diversity to ensure the obtain of the correct correlation.

Procedures

Total RNA extraction

1. We added 0.2 ml of blood and placed it in a 1.5 ml tube then add 1 ml of Lysis buffer to the tube.
2. The mixture mixed strongly using vortex mixer until there are no noticeable clumps at room temperature for 10 s.
3. Then applied with 0.2 ml of Chloroform and mix it using a vortex mixer. The chloroform is added to facilitate the separation of the phenol layer from the aqueous layer, which leads to the isolation of RNA and genomic DNA/protein.
4. After centrifugation of the solution at 13,000 rpm at a temperature of 4 Celsius degrees for 10 min, gently we transferred 0.4 ml of the upper liquid layer into an empty 1.5 mL tube. Centrifugation solution will result two separate phases. The top aqueous phase includes RNA, whereas the lower phenol layer (blue colour) includes denatured protein or cellular residue.
5. Then mixed with 0.4 ml of Binding Buffer and homogenised by turning the mixture 2–3 times up and down.
6. The top solution was transferred into the column, making sure the entire top solution is not transferred to the column reservoirs because it has a maximum volume of 0.8 ml. Once the optimal upper solution is loaded onto the column, centrifuge it at 13,000 rpm for 30 s. The liquid that passes through during centrifugation was disposed and the spin column was returned to the previous 2 ml accumulation tube. Replicate this process once again.
7. 0.7 ml of Washing Buffer A was added into the column. The tubes was gently sealed and applied to centrifugal force of 13,000 rpm for 30 s in order to wash the column. The liquid that passes through was removed and the spin column returned to the previous 2 ml accumulating tube.
8. Washed by adding 0.7 ml of Washing Buffer B into the column and applied to centrifugation at 13,000 rpm for 30 s. Filtered liquid was and the centrifuge column returned to the previous 2 ml accumulating tube. Washing Buffer B was provided in a concentrated form therefor, it was mixed with ethanol.
9. The column membrane was Centrifuged at 13,000 rpm for 1–2 min in order to remove moisture and dry it. Making sure that the column membrane is well dried because any remaining ethanol could potentially disrupt subsequent reactions.
10. The column was Transferred into a sterile 1.5 ml micro-centrifuge tube and 50 μl of Elution Buffer was added directly onto the membrane. The sample was placed at room temperature for 1 min, then a centrifuge was used at 13,000 rpm for 1 min to extract the total RNA.

cDNA synthesis

1. The components in Table 3 added to a thin-walled PCR tube:
2. The tubes then transferred into a Thermal cycler machine using the temperature cycling Protocol in Table 4.

Primers preparation

According to the guidance provided by the primer manufacturer company, the lyophilized primers were dissolved in doubly distilled water to reach a final concentration of 100 mol in which the solution was stored as a stock solution at a temperature of −20 °C (see Table 5). A 10 mol concentration was created by diluting the stock primers which utilised as a functional primer. The primers that used in current study shown in Table 4.

RT-qPCR

1. The thermal cycler was prepared for standard RT-qPCR as in Table 7.
2. The content of the GoTaq® qPCR master mix was kept, the cDNA templates, and the primer pair on ice, at room temperature. Rapidly mixed using a vortex mixer in order to minimise the avoid air flow into the mixture.
3. The cDNA samples was diluted in water.

Table 3. cDNA reaction components.

Item	Volume (μl)
Nuclease-Free H_2O	1
2x Reaction Buffer	10
10 mM dNTP Mixture	2
10x oligo dT20	2
20x AddScript Enzyme Solution	1
RNA template	4
Total reaction volume = 20	

Table 4. cDNA reaction cycles.

Stage	Temperature (°C)	Duration
Priming-stage	25°	10 min
Reverse-transcription	50°	60 min
RT-inactivation	80°	5 min
Hold-stage	12°	∞

Table 5. Experiment primers.

Gene	Primer name	5'-3'	Product	Accession number	Reference
Notch 1	F	GGTGAACTGCTCTGAGGAGATC	150bp	XM_054363009.1	ORI-GENE
	R	GGATTGCAGTCGTCCACGTTGA			
ABCC1	F	CCGTGTACTCCAACGCTGACAT	145bp	NM_004996.4	ORI-GENE
	R	ATGCTGTGCGTGACCAAGATCC			
OCT-4	F	CCTGAAGCAGAAGAGGATCACC	106bp	NM_002701.6	ORI-GENE
	R	AAAGCGGCAGATGGTCGTTTGG			
GAPDH	F	GGAGTCAACGGATTTGGT	206bp	NM_002046.7	Chen et al., 2014
	R	GTGATGGGATTTCCATTGAT			

4. The reaction components shown in Table 6 were mixed together in a sterile tube that is non-stick and kept on ice and mixing it delicately after adding each ingredient. The reaction volumes cautiously transferred on the plate while keeping it on ice.
5. The plate transferred from the ice to the instrument that was already programmed. The run was started instantly.
6. After the run was finished, the data was gathered and the results was analysed.

Statistical analysis

All data examinate by normality distribution test, Continuous variables were expressed as means with standard deviation or Median and inter quartile rang (IQR), and nominal variables (discrete variables) were presented as frequency and percentages (%). Continuous variables were analyzed using Student's for normality distributed, while the abnormality distributed variables were analyzed by Mann Whitney U test for compared between two groups, and Kruskal–Wallis H for comprising among three groups. Categorical variables were analyzed using Likelihood Ratio Tests to obtain on Pearson's chi-square or Fisher's exact test used with limited data (less than 5), in addition to calculated the Relative risk (RR) of gene expression fold change. Prediction of the risk factors for gene expression fold change was performed using binary and Multinominal logistic regression to assay the effect size (B), odds ratio with 95% confidence interval OR (95%CI). All data analyzed were performed by SPSS Statistics version 28.0 (IBM, Chicago IL, USA), and values of less than 0.05 were considered statistically significant.

Table 6. Real-Time PCR components concentrations.

Components	Concentration	Final Volume (20 µl)
GoTaq® quantitive-PCR master mix, 2X	1X	10 µl
Forward-primer	10 µM	2 µl
Reverse-primer	10 µM	2 µl
Nuclease free water	–	4 µl
complementary-DNA template	–	2 µl

Table 7. Real-Time PCR stages.

Stage	Temperature (°C)	Time	Cycles
Hot-start activation-stage	95°	2-min	1X
Denaturation-stage	95°	15-s	40X
Annealing-stage (green light measuring)	60°	1 min	
Extension-stage	72°	30-s	
Dissociation-stage	72°	2-min	1X

The results

The distribution of PCa patients across various clinical features

In Table 8 the distribution of PCa patients based on group grade, Gleason score, cell grade, Stage, chemotherapy status and treatment type. The distribution for the patients showed significant differences based on their group grade with $p = 0.030$, Gleason scores with $p = 0.008$, cell grades with $p = 0.0001$ and stages with $p = 0.0001$. The treatment status and the type of treatment were also assessed, which showed significant differences in the distribution of treatment type with $p = 0.001$. While there were no significant in chemotherapy distribution between treatment status.

The demographic and laboratory characteristics of the PCa patients cohort

Table 9 is a summary for demographic and laboratory characteristics of PC patients. The patients' average age in this study was 67.84 years. The median diagnosis time is 7 years. Laboratory assays measured were red blood cell count, platelet count, white blood cell count, and prostate-specific antigen levels. An average total dose of 1708.28 was received by the patients. These are the data that give some background understanding of the patient population under study.

Table 8. The distribution of PCa patients across various categories.

PCa categories		N	%	Chi-Square	*p*-value
Group grade	Unknown	16	32.0%	12.40	0.030*
	Grade 1	3	6.0%		
	Grade 2	9	18.0%		
	Grade 3	10	20.0%		
	Grade 4	7	14.0%		
	Grade 5	5	10.0%		
Gleason Score	unknown	15	30.0%	17.48	0.008*
	3 + 3	3	6.0%		
	3 + 4	9	18.0%		
	4 + 3	10	20.0%		
	4 + 4	7	14.0%		
	4 + 5	3	6.0%		
	5 + 4	3	6.0%		
Cell Grade	unknown	21	42.0%	38.20	0.0001**
	X	20	40.0%		
	II	2	4.0%		
	III	6	12.0%		
	IV	1	2.0%		
Stage	unknown	27	54.0%	43.40	0.0001**
	X	10	20.0%		
	II	2	4.0%		
	III	1	2.0%		
	IV	10	20.0%		
Treatment status	Treated	29	58.0%	1.28	0.258 NS
	Not-treated	21	42.0%		
Treatment type	Not-Treated	21	42.0%	18.40	0.001*
	Zometa	11	22.0%		
	Taxoter	5	10.0%		
	Zometa & Taxoter	9	18.0%		
	5-f-u, leucovorin 5-f-u bollus, oxaliplatin	4	8.0%		

Significant differences at * p-value <0.05, ** <0.01. NS: non-significant.

Distribution of clinical categories according to treatment status in PCa patients

In Table 10 compares the clinical categories of PCa patients who were treated with chemotherapy versus those who were not. Significant differences were found in the distribution of group grades ($p = 0.023$), cell grades ($p = 0.002$), and Gleason scores ($p = 0.025$) between treated and untreated patients. However, no significant differences were observed in the stage distribution ($p = 0.192$). These results highlight the impact of treatment status on the clinical profiles of PCa patients.

Table 9. The demographic and laboratory characteristics of the PCa patients cohort.

	Mean ± SD	Median (IQR)
All patients (N)	50	
Age (years)	67.84 ± 8.93	68.5 (61–74.25)
Diagnosis time (years)	7.6 ± 1.9	7 (6–8)
Dose-week (frequency) –FTP	43.9 ± 50.95	24 (4–69.5)
Dose-total (mg) –FTP	1708.28 ± 3991.08	480 (16–1508)
RBC Count (10∧12) \L (Ref; 4.7–6.1 ∧12\L)	11.3 ± 3.02	11.75 (10.08–13.83)
PLT Count (10∧9) \L (Ref; 150–450 ∧9\L)	237.28 ± 74.62	228 (183.25–286)
WBC Count (10∧9) \L (Ref; 4–11 ∧9\L)	8.07 ± 3.35	7.2 (5.98–9.98)
PSA (ng\ml) (Ref; 0 –4 ng\ml)	20.26 ± 31.25	4.84 (0.47–25.51)

SD; Standard deviation, IQR; interquartile range, FTP; for treated patients, Ref; reference, RBC; red blood cell, PLT; platelet, WBC; white blood cell, PSA; prostate specific antigen.

Comparison of laboratory analysis in treated and not-treated of PCa patients

In Table 11 presents the comparison of laboratory assay results between PCa patients who received chemotherapy and those who did not. A significant difference was noted in the diagnosis time ($p = 0.011$) between the two groups. Other variables, such as RBC count, PLT count, WBC count, and PSA levels, did not show significant differences. This comparison underscores the role of chemotherapy in potentially influencing the timing of diagnosis in PC patients.

Effects of treatment status on gene expression fold change values in PCa patients

Table 12 examines the effects of chemotherapy on gene expression fold change value for *Notch1* in PCa patients. A highly significant downregulation of *Notch1* expression was observed in treated patients compared to untreated patients ($p = 0.0001$).

Relationship between different chemotherapy regimens and gene expression fold changes in PCa patients

Table 13 illustrated effects of treatment type on gene expression fold change value in PCa patients. This table explores the relationship between different chemotherapy regimens and gene expression fold changes in PCa patients. *Notch1* expression showed highly significant differences across different treatment groups ($p < 0.001$), with Zometa & Taxoter treatments showing lower expression levels compared to more extensive drug regimens.

Distribution of clinical categories of PCa patients according to Notch1 *gene expression fold change (downregulation and upregulation)*

In Table 14 details of the distribution of clinical categories of PCa patients based on the down-regulation and upregulation of *Notch1* gene expression. The distribution is varied across group

Table 10. Distribution of clinical categories according to treatment status in PCa patients.

Clinical Categories		Treatment status				Total		Chi-square p-value
		Treated		Not-treated				
		N	%	N	%	N	%	
Group grade	Unknown	10	34.5%	6	28.6%	16	32.0%	12.347
	Grade 1	0	0.0%	3	14.3%	3	6.0%	0.023*
	Grade 2	4	13.8%	5	23.8%	9	18.0%	
	Grade 3	4	13.8%	6	28.6%	10	20.0%	
	Grade 4	7	24.1%	0	0.0%	7	14.0%	
	Grade 5	4	13.8%	1	4.8%	5	10.0%	
Cell Grade	unknown	6	20.7%	15	71.4%	21	42.0%	13.797
	X	16	55.2%	4	19.0%	20	40.0%	0.002*
	II	2	6.9%	0	0.0%	2	4.0%	
	III	4	13.8%	2	9.5%	6	12.0%	
	IV	1	3.4%	0	0.0%	1	2.0%	
Stage	unknown	13	44.8%	14	66.7%	27	54.0%	5.908
	X	8	27.6%	2	9.5%	10	20.0%	0.192 NS
	II	2	6.9%	0	0.0%	2	4.0%	
	III	0	0.0%	1	4.8%	1	2.0%	
	IV	6	20.7%	4	19.0%	10	20.0%	
Gleason Score	unknown	9	31.0%	6	28.6%	15	30.0%	13.51
	3 + 3	0	0.0%	3	14.3%	3	6.0%	0.025*
	3 + 4	4	13.8%	5	23.8%	9	18.0%	
	4 + 3	4	13.8%	6	28.6%	10	20.0%	
	4 + 4	7	24.1%	0	0.0%	7	14.0%	
	4 + 5	2	6.9%	1	4.8%	3	6.0%	
	5 + 4	3	10.3%	0	0.0%	3	6.0%	

Significant differences at * p-value <0.05. NS: non-significant.

Table 11. Comparison of Laboratory analysis in treated and not-treated of PC patients.

Variables	Treatment status	Mean ± SD	*p*-value
Diagnosis time (years)	Treated	8.17 ± 2	0.011*
	Not-treated	6.81 ± 1.44	
RBC Count (10∧12) ⧹L	Treated	11.09 ± 3.29	0.559 NS
(Ref; 4.7–6.1 ∧12⧹L)	Not-treated	11.6 ± 2.66	
PLT Count (10∧9) ⧹L	Treated	228 ± 76.06	0.306 NS
(Ref; 150–450 ∧9⧹L)	Not-treated	250.1 ± 72.41	
WBC Count (10∧9) ⧹L	Treated	8.49 ± 3.64	0.309 NS
(Ref; 4–11 ∧9⧹L)	Not-treated	7.52.88	
PSA (ng⧹ml) (Ref; 0	Treated	26.04 ± 37.38	0.118 NS
−4 ng⧹ml)	Not-treated	12.27 ± 17.91	

Significant differences at * p-value <0.05. NS: non-significant. Median (IQR) and by Mann–Whitney U test. SD; Standard deviation, Ref; reference, RBC; red blood cell, PLT; platelet, WBC; white blood cell, PSA; prostate specific antigen.

Table 12. Effects of treatment status on gene expression fold change value in PCa patients.

Gene expression fold change	Treatment status	PCa patients		p-value
		Median	IQR	
Notch1	Treated	0.599	0.279–1.447	**0.0001****
	Not-treated	2.676	1.636–7.189	

Significant differences *p < 0.05, **p < 0.01. Mann–Whitney U test. NS: non-significant. PCa; prostate cancer, IQR; interquartile range.

grades, Gleason scores, cell grades, and stages, with a notable proportion of patients exhibiting down-regulation or upregulation of *Notch1* in each category. These distributions highlight the heterogeneity of *Notch1* expression and its potential clinical implications in PCa.

Distribution and Likelihood Ratio Tests of treatment type associated with Notch1 *gene expression fold change*

Table 15 summarizes the distribution of different treatment type and their association with the fold change in *Notch1* gene expression, presenting both the number and percentage of cases showing downregulation and upregulation. The likelihood ratio test results, including the chi-square value and *p*-value, indicate significant differences in the fold change associated with the treatment type, at the same time this table illustrated that Zometa, and combination Zometa with Taxoter have impact effect on the regulation gene of *Notch-1* compared to taxoter and more than two drugs with *p* value 0.001.

Table 13. Relationship between different chemotherapy regimens and gene expression fold changes in PCa patients.

Fold change	Treatment type	Median	IQR		Mean Rank	Kruskal–Wallis H *p*-value
Notch1 gene	Zometa	0.473	0.291	1.149	16.86	**20.368 $p < 0.001$****
	Taxoter	1.173	0.382	2.350	22.80	
	Zometa & Taxoter	0.409	0.204	1.071	14.17	
	5-f-u, leucovorin, 5-f-u bollus, oxaliplatin	1.188	0.413	2.053	23.25	
	Not-Treated	2.676	1.636	7.189	35.95	

Significant differences at * p-value <0.05. NS: non-significant. IQR; interquartile range.

Table 14. Distribution of clinical categories of PCa patients according to Notch1 *gene expression fold change.*

		Notch1 Fold Change			
		Downregulation		Upregulation	
		N	%	N	%
Group grade	Unknown	5	(25.0%)	11	(36.7%)
	Grade 1	1	(5.0%)	2	(6.7%)
	Grade 2	2	(10.0%)	7	(23.3%)
	Grade 3	5	(25.0%)	5	(16.7%)
	Grade 4	5	(25.0%)	2	(6.7%)
	Grade 5	2	(10.0%)	3	(10.0%)
Gleason Score	unknown	5	(25.0%)	10	(33.3%)
	3 + 3	1	(5.0%)	2	(6.7%)
	3 + 4	2	(10.0%)	7	(23.3%)
	4 + 3	5	(25.0%)	5	(16.7%)
	4 + 4	5	(25.0%)	2	(6.7%)
	4 + 5	1	(5.0%)	2	(6.7%)
	5 + 4	1	(5.0%)	2	(6.7%)
Cell Grade	unknown	6	(30.0%)	15	(50.0%)
	X	9	(45.0%)	11	(36.7%)
	II	1	(5.0%)	1	(3.3%)
	III	3	(15.0%)	3	(10.0%)
	IV	1	(5.0%)	0	(0.0%)
Stage	unknown	9	(45.0%)	18	(60.0%)
	X	6	(30.0%)	4	(13.3%)
	II	1	(5.0%)	1	(3.3%)
	III	0	(0.0%)	1	(3.3%)
	IV	4	(20.0%)	6	(20.0%)

Table 15. Distribution and Likelihood Ratio Tests of treatment type associated with Notch1 *gene expression fold change.*

Treatment Type	*Notch1* Fold Change				Likelihood Ratio Tests	
	Downregulation		Upregulation		Chi-square	p-value
	N	%	N	%		
Not-Treated	2	10.0%	19	63.3%	17.862	0.001*
Zometa	7	35.0%	4	13.3%		
Taxoter	2	10.0%	3	10.0%		
Zometa & Taxoter	7	35.0%	2	6.7%		
5-f-u, leucovorin, 5-f-u bollus, oxaliplatin	2	10.0%	2	6.7%		
Total	20	100.0%	30	100.0%		

Significant differences at * p-value <0.05. NS: non-significant.

Logistic regression for prediction the Notch-1 gene expression fold change in PCa patients with different treatment type

This Table 16 presents the results of the logistic regression analysis predicting the likelihood of *Notch-1* gene expression downregulation in PCa patients based on different treatment type. This table summarize that Zometa and the combination of Zometa with Taxoter have strong effect in the downregulation the gene of *Notch-1* with OR 16.625 and 33.250 respectively compared to the Taxoter and more than two drugs' groups with non-significant effects.

Table 16. Logistic Regression for Prediction the Notch-1 *gene expression fold change (downregulation/upregulation) in PCa patients with different treatment type.*

Treatment Type Predictors for *Notch1* Fold Change	*p*-value	OR	95% CI
Not-Treated.	**The categories references**		
Zometa	0.004	16.625 *	2.472–111.799
Taxoter	0.117	6.333 NS	0.630–63.639
Zometa & Taxoter	0.001	33.250 *	3.900–283.454
5-f-u, leucovorin, 5-f-u bollus, oxaliplatin	0.071	9.500 NS	0.826–109.235

Significant differences at *p < 0.05, **p < 0.01. B: Effect size. OR: Odds Ratio. 95%CI: Confidence Interval. NS: non-significant.

Discussion

Prostate cancer is the second most common cancer in men, it remains one of the main causes of mortality worldwide. In this regard, knowledge and awareness about the disease become very important to be informed about its wide occurrence and the consequent negative impact on health. Sadly, one of the major problems when treating prostate cancer is that during the beginnings of its development, the cancer remains asymptomatic, quite obviously resulting in missed opportunities for intervention (Bergengren et al., 2023).

Haematological parameters

Difference noted in diagnosis time ($p = 0.011$) between two groups, other variables RBC count, PLT count, WBC count and PSA levels did not show significant differences. This comparison underscores the role of chemotherapy potentially influencing the timing of diagnosis in PC patients.

Red blood cells

Chemotherapy is known to affect erythropoiesis, and it frequently causes anemia (Beyer et al., 2020). However, this difference in the levels of RBC in our study was not significant, probably due to factors such as the type of chemotherapy regimen, treatment duration, and baseline status of the patient. Recent reports have speculated that some chemotherapy regimens might have less pronounced effects on RBC counts or be followed by supportive treatments that might mitigate this effect, such as erythropoiesis-stimulating agents (Meyer et al., 2022).

White blood cells

It is common for chemotherapy to result in leukopenia due to it myelo-suppressive effects (Smith et al., 2019), but the lack of difference in WBCs in our study may suggest that our cohort had either not undergone deep leukopenia or that leukocyte counts had been managed with growth factors or other supportive measures (Johnson & Patel, 2021).

Platelets

Thrombocytopenia is the other frequent toxicity that could increase bleeding risk, similar to chemotherapy (Brown et al., 2021). No differences in PLT levels could be exsist due to specific chemotherapy regimens or the application of stimuWilson & Lee, 2023lating thrombopoiesis agents that help maintain platelet counts (Wilson & Lee, 2023).

Prostate specific antigen

This is evidenced by the minimal changes in PSA levels, indicating that another biomarker or clinical parameter may be more representative of chemotherapy effectiveness in prostate cancer. Looking at other circulating biomarkers would contribute to a better understanding of treatment response circulating tumor cells or gene expression profiles (Beraldi et al., 2020). Similarly, prostate cancer also shows heterogeneity, and there are differences in tumor biology and PSA production in patients. This heterogeneity in most cases results in variations in response to chemotherapy and hence effects on PSA levels different from one another (Kumar et al., 2017). Some tumours may be highly aggresive and so present with high levels of PSA, while others may not have any significant effect on the PSA levels in spite of cancer presentations.

Associations of fold changes in gene expression variations of *Notch1* with treatment status

Data shows a very remarkable decline in *Notch1* expression after chemotherapy ($p = 0.0001$). *Notch1* is considered one of the major regulators involved in various cellular processes including proliferation and differentiation. It has been associated with the progression of several cancers, including prostate cancer (Zhao et al., 2021).

Mechanism of interaction

Previous studies have shown that *Notch1* signaling can influence tumor growth and resistance to treatment (Kumari et al., 2020). One implication of the observed downregulation may be that it is an

adaptive response to chemotherapy or a decrease in *Notch1* functional contribution to the maintenance of the malignant phenotype as a result of applied treatment pressure. This observation is in agreement with other studies showing that chemotherapy can alter Notch signaling pathways, suggesting that one way through which chemotherapy works is by perturbing tumor cell survival mechanisms (Li et al., 2015).

The effect of chemotherapy on Notch1 *gene expression*

In our results, there were highly significant differences, as in Table 13, in *Notch1* expression across the different treatment groups ($p < 0.001$). *Notch1* expression in patients treated by Zometa & Taxoter was low compared to patients treated by more extensive regimens of drugs. This points to the fact that a certain chemotherapy combination could be better at reducing *Notch1* expression associated with cancer progression and treatment resistance (Wang et al., 2015).

Fold change distribution of Notch-1 *gene expression within the different treatment groups*

Table 14 show a Fold change distribution of *Notch1* gene expression within the different treatment groups, which expresses the extent of down- and up-regulation. *Notch1* gene is known to play a significant role in various cancers, notably prostate cancer and is, therefore an important target in understanding chemotherapy responses (Takebe et al., 2015). This table points to the differential impact on *Notch1* regulation by Zometa, Taxoter, their combination, and multiple drug regimes.

Untreated group

Notch1 in the untreated group was overexpressed in about 63.3%. The baseline upregulation thus is in agreement with previous reports in which the cancer cells that did not undergo treatment normally kept the Notch signaling at higher levels compared to their counterparts undergoing chemotherapy, promoting the progression of tumors and fueling chemotherapy resistance (Bray et al., 2018).

Only 10.0% of the patients in the untreated group demonstrated *Notch1* downregulation, while 63.3% showed upregulation. This, therefore, means that having no treatment significantly goes with a higher chance of *Notch1* upregulation, thereby agreeing with the role of *Notch*-1 in promoting survival and the proliferation of cancer cells (Nowell & Radtke, 2017). In the treated group, 90.0% of patients showed downregulation, while only 36.7% showed upregulation.

Treated group

Treated with Zometa. Treatment with Zometa increased this shift significantly to 35.0%, while only 13.3% cases had up-regulation. Since there was significant down-regulation by Zometa alone, it revealed its possible effectiveness in suppressing *Notch1* activity, which may be critical in reducing growth and mechanisms of resistance in the tumor (Costa et al., 2019).

In this study, the untreated group is the reference category and provides a baseline against which the effects of different drug regimens can be compared. Zometa: There was a positive effect of Zometa on *Notch1* downregulation in this analysis, with an odds ratio of 16.625 (95% CI, 2.472−111.799; $p = 0.004$). This would make the patients treated with Zometa about 16.6 times more likely to downregulate the *Notch1* gene when compared to non-treated patients. The results presented herein corroborate and further extend previous studies that show Zometa's effectiveness in blocking Notch signaling pathways, leading to reduced tumor growth and metastasis (Coleman et al., 2014).

Treated with Taxoter. Taxoter showed a little tendency toward up-regulation, showing a more balanced effect. That means it will probably have some action on *Notch1* but is perhaps not as potent as Zometa in the down-regulation of this gene.

There is no statistically significant effect of Taxoter on *Notch1* downregulation: OR = 6.333, 95% CI: 0.630−63.639, $p = 0.117$. Although the OR might indicate some trend toward downregulation, the wide confidence interval and non-significant p-value set aside the evidence for that. The result may interpret that Taxoter alone cannot effectively depress *Notch1* expression in PC patients.

Treated with Zometa and Taxoter. The most remarkable effect on downregulation was noted in the combination Zometa and Taxoter, 35.0% versus only 6.7% upregulation in *Notch1* expression. This underlines the potential synergistic effect of combined therapy in achieving more substantial gene regulation and concurs with studies that suggested better outcomes through combined therapeutic strategies (Takebe et al., 2015). All regimens involving more than two drugs resulted in equal percentages of down- and up-regulation at 10.0%, indicating mixed

responses which may be attributed to the complexity and variability of multi-drug interactions.

Treated with more than two drugs. In the combination therapy including Zometa and Taxoter, there is a strong effect of *Notch1* downregulation, with OR: 33.250; 95% CI: 3.900–283.454, $p = 0.001$. This combination would give an odds ratio significantly high for downregulating *Notch1* expression compared with no treatment. It could be through synergistic action Zometa and Taxoter are able to carry out a more effective inhibition of the Notch pathway. The finding, therefore, supports results showing that therapies targeting complex cancer signaling networks through combination therapies might be able to ensure better inhibitions (Pal et al., 2017). The use of over two drugs demonstrates an OR of 9.500, showing a positive effect on *Notch1* downregulation; however, the result is not statistically significant with 95% CI: 0.826–109.235, p = 0.071. This could be attributed to variability in the combinations used and their discrete effects on *Notch1* regulation.

Logistic regression analysis of Notch1 *gene expression fold change*

In Table 16, logistic regression analysis showing odds ratio of *Notch1* gene downregulation in prostate cancer patients as a function of various treatment regimens. In this respect, the results is dedicated to assessing the probability of gene downregulation of *Notch1* in PC patients with various treatment modalities. It is aimed at establishing whether some chemotherapy medicines or their combinations have any effect on the regulation of the *Notch1* gene, which is considered an important player in the progress and drug resistance of cancer (Koch & Radtke, 2020).

Logistic regression analysis indicates that for every unit increase in *Notch1* gene expression fold change, there is a roughly 2.734 times greater likelihood of belonging to the not-treated group versus the treated group: OR = 2.734 with its 95% CI, 1.350–5.538, at a *p-value* of 0.005. The result underscores the very strong link between high *Notch1* expression and the likelihood of an individual remaining untreated. *Notch1* is known for its role in maintaining stem-like properties in cancer cells and resistance to therapy (Miele et al., 2017). High levels of *Notch1* could be related to much more aggressive diseases or poor treatment efficacy.

The chi-square value of 14.012 and a *p-value* of <0.001 indicate a highly significant difference in the fold change of *Notch1* expression between treated and untreated groups.

The RR of 2.385 (95% CI: 1.467–3.877) and OR of 15.55 (95% CI: 3.02–80.04) suggest that treatment substantially increases the likelihood of *Notch1* downregulation, highlighting its potential as a therapeutic target (Aster et al., 2017).

Conclusions

The study highlights a significant difference in the timing of prostate cancer diagnosis between patients who received chemotherapy and those who did not Treated patients are more likely to be diagnosed earlier, likely due to better access to healthcare and regular screenings, which facilitates early detection. Chemotherapy significantly downregulated *Notch1* expression in treated patients. The combination of Zometa and Taxoter was particularly effective in downregulating *Notch1* expression, demonstrating a synergistic effect. This combination therapy significantly increases the likelihood of Notch1 downregulation compared to monotherapy or untreated patients. Variability in Chemotherapy Impact: The study observed variability in gene expression responses across different chemotherapy regimens, highlighting the complex interactions and individual differences in treatment response.

Ethics information

This study was performed after taking the ethical approvals for conducting the research from the Najaf Health Department and the approval of the Scientific Committee for Research in the center of the Najaf Health Department with full compliance with the instructions for biosafety and ethical controls and obtaining the approval of the participants before proceeding to search and maintain their privacy and not disclose the data.

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Author contribution

My contribution includes samples collection, patients' data collection, samples storage, financial coverage, and in the experiment practical side.

Conflict of interest

None.

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