

SUPPLEMENTARY FIGURES

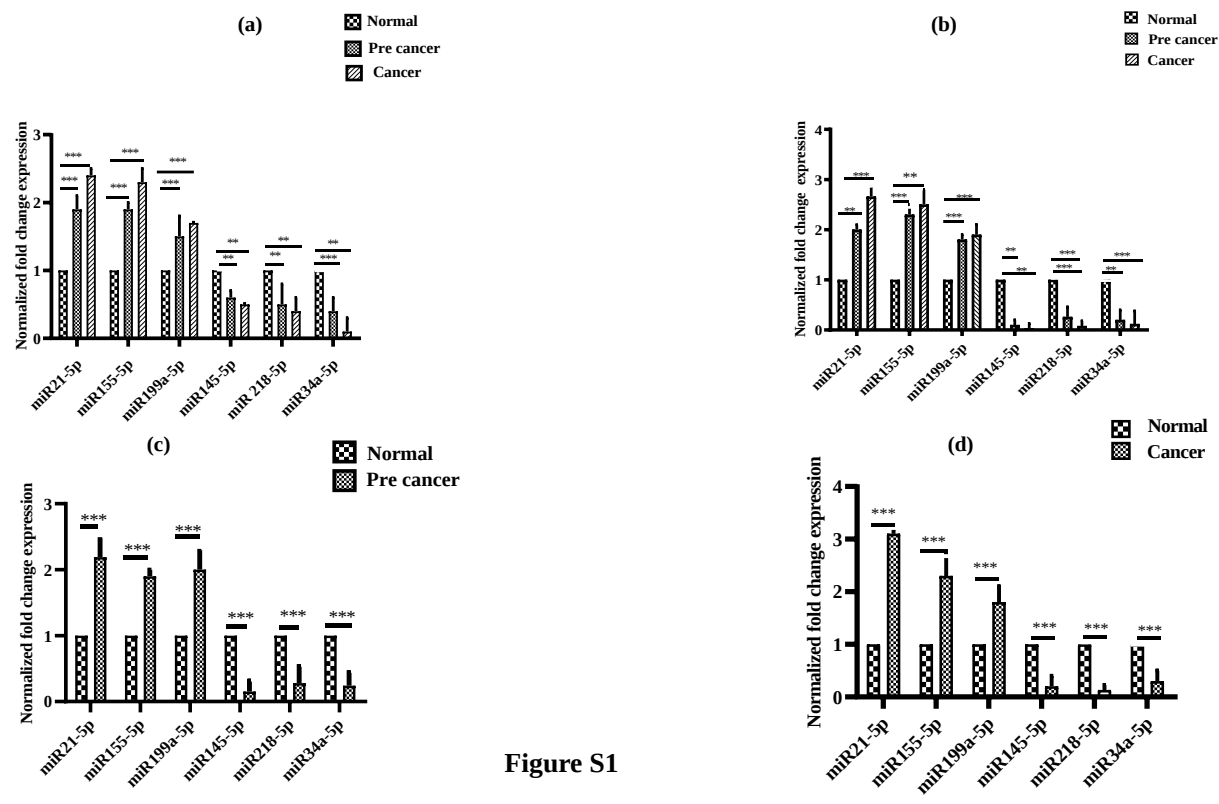


Figure S1

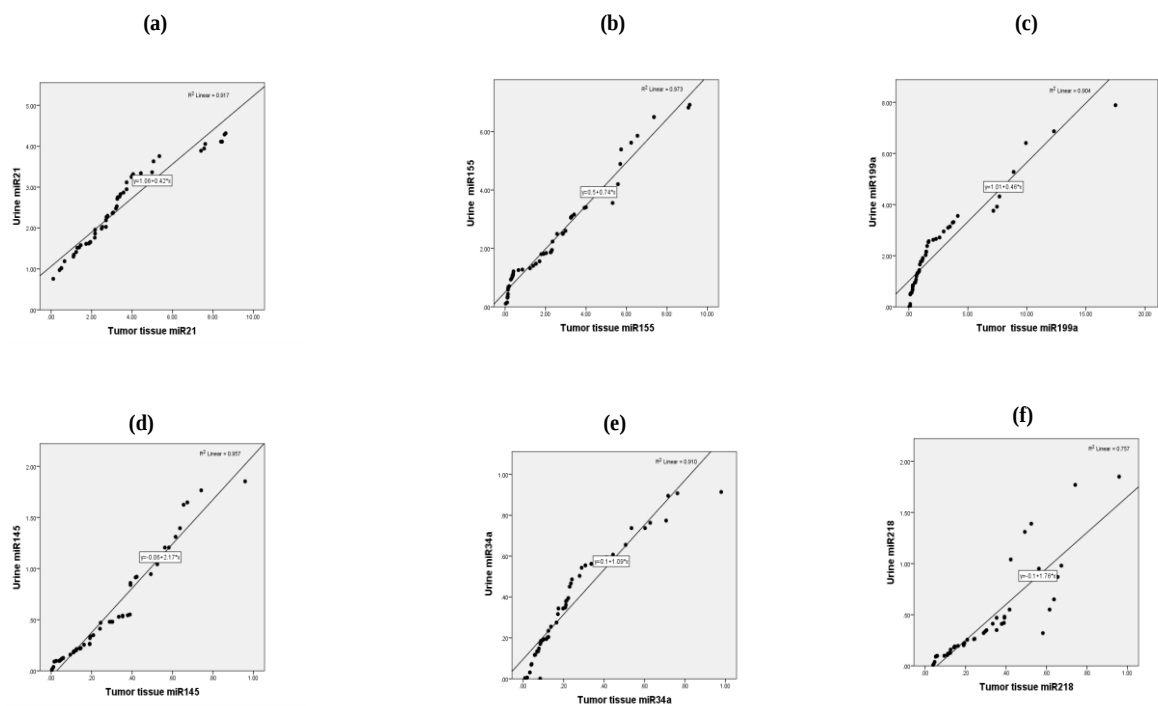


Figure S2

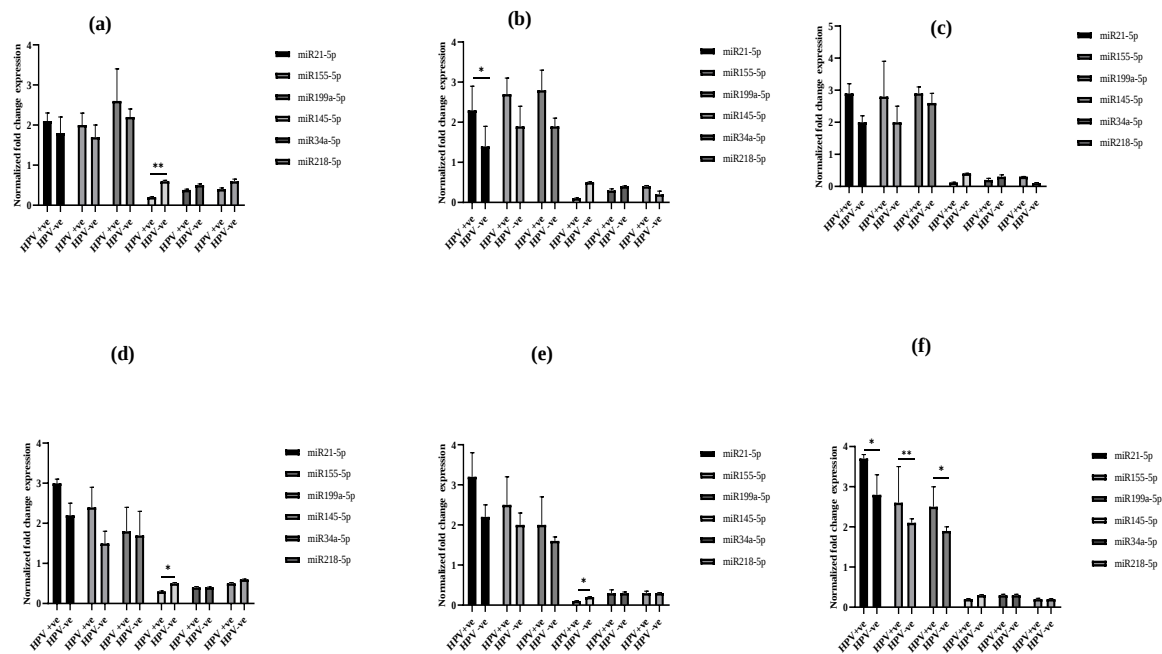


Figure S3

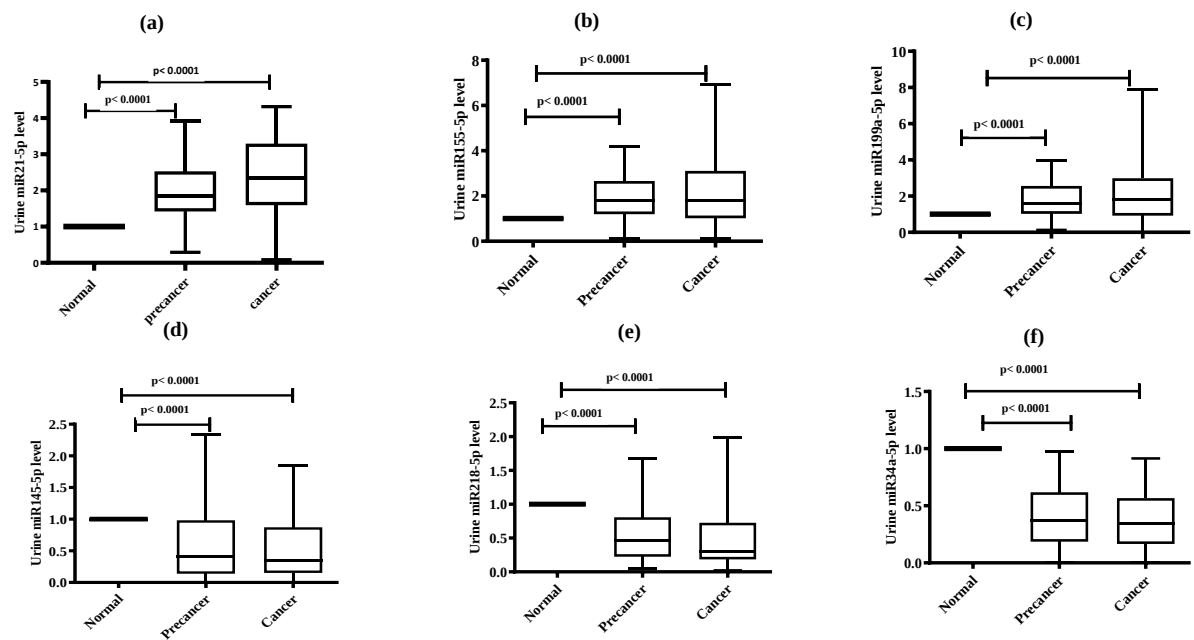


Figure S4

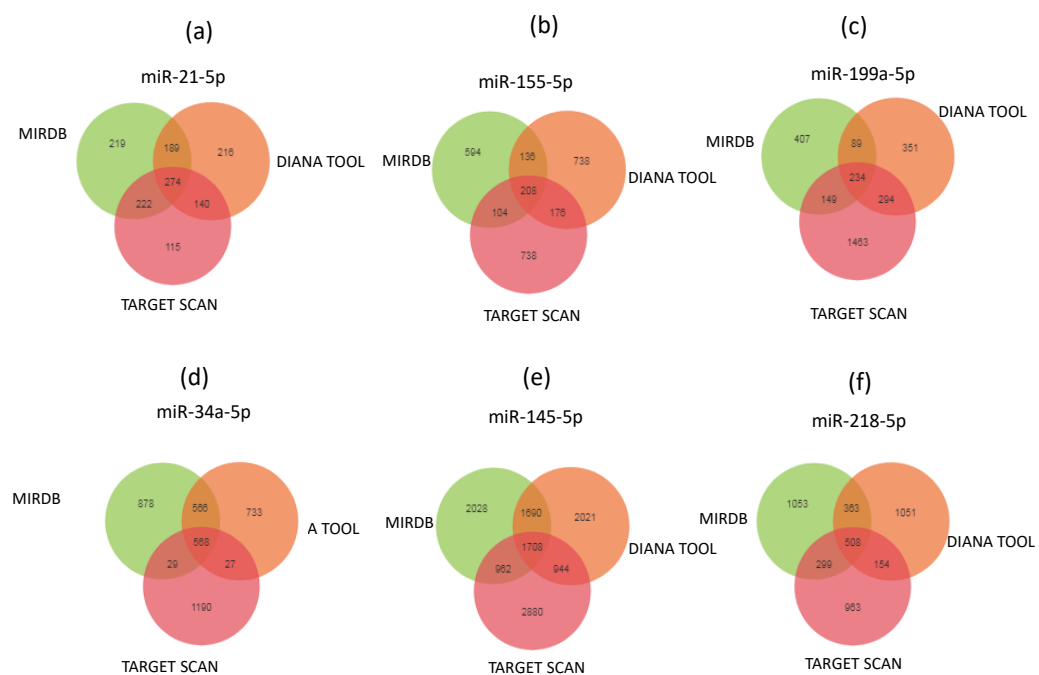
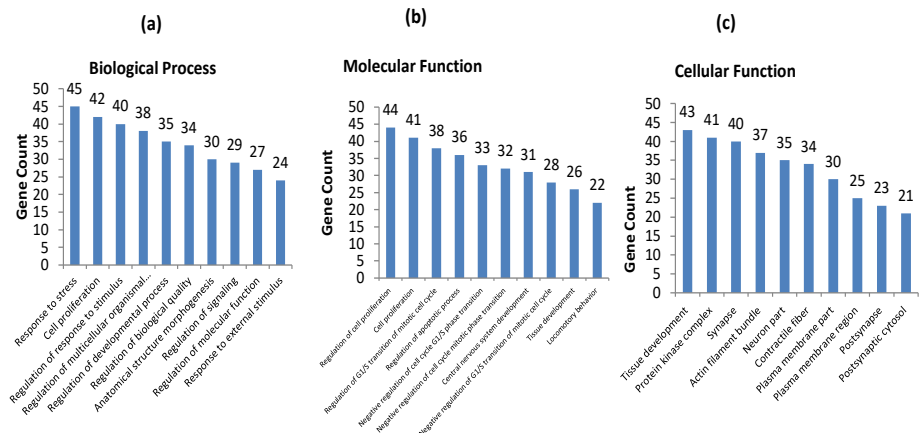


Figure S5

(A) Gene Ontology Enrichment



(B) Pathways

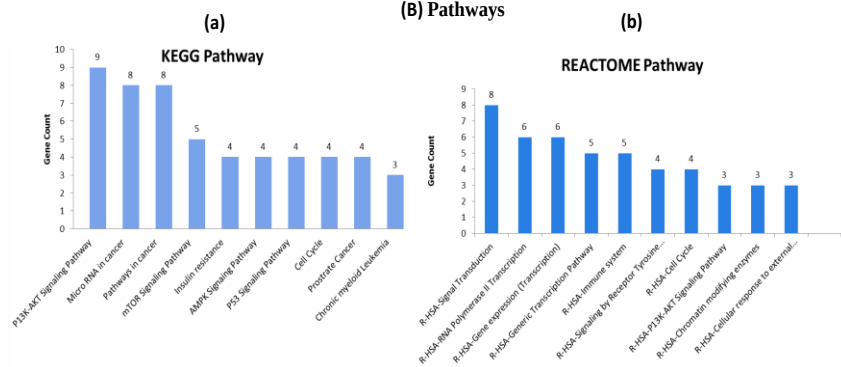


Figure S6

SUPPLEMENTARY TABLES

Table S1: The expression of miRNAs in HPV positive and HPV negative cervical cancer cell line

	SiHa (HPV16 positive)		HeLa (HPV18 positive)		C33a (HPV negative)	
miRNAs	FC±SD	<i>p</i> value	FC±SD	<i>p</i> value	FC±SD	<i>p</i> value
miR-21-5p	4.98±1.5	<0.0001	2.95±1.2	<0.0001	1.36±0.8	<0.001
miR-155-5p	3.86±0.9	<0.0001	2.43±2.0	<0.0001	1.58±1.0	0.001
miR-199a-5p	3.03±0.5	<0.0001	2.07±0.9	<0.0001	1.09± 0.6	0.01
miR-145-5p	0. 18±0.6	<0.0001	0.34±0.1	<0.0001	0.42±0.1	<0.0001
miR-218-5p	0. 26±0.4	<0.0001	0.59±0.1	<0.0001	0.65±0.4	0.001
miR-34a-5p	0.45±0.2	<0.0001	0.87±0.2	0.01	0.75±0.1	<0.01

FC: Fold change; SD: standard deviation

Table S2: The expression of miRNAs according to HPV infection status in urine, serum, cervical scrape, and tissue biopsies derived from cervical pre-cancer and cancer patients

Sample Types	Pathology	miR-21-5p (NFE) FC±SE	<i>p</i>	miR-145-5p (NFE) FC±SE	<i>p</i>	miR-218-5p (NFE) FC±SE	<i>p</i>	miR-34a-5p (NFE) FC±SE	<i>p</i>	miR-155-5p (NFE) FC±SE	<i>p</i>	miR-199-5p (NFE) FC±SE	<i>p</i>
Urine (n=75) HPV16/18 positive	Pre-cancer (n= 35)	2.1±0.2	0.64	0.2±0.01	0.007	0.4±0.03	0.81	0.4±0.02	0.851	2.0±0.3	0.40	2.6±0.8	0.75
	Cancer (n= 40)	3.0±0.1	0.68	0.3±0.01	0.02	0.5±0.02	0.15	0.4±0.02	0.773	2.4±0.5	0.54	1.8±0.6	0.16
Urine (n=25) HPV16/18 negative	Pre-cancer (n= 15)	1.8±0.4		0.6±0.02		0.5±0.03		0.6±0.05		1.7±0.2		2.2±0.2	
	Cancer (n= 10)	2.2±0.3		0.5±0.02		0.6±0.01		0.4±0.1		1.5±0.3		1.7±0.6	
Serum (n=75) HPV16/18 positive	Pre-cancer (n= 35)	2.3±0.8	0.04	0.1±0.01	0.77	0.3±0.04	0.64	0.3±0.02	0.231	2.7±1.0	0.47	1.7±0.5	0.47
	Cancer (n= 40)	3.2±0.6	0.47	0.12±0.1	0.02	0.32±0.05	0.86	0.32±0.08	0.551	2.5±1.5	0.41	2.0±0.7	0.90
Serum (n=25) HPV16/18 negative	Pre-cancer (n=15)	1.4±0.5		0.5±0.01		0.2±0.08		0.4±0.01		1.9±0.5		1.9±0.2	
	Cancer (n= 10)	2.2±0.3		0.24±0.01		0.3±0.01		0.26±0.03		2.0±0.6		1.6±0.1	
Scrape (n=35) HPV16/18 positive	Pre-cancer (n=35)	2.9±0.3	0.86	0.12±0.01	0.28	0.3±0.06	0.33	0.2±0.05	0.876	2.8±1.1	0.31	2.9±0.2	0.57
Scrape (n=15) HPV16/18 negative	Pre-cancer (n=15)	2.06±0.2		0.4±0.01		0.1±0.02		0.3±0.06		2.0±0.5		2.6±0.3	
Biopsy (n=36) HPV positive	Cancer (n=36)	3.69±0.1	0.01	0.2±0.01	0.53	0.2±0.02	0.93	0.27±0.02	0.98	2.6±0.9	0.001	2.5±0.5	0.01
Biopsy (n=4) HPV negative	Cancer (n=4)	2.88±0.5		0.3±0.01		0.2±0.01		0.3±0.02		2.1±0.1		1.9±0.1	

FC: Fold change; SE: standard error

Table S3: The correlation of the expression of urine, serum and cervical scrape miRNAs with the clinicopathological parameters of cervical pre-cancer patients

Variables	Sample Type	miR-21-5p FC±SD	p value	miR-145-5p FC±SD	p value	miR-218-5p FC±SD	p value	miR-34a-5p FC±SD	p value	miR-155-5p FC±SD	p value	miR-199a-5p FC±SD	p value
Age (years)	Urine	2.23±1.2	0.01	0.61±0.1	0.01	0.57±0.1	0.02	0.4±0.1	0.06	2.95±0.02	0.002	2.87±1.8	0.001
	Serum	2.82±1.6	0.01	0.19±0.01	0.03	0.27±0.1	0.0002	0.39±0.1	0.07	2.43±0.05	0.0004	3.27±1.9	0.671
	Cervical scrape	3.58±1.3	0.46	0.12±0.01	0.03	0.25±0.1	0.001	0.21±0.02	0.01	2.03±0.1	0.06	3.74±1.5	0.0001
>40 years (n=24)	Urine	1.98±0.8		0.86±0.1		0.38±0.2		0.58±0.2		2.09±0.04		2.04±0.6	
	Serum	2.4±1.0		0.23±0.01		0.65±0.1		0.76±0.4		2.08±0.05		3.1±1.2	
	Cervical scrape	3.0±0.5		0.31±0.02		0.81±0.3		0.43±0.02		1.9±0.8		2.12±0.5	
<40 years (n=26)	Urine	1.98±0.8		0.86±0.1		0.38±0.2		0.58±0.2		2.09±0.04		2.04±0.6	
	Serum	2.4±1.0		0.23±0.01		0.65±0.1		0.76±0.4		2.08±0.05		3.1±1.2	
	Cervical scrape	3.0±0.5		0.31±0.02		0.81±0.3		0.43±0.02		1.9±0.8		2.12±0.5	
Age of marriage <18 (n=31)	Urine	2.84±0.2	0.01	0.5±0.2	0.01	0.49±0.1	0.08	0.38±0.01	0.74	1.71±0.09	0.77	2.8±1.6	0.01
	Serum	2.23±1.1	0.02	0.1±0.01	0.57	0.26±0.1	0.77	0.25±0.01	0.95	1.54±0.6	0.01	2.38±2.0	0.01
	Cervical scrape	2.1±1.6	0.01	0.16±0.03	0.06	0.31±0.1	0.692	0.41±0.01	0.527	2.1±1.7	0.01	3.81±2.3	0.487
>18 (n=19)	Urine	2.05±1.4		0.73±0.01		0.63±0.1		0.44±0.02		2.11±1.6		1.81±0.4	
	Serum	1.93±0.7		0.18±0.02		0.24±0.1		0.4±0.01		2.47±1.9		2.13±1.0	
	Cervical scrape	2.43±1.8		0.09±0.01		0.26±0.2		0.23±0.03		2.37±1.0		3.98±2.1	
Parity >5 (n=35)	Urine	1.7±0.1	0.04	0.64±0.3	0.16	0.51±0.2	0.06	0.43±0.1	0.07	1.96±0.9	0.10	1.67±1.3	0.001
	Serum	1.99±0.6	0.01	0.14±0.03	0.01	0.27±0.01	0.05	0.24±0.2	0.05	2.3±1.0	0.73	2.93±1.7	0.0001
	Cervical scrape	2.24±1.3	0.04	0.15±0.01	0.15	0.3±0.1	0.06	0.22±0.1	0.08	2.09±0.7	0.01	1.71±0.8	0.01
<5 (n=15)	Urine	2.34±1.9		0.59±0.2		0.65±0.2		0.35±0.01		2.02±0.5		2.55±1.8	
	Serum	2.22±0.9		0.25±0.3		0.21±0.02		0.38±0.1		2.27±0.9		1.91±1.6	
	Cervical scrape	3.08±1.9		0.12±0.02		0.23±0.01		0.33±0.1		1.37±0.5		1.49±1.2	

FC: Fold change; SD: standard deviation

Table S4: The correlation of the expression of urine and serum miRNAs with the clinicopathological parameters of cervical cancer patients

Variables	Sample Type	miR-21-5p FC±SD	p value	miR-145-5p FC±SD	p value	miR-218-5p FC±SD	p value	miR-34a-5p FC±SD	P value	miR-155-5p FC±SD	P value	miR-199a-5p FC±SD	p value
Age(years)	Urine	2.51±0.3	0.09	0.34±0.1	0.4	0.62±0.2	0.001	0.44±0.1	0.10	2.15±1.2	0.01	1.98±0.8	0.28
	Serum	3.28±0.7	0.001	0.24±0.1	0.05	0.39±0.1	0.02	0.23±0.1	0.06	2.41±1.1	0.001	2.77±1.3	0.01
	Tumor biopsy	3.78±2.0	0.02	0.33±0.1	0.3	0.23±0.02	0.02	0.22±0.1	0.07	2.75±0.9	0.01	3.01±1.7	<0.0001
<40yr (n=27)	Urine	2.32±0.7		0.51±0.2		0.98±0.2		0.42±0.01		1.98±0.6		1.87±1.2	
	Serum	2.52±0.2		0.13±0.02		0.45±0.1		0.20±0.01		2.00±1.1		2.15±1.1	
	Tumor biopsy	3.08±1.2		0.29±0.2		0.36±0.1		0.23±0.1		2.01±1.6		1.65±0.5	
Age of marriage >18 (n=29)	Urine	1.9±1.2	0.01	0.69±0.3	0.6	0.56±0.2	0.8	0.72±0.3	0.08	2.34± 1.2	0.01	2.15±0.01	0.02
	Serum	2.08±0.8	0.04	0.51±0.2	0.03	0.43±0.1	0.01	0.37±0.2	0.36	2.98±1.3	0.02	2.49±0.4	0.01
	Tumor biopsy	3.26±2.1	0.001	0.34±0.1	0.01	0.12±0.01	0.58	0.23±0.1	0.10	3.62±1.5	<0.0001	3.98±1.2	0.001
≤18 (n=21)	Urine	2.64±1.6		0.5±0.01		0.49±0.1		0.38±0.1		3.71±1.2		3.8±0.9	
	Serum	2.90±1.4		0.16±0.03		0.26±0.1		0.31±0.2		4.1±0.9		4.01±0.3	
	Tumor biopsy	3.78±1.9		0.12±0.02		0.11±0.01		0.25±0.01		3.4±1.1		4.15±2.5	
Parity >5 (n=36)	Urine	2.89±1.8	0.1	0.62±0.2	0.8	0.42±0.1	0.5	0.35±0.1	0.08	2.91±0.9	0.0009	1.96±1.3	<0.0001
	Serum	3.2±0.9	0.1	0.12±0.02	0.04	0.31±0.1	0.02	0.29±0.1	0.3	2.74±0.4	0.0007	1.94±0.6	<0.0001
	Tumor biopsy	3.74±2.1	0.02	0.22±0.01	0.02	0.1±0.01	0.04	0.19±0.01	0.001	1.16±0.3	0.0001	2.53±1.1	<0.0001
≤5 (n=14)	Urine	3.01±1.9		0.58±0.3		0.38±0.1		0.39±0.2		3.53±0.3		2.98±0.7	
	Serum	3.5±2.3		0.16±0.2		0.46±0.2		0.27±0.3		4.01±1.1		3.7±1.5	
	Tumor biopsy	3.36±1.9		0.32±0.1		0.28±0.01		0.16±0.01		3.78±1.3		4.25±2.3	
Stage I-II (n=22)	Urine	2.1±0.5	<0.0001	0.85±0.4	0.001	0.65±0.2	0.01	0.89±0.2	0.01	1.5±1.0	0.02	1.9±0.8	0.001
	Serum	2.8±0.4	<0.0001	0.54±0.2	0.0009	0.42±0.1	0.01	0.79±0.3	0.01	2.0±1.2	<0.0001	2.5±1.9	0.001
	Tumor biopsy	3.5±1.9	<0.0001	0.11±0.01	0.001	0.16±0.04	0.001	0.12±0.01	0.001	3.0±1.5	0.01	3.7±1.8	<0.0001
III-IV (n=28)	Urine	3.2±1.7		0.14±0.01		0.33±0.1		0.36±0.2		1.8±0.9		2.2±0.9	
	Serum	3.5±1.9		0.17±0.01		0.21±0.01		0.48±0.1		2.9±0.8		1.9 ±1.0	
	Tumor biopsy	5.3±2.5		0.01±0.002		0.18±0.02		0.11±0.01		3.40±1.8		4.23±2.1	

FC: Fold change; SD: standard deviation

Table S5: Primers for amplification of human papilloma virus gene sequence

S. No.	Description	Primer Sequence
1.	Beta – Globin	FORWARD - 5'-GAAGAGCCAAGGACAGGTAC-3' REVERSE - 5'-CAACTTCATCCACGTTACACC-3'
2.	HPV L1	MY09 - 5'-GCMCAGGGWCATAAAYAATGG-3' MY11- 5'-CGTCCMARRGGAWACTGATC-3'
3.	HPV 16	FORWARD – 5'-AAGGCCAACTAAATGTCAC-3' REVERSE - 5'-CTGCTTTTATACTAACCGG-3'
4.	HPV 18	FORWARD - 5'-TGAGGTACCATTGGATATTT-3' REVERSE - 5'-TAGCAAAAAGCTGCTTCACGC-3'
5.	HPV 6	FORWARD- 5'-TAGTGGGCCTATGGCTCGTC-3' REVERSE - 5'-TCCATTAGCCTCCACGGGTG-3'
6.	HPV 11	FORWARD - 5'-CGAGCAGACGTCCGTCCTCG-3' REVERSE - 5'-GGAATACATGCGCCATGTGG-3'

Key to Symbols: R=A+G, Y=C+T, M=A+C, K=G+T, S=G+C, W=A+T, H=A+T+C, B=G+T+C, D=G+A+T, N=A+C+G+T, V=G+A+C.

Table S6: miRNA primer sequences used for Real-time PCR

S.No.	Genes	Stem Loop Sequence for RT	qPCR Forward Primer	qPCR Reverse Primer
1	U6	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGC ACTGGATACGACAAAATA	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGCAT
2	miR145-5p	ACTTCGCAACTACCGTTTGAAGGGATTC	TCG GTC CAG TTT TCC CAG	TGAACTTCGCAACTACCGTTTG
3	miR218 -5p	GGTCGAACGCCTAACGTCACATGGTTAG	CGAGTGCATTTGTGCTTGATCTA	TAATGGTCGAACGCCTAACGTC
4	miR34a -5p	GCAGCGAATCCACGATTAGAACAACCAG	GAATGGTGGCAGTGTCTTAGC	GGAGCAGCGAATCCACGATTAG
5	miR21-5p	CACTGTCTAGCAGCAGACTAATCAACATCAG	GCCCCTAGCTTATCAGACTGATG	CAGTGCAGGGTCCGAGGT
6	miR199a-5p	GTCAGAAGGAATGATGCACAGCCAACA	ACAACCTGCGTAGGTAGTTTCATGT	CGTCAGAAGGAATGATGCACAG
7	miR155-5p	CCAGAAACCGATCAGAGTGTCCCCTATCA	CGCCATGTTTAATGCTAATCGTGA	TTCCAGAAACCGATCAGAGTGT