Results

Our study was performed on twenty women had repeated spontaneous miscarriages and twenty healthy women as normal control group for mutation detection of the three thrombophilic genes, factor V (FV), prothrombin (PTH) and methylenetetrahydrofolate reductase (MTHFR) genes.

Table (1): Clinical profile of repeated spontaneous miscarriage (RSM) cases and normal control group.

Studied Groups / **Parameters**		**Normal Control Group (n.=20)**	**RSM Cases (n.=20)**
Maternal age (years)		29.0 ± 4.80 (20-39)	31.4 ± 6.82 (22-45)
No. of repeated spontaneous miscarriages	≤3	– 0%	12 (60%)
	>3	– 0%	8 (40%)
Previous live births		20/20 100%	5/20 25%
Other obstetric complications		– 0%	3/20 15%
Family history of RSM		– 0%	4 20%
Drug intake		No 0%	No 0%
Immobility		1 5%	2 10%
Smoking		No 0%	No 0%
Contraception		No 0%	No 0%
Diseases		No 0%	No 0%

The table shows description of the clinical data of repeated spontaneous miscarriage cases and normal control group. These data include maternal age, number of repeated spontaneous miscarriages, previous live births, other obstetric complications (i.e. stillbirth), family history of RSM, drug intake, immobility, smoking, contraception and diseases.

Figure (1): Maternal age among repeated spontaneous miscarriage (RSM) cases and normal control group.

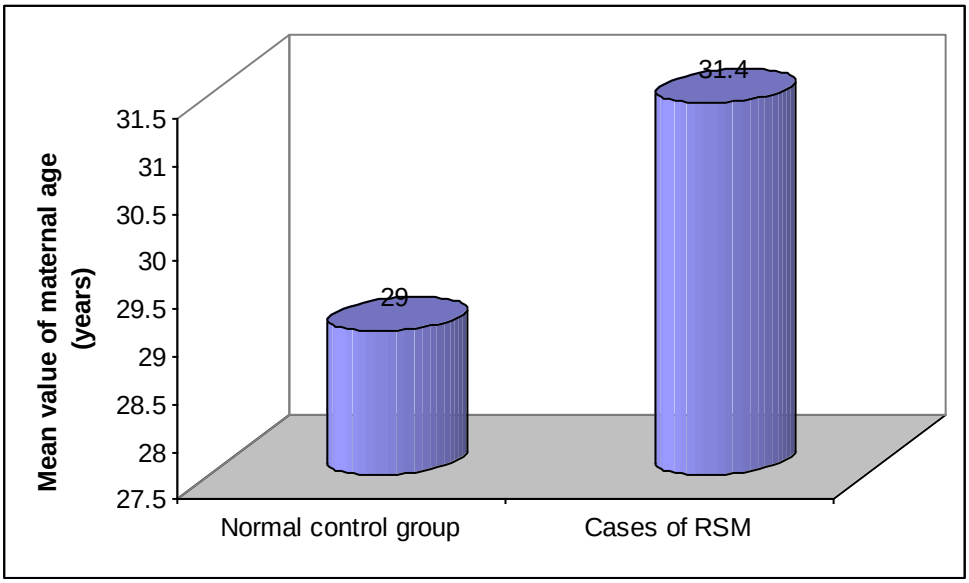

Figure (2): Number of repeated spontaneous miscarriages among repeated spontaneous miscarriage (RSM) cases.

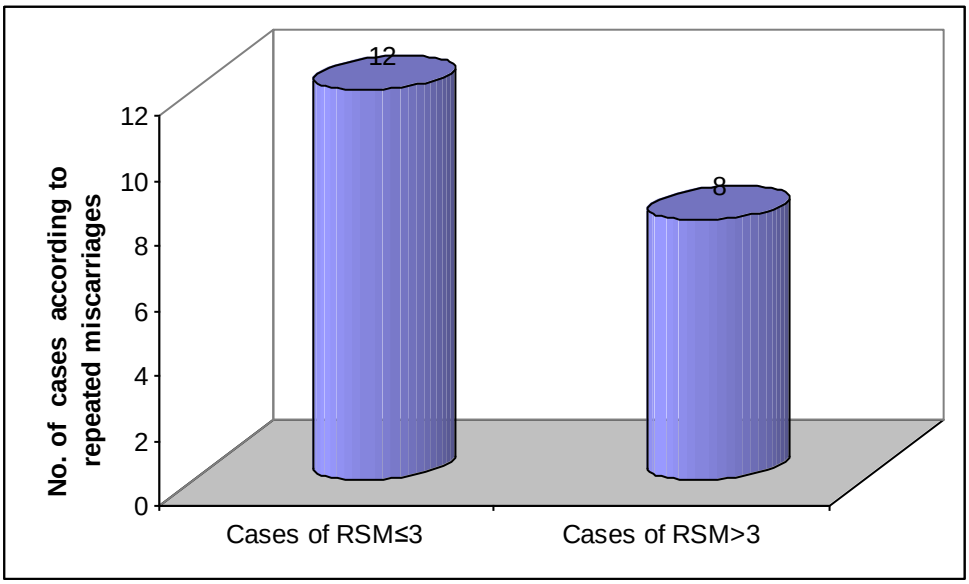

Table (2): Family history of repeated spontaneous miscarriage and number of previous live births among repeated spontaneous miscarriage (RSM) cases and normal control group.

Parameters / Studied Groups			Normal Control Group (n.=20)	RSM Cases (n.=20)	Total (n.=40)	X^2	p
Family history of RSM	+ve	No. %	_	4 (20%)	4 10%	2.5	> 0.05
	-ve	No. %	٢٠ (100%)	16 (80%)	36 90%		
History of previous Live births	+ve	No. %	20 100%	5 25%	25 62.5	209	<0.001**
	-ve	No. %	_	15 75%	15 37.5%		

p> 0.05 not significant and ** p<0.001 high significant

Figure (3): Family history of repeated spontaneous miscarriage among repeated spontaneous miscarriage (RSM) cases and normal control group.

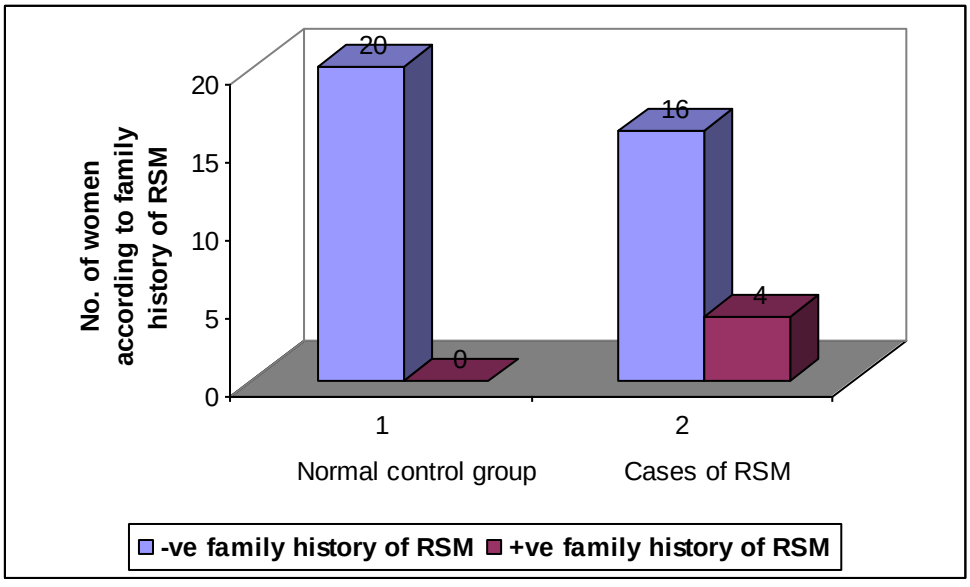

Figure (٤): Number of previous live births among repeated spontaneous miscarriage (RSM) cases and normal control group.

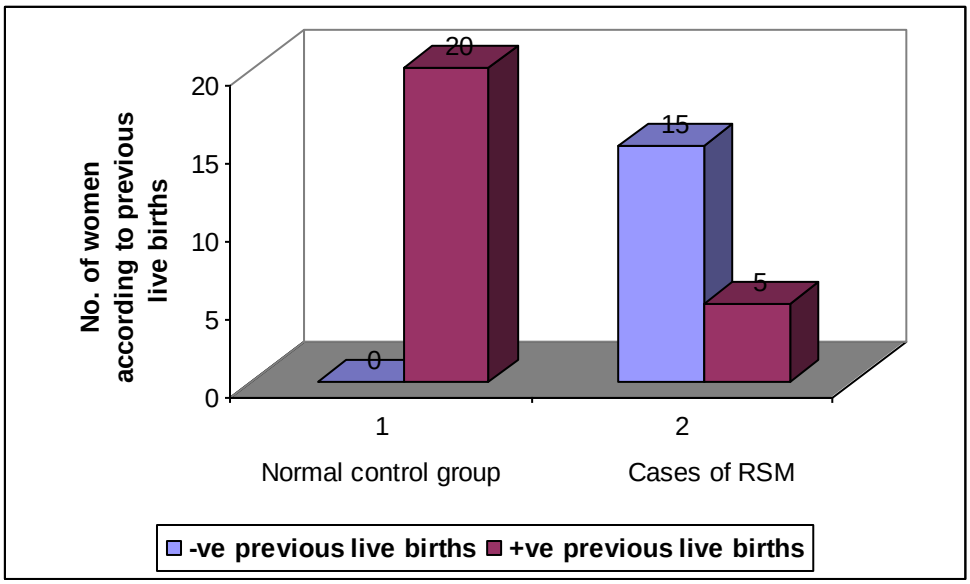

Table (2), figures (3) and (4) show that there is an increase in the number of cases of with family history of RSM as compared to normal controls but this increase does not reach to a statistically significant level, ($p>0.05$). However, there is a high statistically significant decrease in the number of previous live births among cases of RSM as compared to normal controls, ($p <0.001$).

Table (3): Mean values ± SD and confidence interval (CI) of prothrombin time (PT), prothrombin concentration (PC) and activated partial thromboplastin time (APTT) among repeated spontaneous miscarriage (RSM) cases and normal control group.

Studied Groups / **Parameters**	**Normal Control Group (n.=20)**	**RSM Cases (n.=20)**	**"t"**	**p**	**95% CI**
	± SD $\overline{X}$ (Range)				
Prothrombin time (PT) in sec.	12.65 ± 0.51 (12.0-13.6)	12.56 ± 0.55 (11.8-14)	-0.51	>0.05	(-0.43-0.26)
Prothombin concentration (PC) in %	88.5 ± 7.50 (75-100)	89.4 ± 7.90 (70-100)	0.37	>0.05	(-0.43-0.26)
Activated partial thromboplastin time (APTT) in sec.	33.7 ± 4.1 (29.0-41.2)	36.1 ± 4.4 (28.5-45.0)	1.79	>0.05	(-0.3-5.1)

p> 0.05 not significant

The table shows that there are non significant differences neither in prothrombin time, prothrombin concentration nor activated partial thromboplastin time among repeated spontaneous miscarriage cases as compared to normal controls, (p> 0.05 for all).

Table (4): Mean ± SD of maternal age, number of repeated spontaneous miscarriages, prothrombin time (PT), prothrombin concentration (PC) and activated partial thromboplastin time (APTT) in relation to factor V (FV) gene mutation among cases of repeated spontaneous miscarriage (RSM).

Studied Groups / **Parameters**	**RSM Cases with Normal FV Gene ± SD $\overline{X}$**	**RSM Cases with Mutant FV Gene ± SD $\overline{X}$**	**"t"**	**p**
	(n.=20)			
	(n.=6)	**(n.=14)**		
Maternal age (years)	27.8 ± 5.7	32.9 ± 6.9	-1.59	>0.05
Number of repeated spontaneous miscarriages	3.2 ± 0.4	4.6 ± 1.9	1.85	>0.05
Prothrombin time (PT) in sec.	12.60 ± 0.56	12.50 ± 0.65	-0.398	>0.05
Prothombin concentration (PC) in %	88.9 ± 7.9	90.5 ± 8.4	0.418	>0.05
Activated partial thromboplastin time (APTT) in sec.	36.9 ± 4.8	34.2 ± 2.2	-1.3	>0.05

p> 0.05 not significant

Figure (٥): Maternal age, number of repeated spontaneous miscarriages, prothrombin time (PT), prothrombin concentration (PC) and activated partial thromboplastin time (APTT) in relation to factor V (FV) gene mutation among cases of repeated spontaneous miscarriage (RSM).

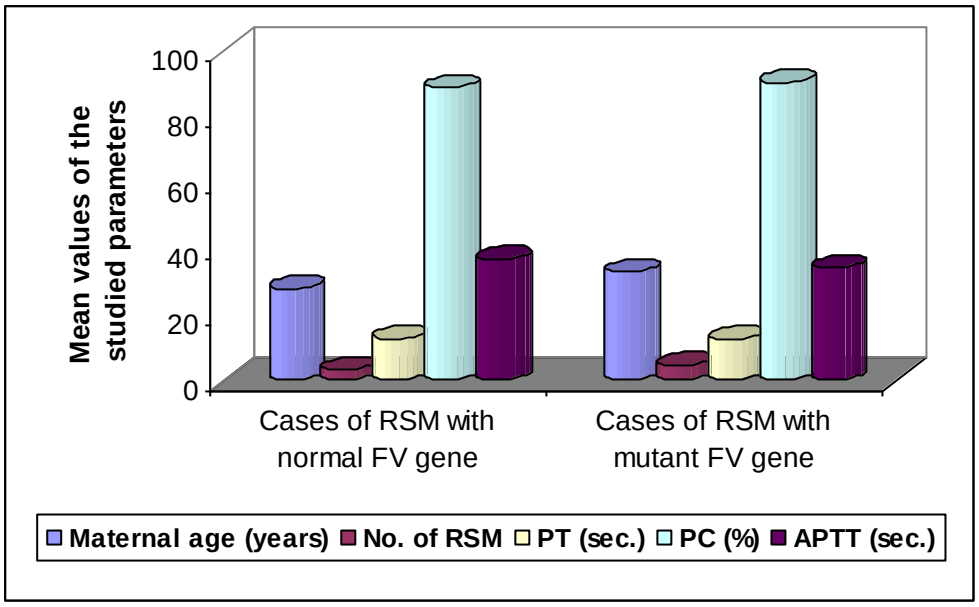

Table (4) and figure (5) show that there are increases in maternal age, number of repeated spontaneous miscarriages and prothombin concentration and decrease in activated partial thromboplastin time in cases with FV gene mutation as compared to those with normal gene but these differences do not reach to statistically significant levels, (p> 0.05 for all).

Table (5): Mean ± SD of maternal age, number of repeated spontaneous miscarriages, prothrombin time (PT), prothrombin concentration (PC) and activated partial thromboplastin time (APTT) in relation to prothrombin (PTH) gene mutation among cases of repeated spontaneous miscarriage (RSM).

Studied Groups / **Parameters**	**RSM Cases with Normal Prothrombin Gene ± SD** $\overline{X}$	**RSM Cases with Mutant Prothrombin Gene ± SD** $\overline{X}$	**"t"**	**p**
	(n.=20)			
	(n.=7)	**(n.=13)**		
Maternal age (years)	28.7 ± 5.3	32.8 ± 7.3	-1.32	>0.05
Number of repeated spontaneous miscarriages	3.6 ± 1.5	4.5 ± 1.8	1.2	>0.05
Prothrombin time (PT) in sec.	12.50 ± 0.57	12.60 ± 0.56	0.15	>0.05
Prothombin concentration (PC) in %	89.8 ± 8.0	88.6 ± 8.3	-0.32	>0.05
Activated partial thromboplastin time (APTT) in sec.	36.1 ± 4.3	36.1 ± 4.7	-0.001	>0.05

p> 0.05 not significant

Figure (٦): Maternal age, number of repeated spontaneous miscarriages, prothrombin time (PT), prothrombin concentration (PC) and activated partial thromboplastin time (APTT) in relation to prothrombin (PTH) gene mutation among repeated spontaneous miscarriage (RSM).

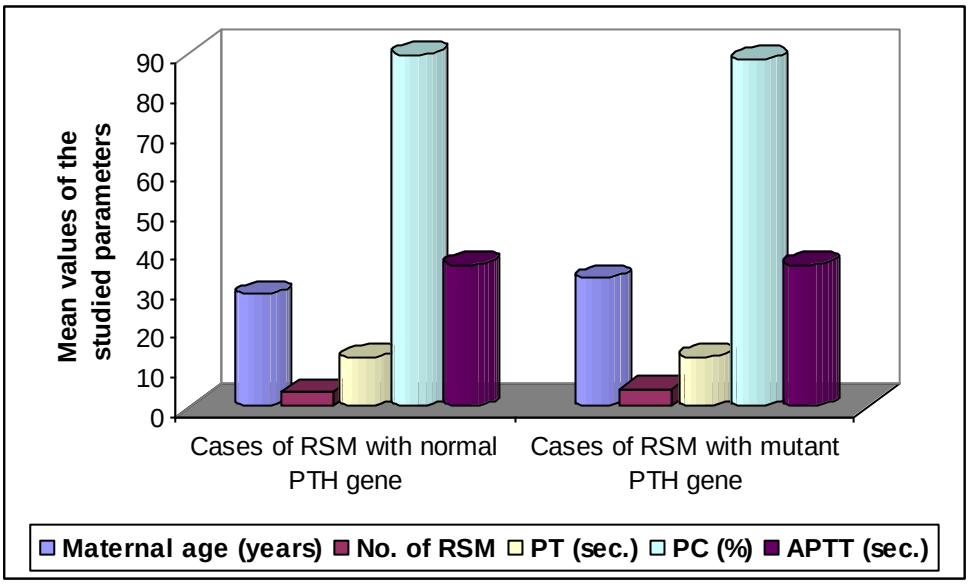

Table (٥) and figure (٦) show that there are increases in maternal age and number of repeated spontaneous miscarriages, and decrease in PC in cases with PTH gene mutation compared to those with normal gene but these differences do not reach to statistically significant levels, (p> 0.05 for all).

Table (6): Mean ± SD of maternal age, number of repeated spontaneous miscarriages, prothrombin time (PT), prothrombin concentration (PC) and activated partial thromboplastin time (APTT) in relation to methylenetetrahydrofolate reductase (MTHFR) gene mutation among cases of repeated spontaneous miscarriage (RSM).

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Studied Groups / **Parameters**	**RSM Cases with Normal MTHFR Gene ± SD $\overline{X}$**	**RSM Cases with Mutant MTHFR Gene ± SD $\overline{X}$**	**"t"**	**p**
	(n.=20)			
	(n.=6)	**(n.=14)**		
Maternal age (years)	30.3 ± 6.3	31.9 ± 7.2	-0.448	>0.05
Number of repeated spontaneous miscarriages	4.0 ± 2.0	4.3 ± 1.7	-0.33	>0.05
Prothrombin time (PT) in sec.	12.55 ± 0.57	12.56 ± 0.56	0.12	>0.05
Prothombin concentration (PC) in %	89.6 ± 7.9	88.7 ± 8.4	-0.25	>0.05
Activated partial thromboplastin time (APTT) in sec.	35.6 ± 4.4	37.4 ± 4.4	0.85	>0.05

p>0.05 not significant

Figure (٧): Maternal age, number of repeated spontaneous miscarriages, prothrombin time (PT), prothrombin concentration (PC) and activated partial thromboplastin time (APTT) in relation to methylenetetrahydrofolate reductase (MTHFR) gene mutation among cases of repeated spontaneous miscarriage (RSM).

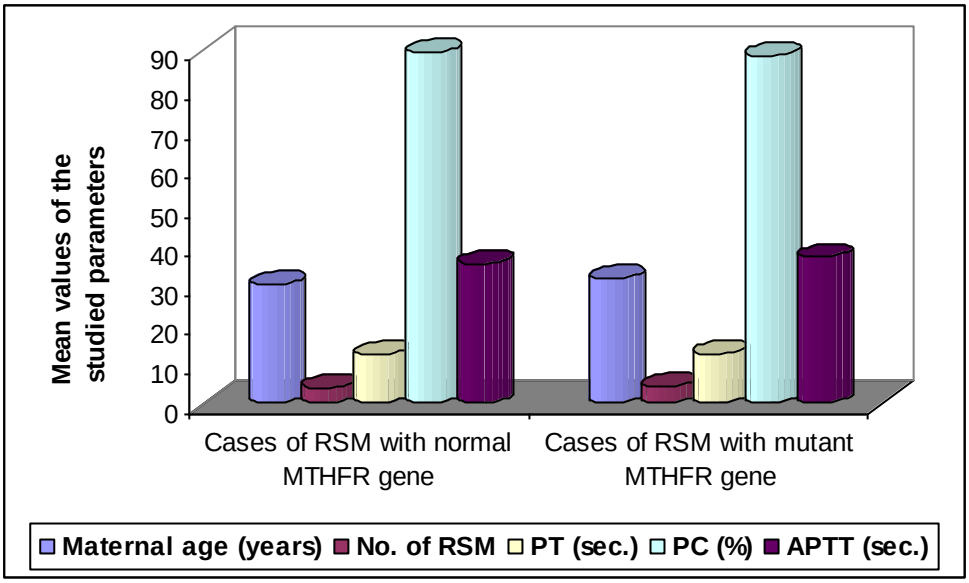


Table (6) and figure (7) show that there are increases in maternal age and number of repeated spontaneous miscarriages, APTT and decrease in PC in cases with MTHFR gene mutation as compared to those with normal gene but these differences do not reach to statistically significant levels, (p> 0.05 for all).

Table (7): Percentage of allele frequencies of factor V, prothrombin and methylenetetrahydrofolate reductase genes mutations among repeated spontaneous miscarriage (RSM) cases and normal control group.

Parameters \ Studied Groups			Normal Control Group (n.=20)	RSM Cases (n.=20)	Total (n.=40)	X^2	p
FV gene	Normal	No. %	19 (95.0%)	6 (30.0%)	25 62.5%	18.03	<0.001**
	Mutant	No. %	1 5.0%	14 (70.0%)	15 37.5%		
PTH gene	Normal	No. %	19 95.0%	7 35.0%	26 65%	15.8	<0.001**
	Mutant	No. %	1 5.0%	13 65.0%	14 35%		
MTHFR gene	Normal	No. %	20 100.0%	6 30.0%	26 65.0%	18.6	<0.001**
	Mutant	No. %	–	14 70.0%	14 35.0%		

**p<0.001 high significant

Figure (٨): Percentage of allele frequency of factor V gene mutation among repeated spontaneous miscarriage (RSM) cases and normal control group.

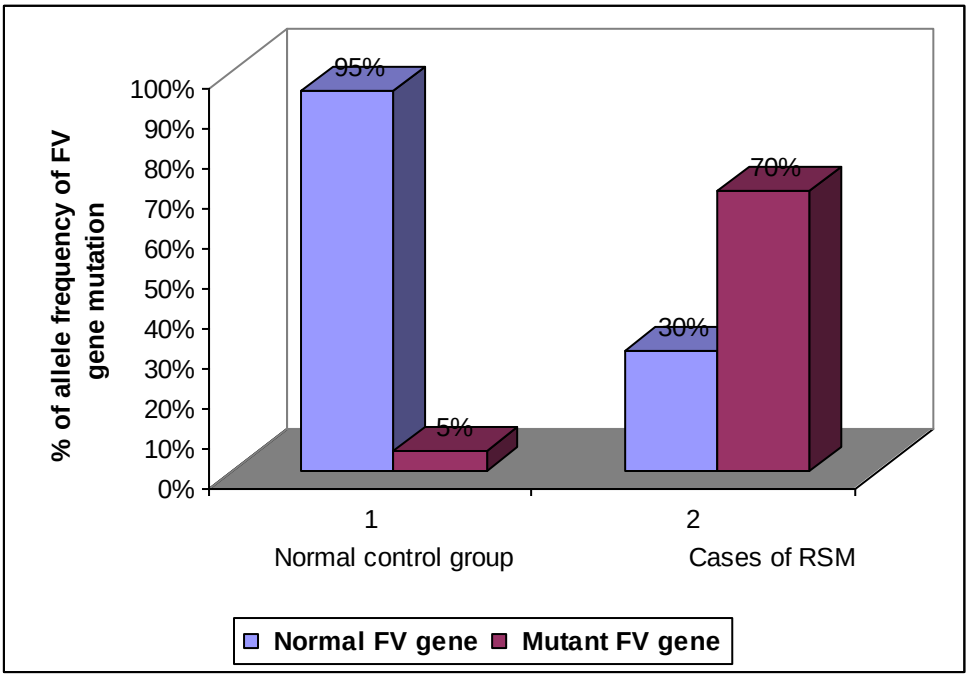


Figure (٩): Percentage of allele frequency of prothrombin gene mutation among repeated spontaneous miscarriage (RSM) cases and normal control group.

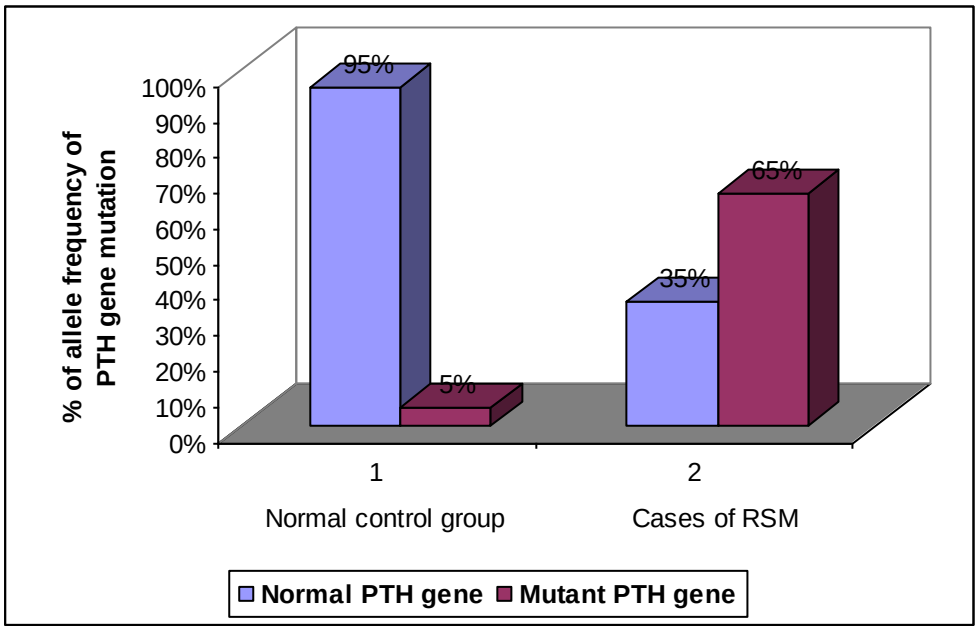


Figure (١٠): Percentage of allele frequency of methylenetetrahydrofolate reductase gene mutation among repeated spontaneous miscarriage (RSM) cases and normal control group.

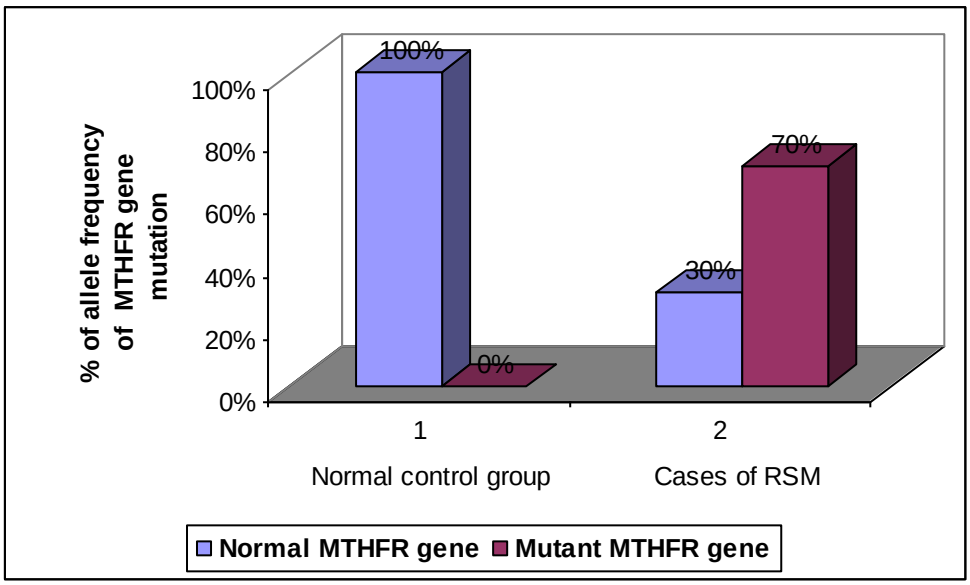


Table (7) and figures (8), (9) and (10) shows that there are statistically high significant increases in the number of cases with factor V, prothrombin

and methylenetetrahydrofolate reductase genes mutations as compared to normal control group (70%, 65% and 70% respectively), ($p<0.001$) for all.

Table (8): Percentage of genotype frequencies of factor V, prothrombin and methylenetetrahydrofolate reductase genes mutations among repeated spontaneous miscarriage (RSM) cases and normal control group.

Parameters / Studied Groups		Normal Control Group (n.=20)	RSM Cases (n.=20)	Total (n.=40)	X^2	p
Genotype of FV	Normal (-/-)	19 95.0%	6 30.0%	25 62.5%	18.1	<0.001**
	Heterozygous (+/-)	1 5.0%	12 60.0%	13 32.5%		
	Homozygous (+/+)	_	2 10.0%	2 5.0%		
Genotype of PTH	Normal (-/-)	19 95.0%	7 35.0%	26 65.0%	16.1	<0.001**
	Heterozygous (+/-)	_	7 35.0%	7 17.5%		
	Homozygous (+/+)	1 5.0%	6 30.0%	7 17.5%		
Genotype of MTHFR	Normal (-/-)	20 100.0	6 30.0%	26 65.0%	21.5	<0.001**
	Heterozygous (+/-)	_	9 45.0%	9 22.5%		
	Homozygous (+/+)	_	5 25.0%	5 12.5%		

** $p<0.001$ high significant

Figure (١١): Percentage of genotype frequency of factor V gene mutation among repeated spontaneous miscarriage (RSM) cases and normal control group.

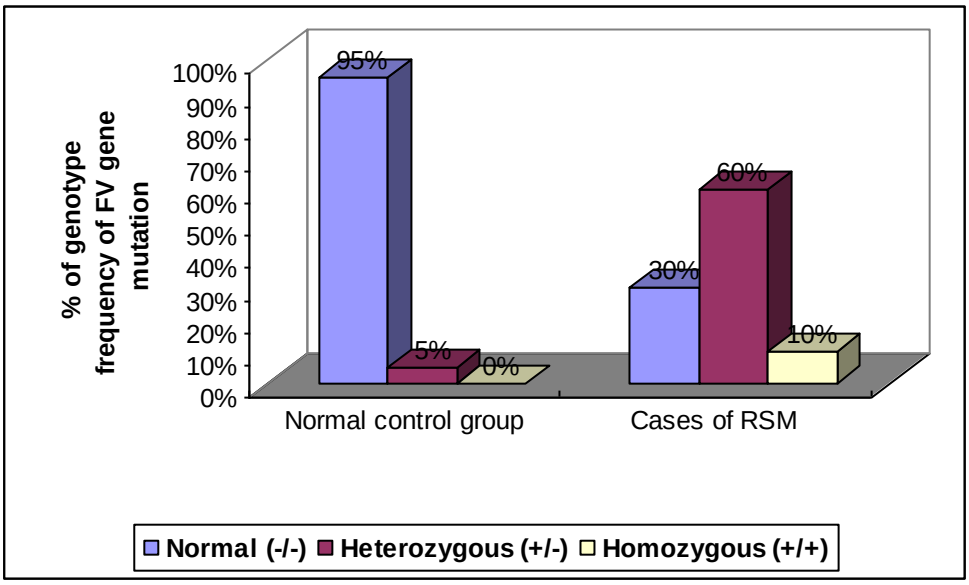


Figure (12): Percentage of genotype frequency of prothrombin gene mutation among the repeated spontaneous miscarriage (RSM) cases and normal control group.

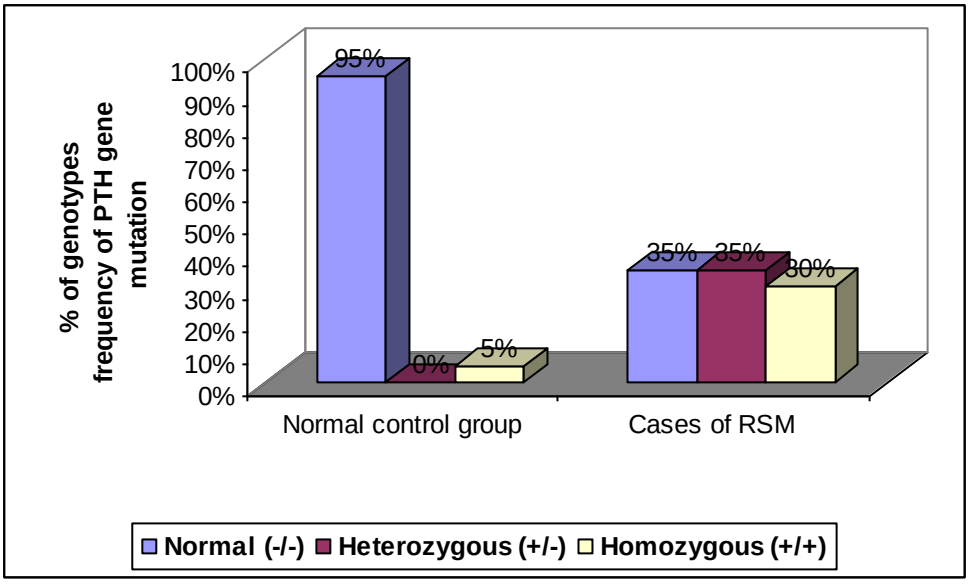


Figure (13): Percentage of genotype frequency of methylenetetrahydrofolate reductase gene mutation among repeated spontaneous miscarriage (RSM) cases and normal control group.

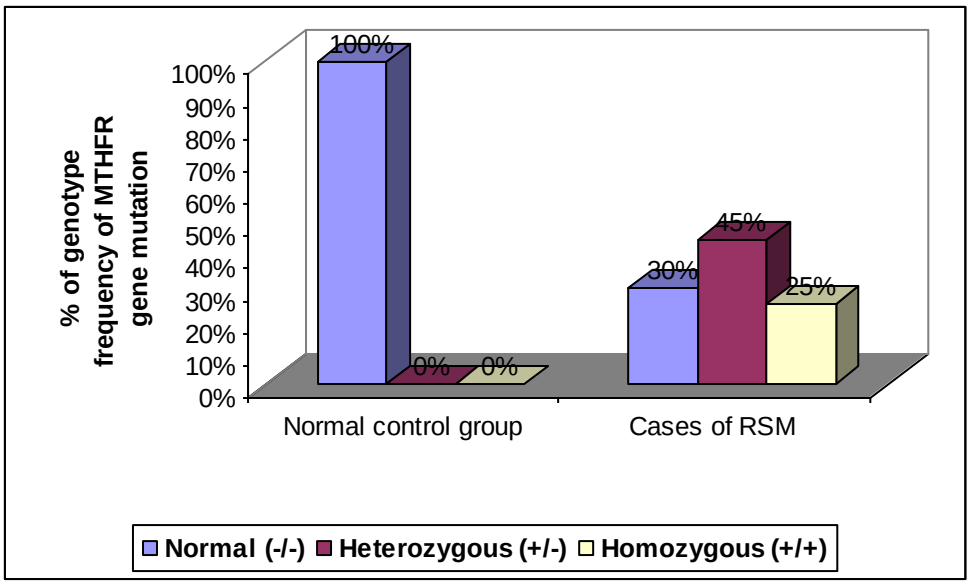


Table (8) and figures (11), (12) and (13) show that there are statistically high significant increases in the percentage of cases with heterozygous and homozygous mutations in factor V gene (heterozygous 60% and homozygous 10%), prothrombin (heterozygous 35% and homozygous 30%) and methylenetetrahydrofolate reductase (heterozygous 45% and homozygous 5%), as compared to normal controls, ($p<0.001$ for all).

Table (9): Percentage of single and multiple gene mutations among repeated spontaneous miscarriage (RSM) cases and normal control group.

Studied Groups / Parameters		Normal Control Group (n.=20)	RSM Cases (n.=20)	Total (n.=40)	X^2	p
No gene mutation (normal gene)	No. %	١٨ 90%	3 15.0%	21 52.5%	26.4	<0.001**
Single gene mutation	No. %	2 10%	4 20.0%	6 15.0%		
Multiple genes mutations	No. %	_	13 65.0%	13 32.5%		

** p<0.001 high significant

Figure (14): Percentage of single and multiple gene mutations among repeated spontaneous miscarriage (RSM) cases and normal control group.

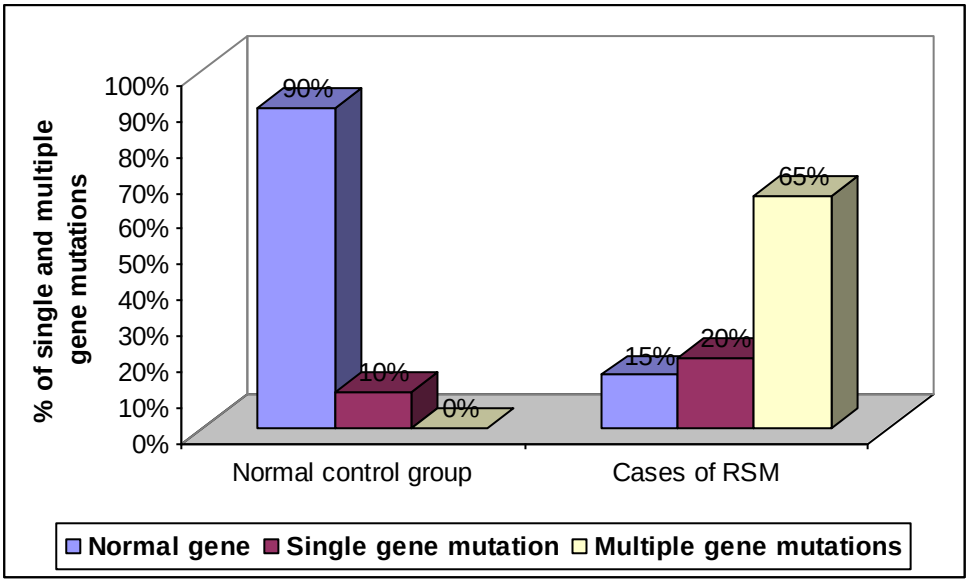


Table (9) and figure (14) show that there are statistically high significant increases in the percentage of cases with single (20%) and multiple gene mutations (65%) as compared to normal controls, (p<0.001 for all).

The following diagram illustrates the wild and mutant probes for each gene:

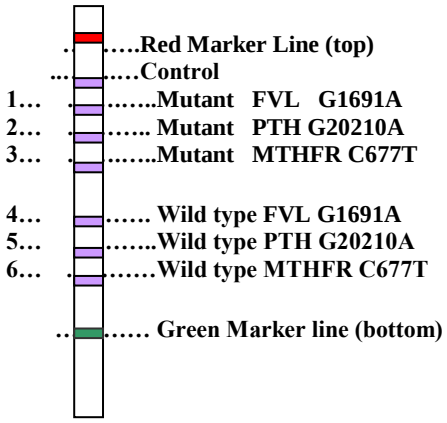
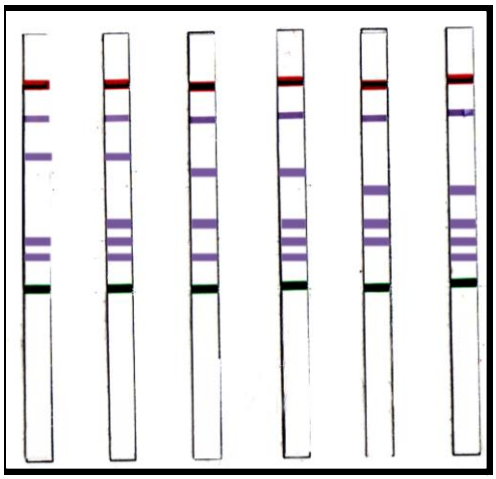


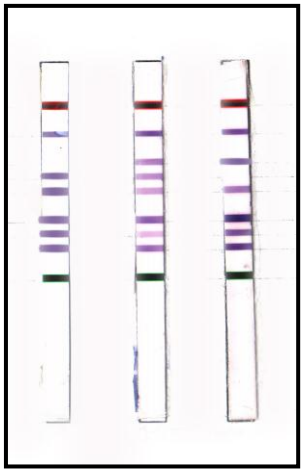
Figure (15): Single gene mutation.



(i) (ii) (iii) (iv) (v) (vi)

Figure (15), [(i), (ii), (iii), (iv), (v) and (vi)] show homozygous and heterozygous mutations of factor V (FV), prothrombin (PTH) and methylenetetrahydrofolate reductase (MTHFR) genes respectively.

Figure (16): Multiple gene mutations.



(i) (ii) (iii)

Figure (16) (i) shows compound heterozygosity of both prothrombin (PTH) and methylenetetrahydrofolate reductase (MTHFR) genes, (ii) shows compound heterozygosity of factor V (FV), prothrombin (PTH) and methylenetetrahydrofolate reductase (MTHFR) genes and (iii) shows compound heterozygosity of both factor V (FV) and methylenetetrahydrofolate reductase (MTHFR) genes.

Figure (١٧): Correlation coefficient between maternal age and number of gene mutations among cases of repeated spontaneous miscarriage.

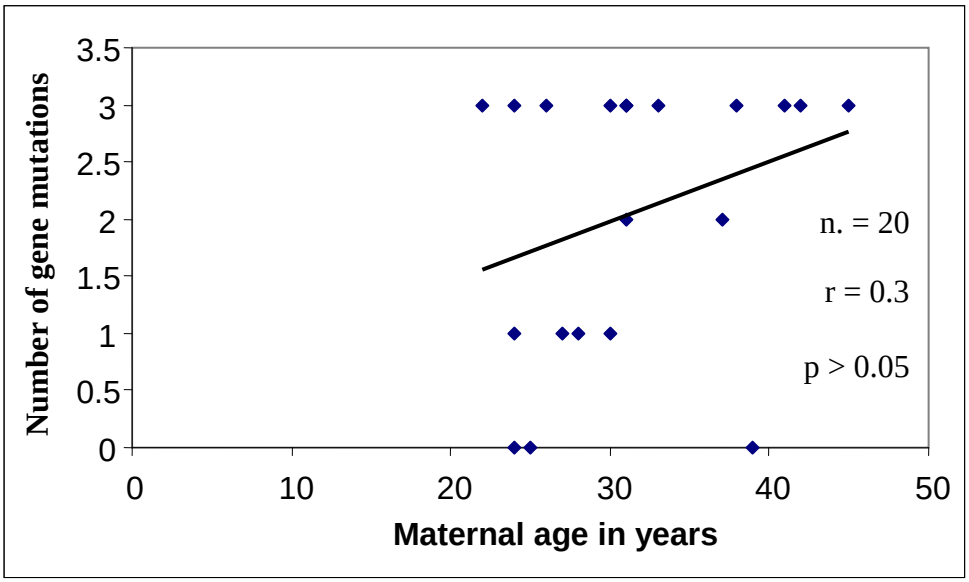
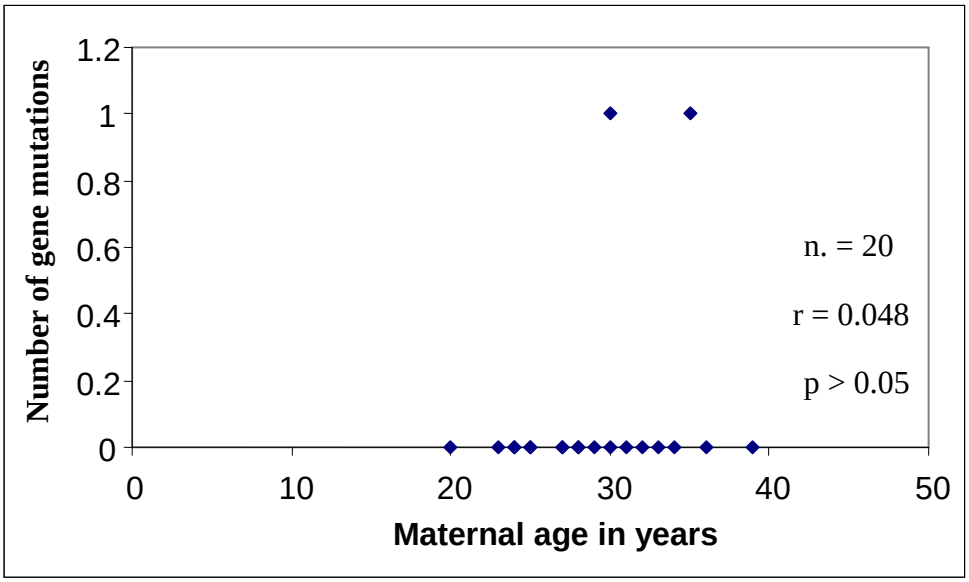


Figure (١٨): Correlation coefficient between maternal age and number of gene mutations among normal control group.



Figures (١٧) and (١٨) show that there is non significant correlation between maternal age and number of gene mutation neither among cases of repeated spontaneous miscarriages nor among normal control group.

Table (10): Mean values ± SD of prothrombin time (PT), prothrombin concentration (PC) and activated partial thromboplastin time (APTT) among repeated spontaneous miscarriage (RSM) cases in relation to number of gene mutations.

Parameters / **Studied Groups**	**Prothrombin Time (PT)** **± SD $\overline{X}$ (Range)**		**Prothrombin Concentration (PC)** **± SD $\overline{X}$ (Range)**		**Activated Partial Thromboplastin Time (APTT)** **± SD $\overline{X}$ (Range)**	
Cases of RSM with normal gene (n.=3)	12.8 ± 0.6429 (12.30-13.50)	p>0.05	85.7 ± 9.238 (75-91)	p>0.05	34.7 ± 2.517 (32.00-37.00)	p<0.05*
Cases of RSM with one gene mutation (n.=4)	12.4 ± 0.4203 (11.90-12.80)		92.5 ± 6.758 (84-100)		35.1 ± 3.255 (32.00-39.00)	
Cases of RSM with two gene mutations (n.=2)	12.4 ± 0.8485 (11.80-13.00)	F=0.38	92.0-11.31 (84-100)	F=0.49	45.0 ± 0.0707 (44.90-45.00)	F=4.99
Cases of RSM with three gene mutations (n.=11)	12.6 ± 0.5752 (12.00-14.00)		88.7 ± 8.026 (70-100)		35.3 ± 3.770 (28.50-41.00)	
Total (n.=20)	12.6 ± 0.5510 (11.80-14.00)		89.4 ± 7.876 (70-100)		36.1 ± 4.359 (28.50-45.00)	

* p< 0.05 significant

The table shows distribution of cases of repeated spontaneous miscarriage (RSM) according to prothrombin time, prothrombin concentration and activated partial thromboplastin time.

There are statistically significant increases in APTT in cases of RSM with single and multiple mutations as compared to cases of RSM with normal genes, (p<0.05).

Table (11): Mean values ± SD of maternal age and number of repeated spontaneous miscarriages among repeated spontaneous miscarriage (RSM) cases in relation to number of gene mutations.

Parameters / **Studied Groups**	**Maternal Age (years) ± SD $\overline{X}$ (Range)**		**No. of Repeated Spontaneous Miscarriages ± SD $\overline{X}$ (Range)**	
Cases of RSM with normal gene (n.=3)	29.3 ± 8.386 (24-39)	p>0.05 F=0.87	3.00 ± 0.000	p<0.01* F=5.8
Cases of RSM with one gene mutation (n.=4)	27.3 ± 2.500 (24-30)		3.25 ± 0.500 (3-4)	
Cases of RSM with two gene mutations (n.=2)	34.0 ± 4.243 (31-37)		7.50 ± 0.707 (7-8)	
Cases of RSM with three gene mutations (n.=11)	33.0 ± 7.629 (22-45)		4.27 ± 1.618 (3-8)	
Total (n.=20)	31.4 ± 6.816 (22-45)		4.20 ± 1.735 (3-8)	

p> 0.05 not significant

* p<0.01 significant

This table shows distribution of cases according to number of gene mutation and both maternal age and number of repeated spontaneous miscarriages.

There is statistically significant increase in number of repeated spontaneous miscarriages in cases with single and multiple mutations as compared to those with normal genes (p<0.01).