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In the Name of Allah Most Compassionate, Mort Merciful

Edition Word

O Allah, my Lord

Cast felicity in me, facilitate my cause and unknot my tongue to perceive my speech, thanks be upon Him the Evolver of the universe and peace be upon Mohammad and his immaculate and benevolent progeny.

A fledged edition of Al-Bahr , peer reviewed scientific journal, embraces a constellation of research studies pertinent to engineering and natural sciences we do hope to overlap a scientific gap the specialists observe as an academic phenomenon worth being under the lenses of the researchers, that is why there is diversity in the studies to meet the requirements of the journal readership . For the journal, now, comes to the fore , at the efforts of the editorial and advisory boards and the researchers who strain every sinew to publish in Al-Bahr, to be global as to be published in an international publishing house in line with the global scientific journals.

On such an occasion we do pledge the promise of fealty and loyalty to those who observe our issues with love and heed in the International Al-`Ameed for Research and Studies , Department of Cultural and Intellectual Affairs in the Holy Al-`Abbas Shrine and the strenuous endeavour to cull whatever invigorates the scientific interaction and academic research in Iraq and worldwide to create a new generation keeping pace with the development of the current scientific phase and to lay the hands of the researchers, nationwide and worldwide, upon the desired missions.

Thanks be upon Him, the Evolver ad infinitum.

Asaad Mohammad Ali Husain, Haider Abbas Abdul AL- Ameer Department of Mathematics, College of education for pure science, University of Babylon, Iraq	Fully Stable Semimodules	13
*Nahida B. Hasan, **Ghusson H. Mohammed and *Mohammed A. Abdul Majeed *Department of physics, College of Science, University of Babylon, Iraq **Department of physics, College of Science, University of Baghdad, Iraq.	Electrical Properties of (CdO) _{1-x} (SnO ₂) xThin Films Prepared by Pulsed Laser Deposition	21
Nadia H. Al-Noor and Suzan F. Bawi Dept. of Mathematics, College of Science, AL-Mustansiriyah University, Baghdad, Iraq.	Minimax and Semi-Minimax Estimators for the Parameter of the Inverted Exponential Distribution under Quadratic and Precautionary Loss Functions	31
Mustafa Shakir Hashim and Reem Saadi Khaleel Physics Department, Education College, Al-Mustansiriya University, Baghdad, Iraq.	Studying some sensing properties of ZnOethanol sensor prepared by two methods	45
Kareema Abed Al-Kadim and Mohannad Mohammad Fadhil Department of Mathematics, College of Education of pure Sciences, University of Babylon, Hilla, Iraq.	Bivariate Generalized Double Weighted Exponential Distribution	53
Muhammed Mizher Radhi Radiological Techniques Department, Health and Medical Technology College – Baghdad, Middle Technical University (MTU), Iraq.	A study of electrochemical behavior for redox peaks of Pb(II) ions in human blood samples using Nanosensor	63
A.A. Omran Department of Mathematics, College of Education for Pure Science, Babylon University, Babylon, Iraq	Inverse Co-Independent Domination of Graphs	75
Hassan MahmoodMousa Abo Almaali College of Pharmacy,Kerbala University, Iraq.	A Study of P 53 codon (72) polymorphism distribution and related risk factors in Kerbala population by PCR	81



A Study of P 53 codon (72) polymorphism distribution and related risk factors in Kerbala population by PCR

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الخلاصة

ان بروتين الورم (53) هو عضو في عائله من البروتينات المسؤولة عن مجموعه من الفعاليات الحيوية مثل منع السرطان والسيطرة على دوره الخلية و الموت المرمج للخلايا والاستجابة الى للإجهاد واصلاح جزيئة DNAوالتعبير الجيني. من اجل هذه الاهمية فأن بروتين الورم P53 حضى بدراسة مستفيضه من خلال العديد من الباحثين حول العالم. اظهرت تلك الدراسات بأن بروتينالورم (53) يمتلك العديد من الشفرات الوراثية codons التي يمكن ان تكون عرضه للطفرة الوراثية التي يمكن ان تقود لظهور العديد الاورام في اجهزه جسم الانسان.ان احد اهم الاماكن المعرضة للطفرة المسؤولة عن ظهور السرطان هو الكودون (72) لأنه يقع على المحور (4) من بروتين الورم P53وهو مكان مرتبط بشكل كبير بالطفرات المؤدية لظهور الاورام. ان الكودون (72) يمتلك ثلاثة اليلات مختلفة وهي ارجنين/ ارجنين/ برولين و برولين/ برولين وهذه الاشكال الثلاثة ناتجه عن استبدال الارجنين بالبرولين في المنطقة الغنية بالبرولين من بروتين الورم (53). في هذه الدراسة نحاول استقصاء توزيع الاشكال الثلاثة للكودون (72) بين المدخنين من سكان مدينه كربلاء المقدسة ومحاوله معرفه تأثير العديد من العوامل والامراض التي تملك بعض الاسس الجينية (مثل مرض السكر وارتفاع ضغط الدم) وعوامل اخرى مثل العمر ومكان الاقامة والتحصيل الدراسي والجنس على انتشار تلك الاشكال. اظهرت نتائج هذا البحث ان هنالك اليلين فقط من اليلات كودون (72) الثلاثه وهما ارجنين / ارجنين وارجنين/ برولين في الحالات التي درسناها. كها اظهرت نتائج البحث ايضا عدم وجود علاقة وثيقه بين اي شكل من اشكال الكودون (72) و العوامل التي درسناها. وكانت قيمة معامل الارتباط (R) بين اليلات كودون (72) وبعض العوامل المدروسة وهي كالاتي قيمه معامل الارتباط بين التدخين وتعدد الاشكال تساوي (0.07141)، وقيمه معامل الارتباط بين معدل التدخين وتعدد الاشكال تساوى (0.0549-)، وقيمه معامل الارتباط التحصيل الدراسي وتعدد الاشكال تساوى (0.10955)، و قيمه معامل الارتباط بين العمر و تعدد الاشكال تساوي- (0.0636)، و قيمه معامل الارتباط بين الجنس و تعدد الاشكال تساوى (0.1).

الكليات المفتاحية

بروتين الورم (35)، تعدد اليلات الكودون (27) والطفرات.



Abstract

Tumor protein (53) P53 is a member of family of proteins responsible for many processes such as preventing cancer by controlling cell cycle, programmed cell death (apoptosis), stress response, DNA repair and gene expression. It's found that p53 have many codons that may be subjected to mutation by many factors and these mutation may lead to cancers in many systems of human body. One of most important sites of mutation responsible for cancer development is codon (72) because it located on exon (4). Codon (72) has three polymorphisms which are R/R, R/P and P/P according to arginine substitution with proline in the proline's rich area. The present study aimsto study the distribution of codon (72) polymorphisms in Kerbala population and impact of many risk factors such as smoking, diseases with genetic extension such as diabetes mellitus and hypertension in addition to another factor such as age, residency, academic achievement and gender on polymorphism of codon (72) of p53. Our study showed there is only two types of codon (72) polymorphisms has been found in our cases, which are R/R and R/P, P/P alleles not found in studied population. The results also revealed that no strong association between one type on codon (72) polymorphisms and the studied risk factors, such as smoking, diseases with genetic causation (such as diabetes mellitus and hypertension) and other factor such as age, residency, academic achievement that we are study. The correlation coefficients (r) were as the following; the smoking status/ polymorphism (r=0.07141);education /polymorphism (r=0.10955); age /polymorphism correlation (r=-0.0636); and gender /polymorphism (r=0.1).

Keywords

P53, codon (72) polymorphisms and mutations.



1. Introduction

The relationship between smoking and cancer firstly noted by the German physician Fritz Linkint in (1929) he found linke between lung cancer and smoking [1]. This relationship had been studied from many sides to discover a possible mechanisms by which smoking may cause cancer and these studies demonstrated two theories. The first theory is the poisons found in cigarette smoke may weaken body immune system, making it hard to kill spontaneously generated cancer cell. When this happen cancer cell keep uncontrolled growing without being stopped[2].

Thesecond theory, is the poison in tobacco smoke may damage or change DNA of cell. More specifically tobacco smoke is the main cause of P53 gene (tumor protein 53) mutation, which is an important gene responsible of preventing cancer cell evolving through cell cycle arrest [3].

According to the second theory the relationship between smoking and cancer has been developed to include several parts of body in addition to lung such as the tongue, mouth, throat, nose, nasal sinus, voice box, esophagus pancreas, stomach, liver, kidney, bladder, ureter, bowel, cervix, ovary, and bone marrow[4].

The aim of current study is to find the distribution of particular mutation in P53 gene in smokers in comparison with non-smokers population of Kerbala city, using molecular techniques. Furthermore, the present study aims to find out how these mutations are af-

fected by many factors such as smoking status, gender, some disease, nationality and many other factors.

2. Materials and Methods

One hundred and thirtythree healthy peoples, collected from Al-Husain medical city and from the College of Pharmacy -KerbalaUniversity. Bloodsamples have been collected from both smokers and non-smokers. The Kerbala city divided to three parts including city center, Jazeera and Hydraya in addition to Alhusynea and outside Kerbala.A full questionnaires were obtained from each participants, the questionnaires contains questions aboutage, sex, present smoking, formerly smoking, residency, diseases, nationality and academic achievement. Whole bloods DNA were extracted using automated method described by Bioneer Company using Exiprogen kit.

Polymerase Chain Reaction of P53 Codon (72)Polymorphism done to detect the presence of codon (72)polymorphisms in the blood samples. Amplification reaction mixture and agarose gel electrophoresis done as demonstrated by Nishino *et al.*, (2014)[5]. Agarose gel was prepared by dissolving (1.5) g of agarose powder in (100) ml of (1x) TBE buffer (pH 8) in boiling water bath.

The statistical analysis of the obtained data was analyzed with Excel software (2010) to calculat the correlation of studied factors with p53 genotypes.



3. Results

The results of current investigation concerning association between p53 codon (72) allelic polymorphism and risk factors are explained in the following Tables. These Tables share same result for homozygous P/P allele which was zero in all cases of the present study.

Table (1): Distribution of P53 codon (72)allelic polymorphism according to smoking status

smoking status	RR n(%)	RP n(%)	PP n(%)	Total
Smokers	47 (41.96)	7(33.3)	0	54
Non smokers	53(47.3)	12(57.1)	0	65
Former smokers	12(10.7)	2(9.5)	0	14
Total	112 (100)	21 (100)	0(100)	133 (100)

Table(1) shows that smoking status has no significant association with codon (72)polymorphisms, so that R/R alleles was (41.9%) and (10.7%) in smoker and former smoker re-

spectively while in non-smoker the result was (47.3%). The R/P alleles results was (33.3%) and (9.5%) in smoker and former smoker respectively and the result in non-smoker was (57.1%).

Table (2): Distribution of P53 codon (72)allelic polymorphism according to gender

Gender	RR n(%)	RP n(%)	PP n(%)	Total
Male	70(62.5)	9(42.8)	0	79
Female	42(37.5)	12(57.1)	0	54
Total	112 (100)	21 (100)	0(100)	(100) 133

that results of R/R alleles (62.5%) and (37.5%) males and females respectively.

Table(2)presented the association between for males and females respectively and the recodon (72) alleles and gender which explained sults of R/P alleles (42.8%) and (57.1%) for

Table (3): Distribution of P53 codon (72)allelic polymorphism according to job

Job	(%)RR n	(%)RP n	(%)PP n	Total
Master degree	(5.3)6	(9.5)2	0	8
Housewife	(26.7)30	(33.3)7	0	37
Student	(5.3)6	(19)4	0	10
Helpless	(1.7)2	(0)0	0	2
Unemployed	(17.8)20	(9.5)2	0	22
Worker	(3.5)4	(0)0	0	4
Wage earner	(16.9)19	(14.2)3	0	22



Office job	(22.3)25	(14.2)3	0	28
Total	(100) 112	(100) 21	(100)0	(100) 133

To show the effect of job on codon (72)alleles, Table(3) give a clear picture about this effect. The R/R alleles results was (5.3%), (26.7%), (5.3%), (1.7%), (17.8%), (3.5%), (16.9%) and (22.3%) for mas-

ter, housewife, student, helpless, unemployed, worker, wage earner and office job respectively while the results of R/P alleles were (9.5 %),(33.3%),(19%),(0%),(9.5%),(14.2%) and (14.2%) by same arrangement above.

Table (4): Distribution of P53 codon (72)allelic polymorphism according to residency

Residency	(%)RR n	(%)RP n	(%)PP n	Total
City center	(14)16	(14)3	0	19
Jazeera	(34)38	(38)8	0	46
Hydarya	(30)34	(24)5	0	39
Alhusynea	(17)19	(19)4	0	23
Outside kerbala	(4)5	(5)1	0	6
Total	(100) 112	(100) 21	(100)0	(100) 133

The geographic distribution impact on codon (72)polymorphisms was explained by Table(4) and where Kerbala city divided to three parts city center, Jazeera and Hydarya in addition to Alhusy-

nea and outside Kerbala. The R/R alleles results were (14%), (34%), (30%), (17%) and (4%) respectively while the results of R/P alleles (14%), (38%), (24%), (19%) and (5%) respectively.

Table(5): Distribution of P53 codon (72)allelic polymorphism according to disease status

Disease status	(%)RR n	(%)RP n	(%)PP n	Total
No disease	(59.8)67	(80.9)17	0	84
Diabetes mellitus	(6.25)7	(0)0	0	7
Hypertension	(24.1)27	(14.2)3	0	30
Diabetes mellitus and hypertension	(9.8)11	(4.7)1	0	12
Total	(100)112	(100)21	(100)0	(100)133

Some chronic diseases association with codon(72)polymorphism in comparison with healthy status was explained by this Table and Fig. and the results of R/R alleles were (59.8%) for healthy person and (6.25%), (24.1) and (9.8%)

for diabetes mellitus, hypertension and for diabetes mellitus and hypertension respectively while the results for R/P alleles were (80.9%) for healthy person and (0%),(14.2%) and (4.7%) respectively according to above arrangement.



Age	(%)RR n	(%)RP n	(%)PP n	Total
≤ years 15	(4.4%)5	(4.7%)1	0	6
years 16-30	(34.8%)39	(28.5%)6	0	45
years 31-50	(36.6%)41	(52.3%)11	0	52
years 51-80	(24%)27	(14.2%)3	0	30
Total	112	21	(100)0	133

Table(6): Distribution of P53 codon (72)allelic polymorphism according to age

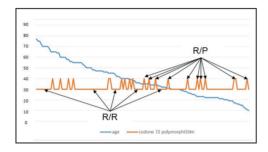


Fig. (1): Distribution of P53 codon (72)allelic polymorphism according to age.

The association between the age and codon

(72)polymorphism shown in Table (5) and Fig. (1), the population classified into four groups of age and the results were as the following the R/R alleles (4.4%), (34.6%), (38.8%) and (24%) for (15) years, (16-30) years, (31-50) years and (50-80) respectively while the results for R/P alleles was as following (4.7%), (28.5%), (52.3%) and (14.2%) for (15) years, (16-30) years, (31-50) years and (50-80) respectively.

Table(7):distribution of P53 allelic polymorphism according to tobacco consumption

Tobacco consumption	RR n(%)	RP n(%)	PP n(%)	Total
Non smokers	66(58.9%)	11(52%)	0	77
Heavy smokers	19(16.9%)	4(19%)	0	23
Very heavy smokers	27(24%)	6(28%)	0(100)	33
Total	112	21		133

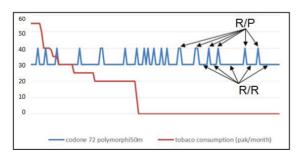


Fig. (2): Distribution of P53 codon (72)allelic polymorphism according to tobacco consumption

By the Table (2) and Fig. (2) the relationship between the tobacco consumption and codon (72)alleles resulted with R/R alleles were as the following (58.9%), (16.9%) and

(24%) for non-smokers, heavy smoker and very heavy smokers respectively,the results for R/P alleles were (52%),(19%) and (28%) for similar arrangement above.

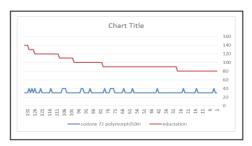


Fig.(3): Distribution of P53 codon (72)allelic polymorphism according to education



Table(8): Correlation coefficient of several studies risk factors with allelic polymorphism of p53 codon (72)

Risk factors	Correlation coefficient
Tobacco consumption	-0.0549
Education	0.10955
Age	-0.0636
Smoking status	0.07141
Gender	0.14585

4. Discussion

The p53 protein has many fields to show its roles in apoptosis, cell cycle regulation, stress response, DNA repair and gene expression [6]. A three polymorphisms of codon (72)have been found around the world which are Arginine/Arginine, Arginine/Proline and Proline/proline the later one have small percentage when compared with the two former polymorphisms. This polymorphism resulted from the substitution of amino acid arginine to proline in the proline rich area of p53 [7].

In addition, Iranian researcher tried to find out the association between stomach cancer and codon (72)polymorphism and they showed that the distribution of P/P polymorphism is less than of distribution of R/R and R/P polymorphism their results was (10%) for P/P, (36%) for R/P and (54%) for R/R[8].

Furthermore, the fact of low distribution of Pro/Pro polymorphism also demonstrated by Japanese study tried to bond pancreatic cancer with codon (72)polymorphisms, where the results showed only (3%) of cases studies is P/P alleles in (446) cases (7). The differences of

distribution in polymorphism may be caused by ethnic extension [9].

However, the current investigation demonstrated that the percentage of P/P alleles is zero the reason behind this result may be the number of cases under the study is relatively small (133 cases) when compared with other studies around the world. Other researchers demonstrated results differ from current study, where the percentage of P/P alleles high such as [10], the result was (41%) from (142) cases. In addition, the results of current investigation showed weak association between codon (72) polymorphisms and studied variables such as age, gender, smoking, tobacco consumption, residency and diseases.

In addition, the result of correlation between smoking and non-smoking with codon (72)polymorphism were explained by Table (1), from this Table it is obvious that the distribution of codon (72)polymorphism between smokers and non-smoker is random and there is no strong association between one type of polymorphisms and smoking status these results have been confirmed by other studies around the world such as [7], the results refer to randomly distribution of codon (72)polymorphism between control cases, a similar results have been noted by Indian researches [11].

In other hand, the association between codon (72)polymorphisms and gender showed by Table (2), two types of polymorphism are distributed randomly between males and females these results have been confirmed by



many studies around world such as [12]. In Table (3) the impact of life style throughout job on codon (72)polymorphisms were explained, no one type of job have a strong relationship with one type of polymorphisms, this relationship have not been studies by other researchers so there is no result can be compared with current study. By investigation the residency of cases to find out the relationship between social life impact on distribution of codon (72)polymorphisms so the Kerbala city divided to three parts including city center, Jazeera and Hydraya in addition to Alhusynea and outside Kerbala. And afew number of cases from outside Kerbala and most of the cases was from city so this reflect on results in Table (4) which shows the association between residency and codon (72)polymorphism, many studies tried to find out the association between geographic distribution and codon (72)polymorphisms such as [13] the geographic distribution linked to some type of codon (72)polymorphism attributed to ethnic differences. These results confirmed the result of current study that lead to absence of P/P polymorphism because it done on small scale included only the people they lives at Kerbala city where, most of the samples taken belong to same ethnic groups.

The results in Table (5) which shows the association between some chronic disease such as diabetes mellitus, hypertension and distribution of codon (72) polymorphisms in comparison with healthy individual, because most cases studied by current study was healthy in-

dividual so that the results referred to that there is no strong association between codon (72) polymorphism and disease status, the results from other studies such as relationship of codon (72)polymorphisms with change in blood pressure by Reilinget al., (2012) [14], shows that P/P polymorphism have a good relationship with high blood pressure while there is no significant correlation between blood pressure and R/R or R/P polymorphisms. The correlation between diabetes mellitus and codon (72)polymorphism have been studies by many researchers [15], when they tried to find out a role of p53 codon (72)polymorphism in the susceptibility to type (2) diabetes in overweight subjects. A study in patients with cardiovascular diseases the referred to good correlation between diabetes with R/R polymorphism while no significant correlation with P/P or R/Rpolymorphism while there is different results similar to current study have done by (16), when they studies the correlation between coronary artery disease and diabetes with codon (72)polymorphisms these study shows no significant correlation between any polymorphism and diseases.

In addition, Fig. (3) shows the relationship between the codon (72)polymorphisms and education because most cases of current investigation have low level of education so the results showed deviation of two type of polymorphism toward this side of chart and no one of education level have significant correlation with any one of polymorphisms. And this variable has not been investigat-



ed by other researchers yet to compare with Kerbalapopulation>s results.

In other hand the association between the codon (72)polymorphisms and age explained by Table (6), the results shows randomly distribution of polymorphism and no one of polymorphism have strong correlation specific age group and such results have been confirmed by other researchers around the world [12,15].

Table (7) shows the relationship between the codon (72)polymorphism and tobacco consumption and the results shows a random distribution codon (72)polymorphism between nonsmoker, heavy smoker and very heavy smokers these results have been confirmed by other studies around the world such as [7, 11].

In Conclusion, according to current study results it's concluded that P53 codon (72) polymorphisms distributed randomly according to studied risk factors. And the P53 proline/prolineallele's ratio in Kerabla population is low as several other parts of worlds. It is not likely that P53 codon (72) polymorphisms participate directly in studies diseases including (diabetes mellitus and hypertension). It's recommended to study the association of other polymorphisms in P53 with current risk factors and diseases.

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