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The relation of insulin like growth factor gene (IGF-1) with some physiological characteristics of common CARP (*Cyprinus carpio*)

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Abstract
The present study was conducted to identify genotypes of insulin- like growth factor (IGF-I) and its relation with some of the physiological characteristics (glucose, AST & ALT) of common carp *Cyprinus carpio* in Al-Radhwaniyah Fish reservoir (Baghdad). It has been identified genotypes of insulin like growth factor by using restriction fragment length polymorphism (RFLP) in specified mutation in the site 3759T > G. Results of physiological characteristics showed that the blood glucose rate was affected significantly (p<0.05) by different genotypes as which glucose concentration in TT genotype was 87.87 mg/100, whereas about 67.21mg/100 in TG genotype. Regarding values of transaminase enzymes (AST & ALT) they did not affect significantly by any of genotypes. ALT Value in TT genotypes was 52.66 IU with 46.33 IU in TG genotypes, as for AST, the results were 87.66, 88.33 IU in TG, TT genotypes respectively. The present study conclude that the possibility of credibility upon polymorphism in insulin like growth factor as assign for physiological performance prediction of fish body and select them to be parents for coming generation.

Keywords: Insulin like growth factor gene, physiological characteristics, common carp

1. Introduction
Molecular genetics has played important role in breeding of the aquatic organisms through application of genetically modified technology to change fish gene in order to promote important production characteristics commercially such as growth rate and disease resistance khan *et al* [1]. Genetic technology has used growth hormones by using genetic modification because production costs in aquaculture projects depended upon time, which will reduce these costs, obtaining healthy animals, reduce the exposing to dangerous diseases and predators, whereas the traditional methods of selection were responsible for great improvement in growth rate, but with slow progress relatively [2].

Insulin like growth factor (IGF-I) is known under the name Somatomedin C which is Peptide multi-hormone plays focal role in growth and development of vertebrates [3]. IGF-I consisted of 70 essential peptide amino acids, and contains three internal chains of disulfate [4]. Insulin like growth factor (IGF-I) is mainly manufactured in liver by stimulation of growth hormone (GH), it flows to blood stream and reach all targeted tissues in order to perform its function effectively, by manufacturing of growth hormone - releasing hormone (GHRH) from hypothalamus, then flow to the pituitary gland by receptors located on the surface secreting growth hormone which directed towards the receptors in all parts of the body such as hepatic glands which enriched with GH receptors, then when it is reaching liver it will start to manufacture IGF-I [5]. But there is general agreement that the concentration of IGF-I in serum effects on the releasing of GH from anterior pituitary gland by feedback mechanism for definite inhibition of genetic transcription and secreting of growth hormone (GH) [6].

Insulin like growth factor (IGF-I) played an important role in cells reproduction, differentiation and functional formation of fish immunity system [7]. Most studies of last decade on fish focused on revealing identity of IGF in different species of fish, improving the blood properties or Peptide tissue levels (mRNA or IGF-I), variables measurement of IGF-I in blood and IGF-I tissues, its response to different nutrition conditions, season and control evaluation upon production of IGF-I by other endocrine gland such as thyroid [8] or estrogen [9]. Furthermore, IGF-I in fish did not only related with growth, but also with metabolic process [10], development [11], reproduction [12] and osmoregulation in marine fish [13].

The objective of the present study was to shed light the several axes: determination genetic morphology, polymorphism in the insulin-like growth hormone gene in a common carp fish sample and the allele frequency calculation with a number of physiological traits that have a direct impact on fish health and growth.

2. Materials and Methods

2.1 study area details

The present study was performed in Al-Radhwaniyah fish reservoir in Baghdad, For the period from 15/10/2016 until 5/2/2017 as 95 samples of *C. carpio* were collected and reared in concrete basins for 110 days with 7*3* 1.2 meter. Fish were fed on commercial diet containing %26.8 proteins, %1.5 Fats and energy of 3165 Kilo calorie. Fish were numbered the number was fixed near to pectoral fin for each fish.

2.2 Extraction of DNA and Polymerase Chain Reaction

One ml of blood was collected from the heart muscle of all trial fish. These samples were collected in EDTA tubes and kept in freezer (-18 ºC). for DNA extraction by using DNA extraction kit (Geneaid,korea). They adopted method of Sambrook *etal*. [14] for Electrophoreses, it specimen transferred by electricity power of 70 Volt and current of 40mA for one hour. Results were filmed by photo documentation system.

Primer was selected for molecular detection and identifying polymorphism for genes and mutation in IGF-I gene according to Feng *et al*. [15]. Primer was provided by Korean pioneer company and its sequences showed in Table 1. Primer works according to specific conditions as in the following:

Initial denaturation at 95° °C, one cycle for 4 minutes, Denaturation at 94° °C, 35 cycle for 30 seconds for single cycle, Annealing at 56° °C, 35 cycle for 30 second for single cycle, Extension at 72° °C,35 cycle for one minutes, Final Extension at 72° °C, single cycle for 5 minutes.

Table 1: Sequence of the primer used and the area covered by the insulin-like factor growth hormone gene

Gene	Sequence region	
IGF-I	Second Intron	F:5 GCACAATGGCTCAAGGAAGT 3
		R:5 GTTTGTATCTGGGGAATGGG 3

2.3 Blood Analysis

One ml from fish blood were collected by drawing the blood from heart muscle, and then put in tube of 5 ml capacity with EDTA. Plasma was centrifuged (3000R/M) for fifteen minutes, and then put in sterile tubes of biochemical analysis and plasma analysis which included Glucose, Aspartate Transaminase (AST) and Alanine Transaminase (ALT).The tests were conducted based on the Indian agappe company [16].

2.4 Statistical analysis

Statistical Analysis System was used in data analysis SAS [17] according to complete randomized design (CRD) using the general linear model (GLM) and Duncan multiple range test [18] was used to compare the means between genotypes at a significant level (p<0.005).

3. Results and Discussion

3.1 Polymerase chain reaction – PCR

Fig. 1: refers to the extracted fragment from IGF-I gene, results of electrophoresis showed the required fragment which amounted 1061 bp after amplifying by Polymerase Chain Reaction (PCR), a sample of 5 microliters was relayed migrate loading pigment during 1 hour, voltage 70, 40 m. Amber, then the result was filmed by photographic documentation devise with the existence of DNA fragments of specified size marker to ensure the extraction process success, and these results are agree with the primer designed according to Feng *et al* [15] in terms of the required bundle appearance which amounted 1061 bp in his study on common carp.

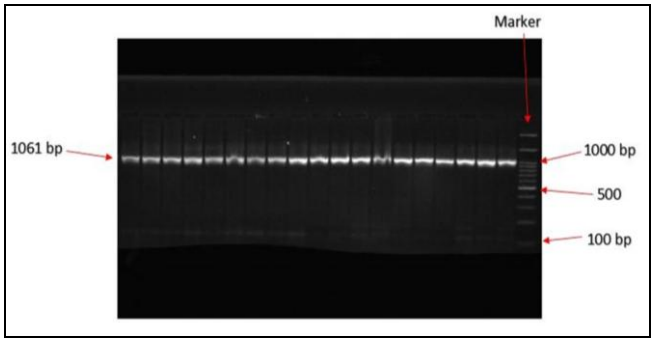

Fig 1: Extracted fragment from insulin like growth factor of hormone gene

3.2 Genotypes in insulin like hormone gene using RFLP Technique

Genotypes in insulin like hormone gene in carp were identified by Restriction Fragment Length Polymorphism(RFLP) with the existence of the Restriction enzyme BstEll (Eco9ll) and the product was migrated by agarose gel 1.5% for one hour and half and adjust the voltage 70 volt, 40 m. Am with marker (100 – 3000 bp) as in Fig. 2.

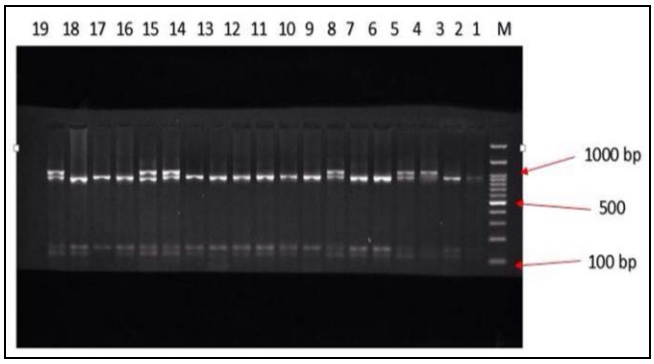

Fig 2: Bundles formed after the BstEII enzyme restriction process to determine the genotypes of IGF-I gene.

Restriction was performed with BstEll, enzyme, where identifying the sensitive location within a specific sequence of cutting location, cutting is done in 3759 T>G location of the second Intron (accession no. AF465830,1) where indicates of two bundles the first was 913 bp and the second 148 bp in size followed the wild genotype (TT), where cutting was performed in both Alleles (no mutation in both Alleles), as for existence of three bundles (19061,913,148 bp) implies the Heterozygous genotype (TG), existence of mutation in one Allele (changing the nitrogenaes base T to G in one Allele) and there existed one bundles of 1061 bp in size, that implies symmetric homozygous genotype (GG) containing the mutation in both Alleles.

3.3 Distribution of IGF-I gene genotypes in common carp

Table 2 demonstrated numbers and percentages of fish genotypes distribution, where they were amounted 69.47% for fish carrying TT genotype, 29.47% for fish carrying TG genotype and 1.06% for fish carrying GG genotype with significant difference between genotypes.

Table 2: Numbers and percentages of genotypes and the allele frequency of IGF-I gene in common carp.

Percentage (%)	the number	Genotype
69.47	66	TT
29.47	28	TG
1.06	1	GG
100%	95	Total
**10.735	---	(χ^2) Chai square value

Fig. 3 indicates the values of glucose, AST and ALT, where noticed significant increase in (P<0.05) blood glucose concentration and recorded 87.87mg/100 ml in TT genotype with 67.21 mg⁄100 ml in TG genotype, where ALT Value in TT genotype was 52.66 IU with 46.33 IU in TG genotype, as for AST, the results were 87.66, 88.33 IU in TG, TT genotypes respectively. It has observed absence of GG genotype, where there is only one fish within genotype, so this sample was neglected from the statistical analysis.

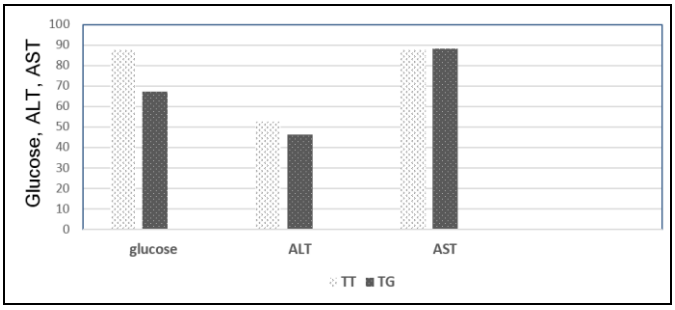

Fig 3: Values of glucose (mg / 100 ml), AST and ALT (IU) in the blood of common carp fish.

Biochemical blood characteristics are considered as important tool could be used as an effective and sensitive indicator to monitor the physiological and pathological changes in fish [19]. It has proved that the analysis health status for the cultivated fish provide reliable data of metabolism disorder and the chronic stress before they being existed in the clinical symptoms.

Insulin mainly contributes in metabolism and regulation the glucose and fat in mammals also control growth and differentiation in fatty tissues [20], whereas the effect of insulin and IGF-I on fatty tissues is not well known in fish, but the relative significance of its effects are on fish muscles and they are seemed to be different from mammals [21]. Dagger *et al* [22] stated in their study which conducted on *lates calcarifer* that IGF-I and the insulin stimulate amino acids absorption in muscles tissues where is more effective than insulin hormone to motivate glucose absorption in fish muscles.

The mechanism by which the insulin works and IGF-I to transfer glucose are completely unclear in fish. Many studies illustrated insulin work and IGF-I in fish for Glucose and Amino acids, where it has described IGF-I and the insulin for the first time on deoxyglucose-2 absorption in salmon and IGF-I was more effective to stimulate Glucose absorption comp owed to insulin, that indicates that IGF-I plays an essential role in metabolism in fatty cells [23]. Liver enzymes (AST and ALT) are considered the most important dominated enzymes for amino acids formulation and are affected largely by IGF-I secretion from liver [24]. It has observed decreasing of enzyme protein metabolism effectiveness (AST and ALT) when the available amino acids are negligible [25]. Also IGF-I has effects on decreasing the osmotic pressure and sodium levels, modifying liver glucose and fat metabolism [26], besides IGF-I regulates most body activities such as cellular growth and differentiation [27], and transcription of DNA and protein production [28], carbohydrate and fat metabolism [29]. Glucose level in blood could be decreased with increasing of water temperature and could differ according to season temperature, and glucose level in fish blood affects by age and size [30].The great increasing in blood glucose concentration in fish carrying genotype TT related to the essential role which the hormone plays to regulate blood glucose, the mutation occurs in 3759 T<G effected largely on IGF-I expression in liver and in locations specified to control blood Glucose that reflects negatively on fish carrying the mutation, age, environmental conditions and fish activity has great effect on glucose concentration in blood. This is agreeing with results presented by Marta *et al* [31] that IGF-I and IGF-II stimulate glucose absorption in salmon *Oncorhynchs mykiss.*

4. Conclusion

The study results revealed polymorphism features in the insulin-like growth factor gene in common carp fish using the BstE11, enzyme, in the SNP identified at site T <G 3759. The values of glucose were significantly affected (P<0.05). TT showed the highest values compared to the hybrid structure. AST and ALT concentrations were not affected by different genotypes.

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