Study of polymorphism in the BDNF gene and its relationship to growth hormone, sodium and potassium levels in children with autism spectrum disorder in Anbar Governorate

1.Haneen Fadhil Jasim

2.Dr. Samir Mishrif Khalaf

3.Hussein Ali Jasim

1- Graduate student in the College of Education for Pure Sciences

2- Lecturer in the College of Education for Pure Sciences

3- Doctor of Pediatrician in Anbar Health Department

Abstract:

Autism spectrum disorder is a neurodevelopmental disorder that is usually diagnosed between (2 - 6) years of age and is characterized by poor social communication, repetitive behaviors, or stereotypes, and low interest in environmental stimuli. and sensory processing or restricted interests.

The current study was conducted for the purpose of studying the relationship of growth hormone and some physiological factors to polymorphism in the Brain Derived Nuetrophic Factor (BDNF) gene in a sample of 40 children with Autism spectrum Disorder (ASD) as well as a control sample of 20 children without autism spectrum disorder for the period from 1/10/2021 to 1/12/2021.

The current study showed an insignificant decrease in p≤0.05 in the concentration of potassium ion in the blood serum of the study sample individuals, which amounted to (4.42 (±0.43) mg/dl) compared to its percentage in the blood serum of the members of the control sample, where it amounted to (5.09(±1.57) mg/dl.The study also indicated that there was no correlation between the concentration of growth hormone and the concentration of potassium ion between the blood serum of the study sample members as the value of the correlation coefficient (-0.12955). The study also indicated a significant difference ≤0.05 in the concentration of sodium ion, as its concentration in the blood serum of the study sample members (123.67 (± 9.57) mmol/l compared to its concentration in the serum of the control sample members was 135.05 (± 9.43) mmol/l). It was also found that there was a significant correlation of p≤0.05 between growth hormone and the concentration of serum sodium ion in the study samples, where the value of the correlation coefficient was (0.273364). The results of the current study also show the occurrence of multiple forms or locations of nitrogen bases (SNPs), as the site rs1048221 showed the occurrence of multiplicity in the base site (A(70bp)) in which the number of beams appeared 20 (50%) in the members of the study sample compared to 18 (90%) in the individuals of the control sample, while the study showed the occurrence of multiplicity in the form of nucleotide above as it showed two additional forms, C(102) and CA (70+102).) and the number of impressions and frequency reached 4 (10%) and 11 (36.36%) respectively compared to the number of times they appeared in the members of the control sample, which amounted to 0(0%) and 2(10%), and the occurrence of multiple in the forms or locations of nitrogen bases (SNPs), where the site rs1189205506 showed the occurrence of multiple in the base site (C(100bp)) in which the number of beams appeared 28 (70%) in the individuals of the study sample compared to 0 (0%) in the control sample, while the study showed the occurrence of multiple in the The form of nucleotides above showed two additional forms, G(87bp) and CG(100+87bp) and with the number of impressions and frequency of 4(10%) and 8(20%) respectively compared to the number of impressions in the members of the control sample, which amounted to 19 (95%) and 1(5%), as well as the occurrence of multiple forms or locations of nitrogen bases (SNPs), where the site rs1856943620 showed the occurrence of multiple in the base site (G(82bp). The number of beams appeared in it reached 11 (27.5%) in the members of the

study sample compared to 18 (90%) in the control sample individuals, while the study showed the occurrence of multiple in the form of nucleotides above as it showed two additional forms, A(151bp) and GA(82+151bp) and the number of impressions and frequency of 6 (15%) and 21 (52.5%) respectively compared to the number of times they appeared in the control sample members, which amounted to 0(0%) and 2(10%).

Keywords : Sodium, Potassium, *BDNF* Gene

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differentiation of new neurons and synapses (Abbott *et al* .,2020). It is active in the brain in the hippocampus, cortex and primary anterior brain – vital areas of learning, memory and high thinking Yamada K. & Nabeshima T. April 2003)). It is also expressed in the retina, kidneys, prostate, motor neurons and skeletal muscles, and is also found in the saliva of Mandel AL *et al* ., 2009). It considers itself important for long-term memory (Bekinschtein P. et al ., 2008). The BDNF protein is encoded by a gene also called *BDNF*, found in humans on

chromosome 11 (2022*et al* ., Sookaromdee). Structurally, *BDNF* copies are controlled by 8 different mechanisms, each of which leads to different versions containing one of 8 xons divided

into 5 of which are non-translatable 3 translatable, the fourth mechanism is stimulated by the t leading to the

translation of mRNA containing exon IV Strongly powered by calcium and primarily controlled by the regulatory

component of Cre, which indicates the supposed role of the CREB transcription factor and the source of dependent-dependent effects on BDNF activity andthere are multiple mechanisms through neuron activity that can increase the qualitative expression of BDNF exon

IV Kornblihtt et al .,(2013). The activity of sodium and potassium is reduced in a number of pathological disorders of the brain, including ischemia, injury, depression, mood disorders, mania, stress, neurological hyperexcitability and epilepsy (De Lores Arnaiz et al., 2014) as well as in autism spectrum disorders (Ghezzo *et al* ., 2013). NKA is a localized protein in the cell membrane, first described by Skou J. C. , 1957). Described as the main factor responsible for creating the sodium and potassium

Introduction

Autism was first described by Kanner *et al*., 1943. In a detailed report of 11 children with similar unusual tendencies

autism patients suffer from maladjustment in emotional response, anxiety, poor emotional learning, limited attention to the surrounding

environment, and deficits in communication and social interactions (Russo AJ. , 2013). Autism spectrum disorder pathology refers to many of the accompanying symptoms resulting from pathological disorders which often include: seizures, anxiety, mental impairment, hyperactivity, hyper-response to stimuli, sleep disturbance, and aggressive behavior (Pasciuto E. *et al*

., 2015). Autism spectrum disorder is also a complex disorder with genetic components (Castro K. *et al*., 2017),(Hill DS. *et al* ., 2015). Autism spectrum

disorders are a complex neurodevelopmental disease and are characterized by a combination of behavioral and cognitive disabilities and these include impaired or diminished social communication skills, repetitive behaviors, sensory processing or restricted interests Lord C. *et al* . , 2020)).

Brain-derived neurotrophic factor (BDNF), or abrinorin is a protein that is encoded in humans by the BDNF gene (Binder DK).

& Scharfman HE. 2004) isa member of the family of neurodevelopmental factors associated with neuronal factor N Mo NeurotransmitterJ and was first isolated from the pig brain in 1982 by Yves Allan Bardi and Hans Thuinen (Kowiański P. *et al* ., (2018 . *BDNF* acts on some neurons in the central nervous system

and peripheral nervous system, helping to support the survival of existing neurons, and encouraging the growth and

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and then incubate at a temperature of 60 ° C for 10 minutes in the water bath (the sample was stirred every three minutes) and then add to it 200 µl of GSB Buffer solution for each tube, Mixed using the Vortex device for 10 seconds and then incubated at a temperature of 70 ° C for 10 minutes (the sample was stirred every three minutes) During the incubation period the solution of Elution Buffer was placed in the water bath (60 ° C) to heat it and make it ready for use in subsequent steps..

3- 200 µl of absolute ethanol (at a concentration of 99%) was added to each tube with shaking quickly for ten seconds (in case of deposits that must be broken by means of a pipette).

4- The GS Columns were placed inside the ML Collection tube tube 2 pipes, moved the mixture of pipes to the GS Columns, and then centrally expelled at a speed of 14-16,000 r/min for two minutes.

5. The 2 ml Collection tube tubes were removed and the GS Columns were placed in new 2 ml Collection tubes.

6. Add 400 µl W1Buffer solution per GS Column, then centrally expelled at a speed of 14-16,000 r/min for 60-30 seconds.

7-The ML Collection tube tube 2 pipes were removed and the GS Columns were placed in new 2 ml Collection tube pipes, to which 600 µl of Wash Buffer (to which absolute ethanol is added at a concentration of 99%) were added and centrally expelled at a speed of 14-16,000 rpm for 60-30 seconds.

8- The precipitate was neglected and the centrifugal process was repeated at a speed of 14-16,000 cycles / minute and for three minutes.

9. The GS Column columns were placed in 1.5 ml microcentrifuge tube tubes, to which 100 µl of Elution Buffer solution was added (Elution Buffer is added to the center of the columns) and left for three minutes at room temperature to ensure the absorption of the solution, centrally expelled 14-16,000 r/min for thirty seconds.

10- GS Columns were removed and the 1.5 ml microcenterifuge tubes containing

gradient across the cell membrane for each ATP-driven transport cycle, two potassium ions are imported into the cell while three sodium ions are exported from them. The NKA activity allows molecules to pass through the cell membrane via indirect active transport, contributes to the creation of the membrane's voltage and is fundamental to the propagation of nerve impulses. Belong to NKA To P-type ATPases, a family of enzymes with more than 50 members and characterized by the presence of a common intermediate phosphorus (P) enzyme. NKA consists of α and β units, both of which are required for enzyme function, while the third sub-unit, referred to as FXYD , appears to be involved in regulating enzyme activity in a tissue-specific way. The α subunit is referred to as the catalytic unit, as it includes binding sites for ATP and for the blockage of ions, mutations in the subunits α involved in many neurological diseases often clausen *et al* .,(2017). The β subunit is in direct contact with the α subunit, facilitating the correct placement and harmonic stability of the sub-unit α within the plasma membrane. The subunit also works on the convergence and transfer of ions, the hydrolysis of ATP, and the binding of inhibitors to NKA as a whole (Scheiner-Bobis & G. 2002). The pattern of expression of different equal shapes depends on the subunits α and and FXYD on cell type, stage of evolution and specific signals (Arnaiz *et al* ., 2014).

Materials and Methods

Extracting genetic DNA from blood

Genomic DNA Extraction from blood

The DNA was extracted from the blood of the 40 children in the study sample as well as the 20 control sample members according to the extraction method approved by the American processing company Geneaid.

1- Transfer 200 µl of blood for all sample members by means of a fine pipette to tubes of 1.5 ml microcentrifuge tube, then add to it 20 µl of proteinase K solution and mix well with vibrator for 10 seconds

cm from one of the edges of the mold, pour the acarose gel quietly so as to avoid the formation of air bubbles, leave to harden in a horizontal position for 30 minutes.

3- After the acarus hardens, lift the comb and put the gel with the mold in the tank of the electric relay device and filled with TBE 1X diffuser so that it covers the surface of the gel

4. 3 µL of DNA Loading Dye was mixed with 5 µL of DNA extracted per sample and carefully carried into the inside of the drill.

5- In the case of electrical migration to detect DNA multiplied by PCR technology, carry the volumetric guide (DNA Ladder) 100bp to know the molecular weight of the resulting DNA pieces .

6. Then connect the power supply equipped with the relay basin by means of positive and negative wires and electrodes and then turn on the power supply and adjust at (7 v\c 2) for (1-2) hours after which the power supply is stopped and the electrical relay process is finished.

7. Then remove the gel from the electric relay basin and examine with the Gel Documentation device by ultraviolet light and photograph with the digital camera attached

DNA extracted by superfreezing were preserved until the rest of the molecular studies were completed on them.

Electrolyte on the gel of the Agarose gel
Agarose gel electrophoresis

The process of electrical relay of DNA in the acarose gel is one of the methods used in diagnosing and purifying the DNA and separating it, as this method is used to separate a mixture of pieces of DNA with different molecular weights, as well as its usefulness in calculating the location of the DNA in the gel directly and the samples of the extracted DNA and the product of PCR technology were detected using electrolyte on the acaros gel according to Sambrook. 1989) My Agencies:

1- Acarus gel has been prepared at a concentration of 1%. 0 by melting 1. 0 g of acarus in 100 milliliters of TBE buffer 1X solution (Tris base Boric acid EDTA) in order to detect the DNA that has been extracted, as it was dissolved by heating it to a temperature (45-50oC) where it was placed in a microwave for 2-1 minutes, leaving the solution to cool. Then a dye of ethidium bromide was added .

2- Install the tray mold of the electric relay device, and put the comb of the drilling composition (Wells) that is used to load DNA samples and at a distance of (1) to the device.

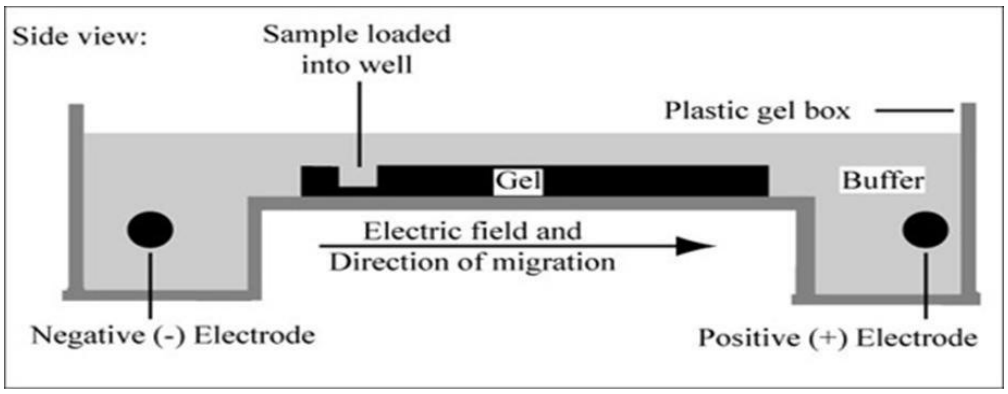

Figure 1-1 showing the basin of the relay device

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Estimation of molecular weights:

The molecular weights of the extracted DNA were estimated based on the distance traveled in the gel compared to the volumetric evidence of the known DNA molecular weight (Genomic marker 1Kbp) Figure 3-2

SiZer DNA Markers (intron)

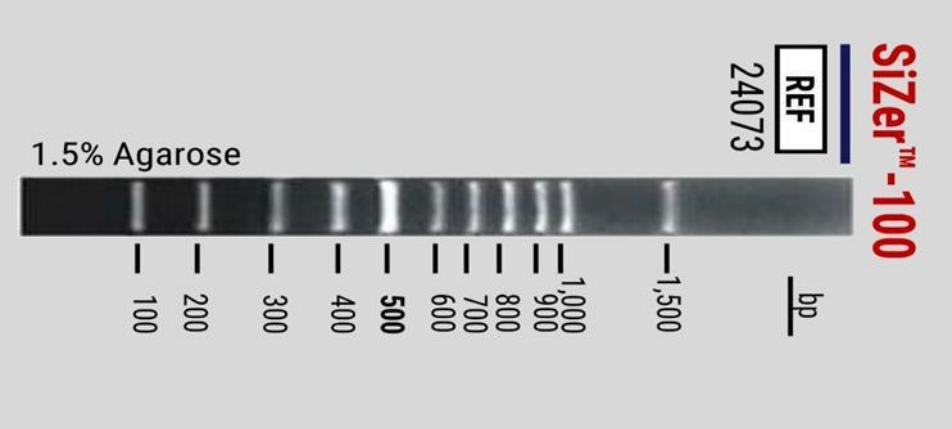

Figure 1-2 shows the volumetric guide used in the migration process.

The molecular weights of the pieces resulting from the PCR technique were estimated in comparison with a special volumetric guide to the PCR product (DNA Ladder 100bp), which in the accarus gel (11) gave a package with known molecular weights (Sambrook, 1989).

Reaction of PCR

The PCR reaction was carried out using the PCR-Premix kit equipped by the Korean company intron with a final size of 25µL. The components of the PCR reaction are illustrated in the following tables:

Table (1-1) showing the components of Maxime PCR PreMix kit (i-Taq) in thePCR reaction

Material	Volume
i-Taq DNA Polymerase	5U/µl
dNTPs	2.5mM
Reaction buffer (10X)	1X
Gel loading buffer	1X

Table (1-2) showing the components of thePCR reaction mix

Components	Concentration
Taq PCR PreMix	5µl
-Forward primer	10 picomols/µl (0.5 µl)
-Reverse primer	10 picomols/µl (0.5 µl)
-Forward primer	10 picomols/µl (0.5 µl)

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-Reverse primer	10 picomols/µl (0.5 µl)
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Components of the reaction of: PCR

1- Specific Primers

Two pairs of specialized prefixes were used for each polymorphism of the SNP nucleotide designed by the researcher according to the T-ARMS-PCR system thermally amplifying the mutation of the mutation hee tetra-primer amplification refractory mutation system–polymerase chain reaction as in Figure (5-3) where the prefixes were equipped in a lyophilized form by the American company Bioneer and dissolved in distilled water, and a final concentration of 10 picomol was prepared / µL.

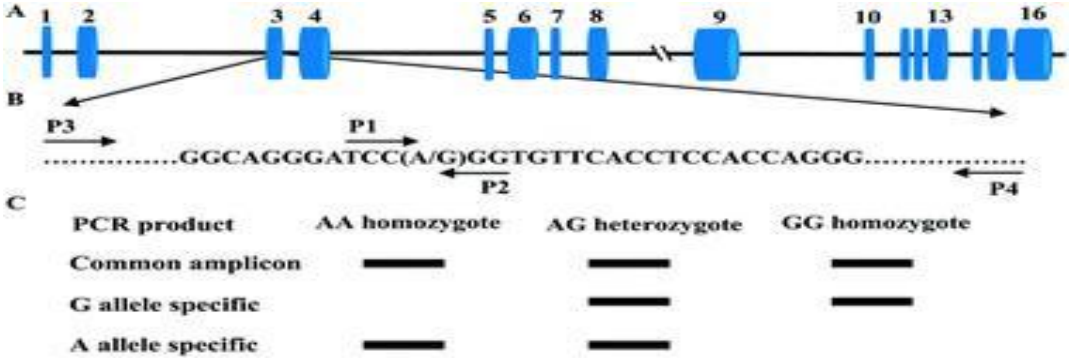

Figure 1-3 shows how prefixes work by T-ARMS(Li *et al.,* 2014)

Table 1-3 shows the primers used in this study with their sequences and expected size.

1)The specific primer ***HS-1*** of gene (***rs1048221***)

Primer	Sequence	Tm (°C)	Product size
HS1OF	5'- ACTAATACTGTCACACACGCTC - 3'	58.17	102bp
HS1OR	5'- GAATACAAAAATTACCTAGATG - 3'	48.47	
HS1I F	5'- GCGGGCAGGGTCAGAGTGGCGC - 3'	59.21	70bp
HS1I R	5'- AAACATGTCCATGAGGGTCCGA - 3'	57.02	

2)The specific primer ***HS-2*** of gene (***rs1189205506***)

Primer	Sequence	Tm (°C)	Product size
HS2OF	5'- AATTACATGTCTTGGGTTTGCT - 3'	56.50	100 bp
HS2OR	5'- GCAAGACAAGCCAACATTCAAA - 3'	58.54	
HS2I F	5'- CTTCTTGAGGGGTATCTTTGTG -3'	59.72	87bp
HS2I R	5'-TTCAGGAAATACAGAGAACACT-3'	54.60	

3)The specific primer ***HS-3*** of gene (***rs1856943620***)

Primer	Sequence	Tm (°C)	Product size
HS3OF	5'- ATTCAAGGGTAATTCTGATGCT - 3'	55.47	151bp
HS3OR	5'- AACAAACCTGCACGTTCTGCAC - 3'	62.64	
HS3I F	5'- CACTGCTGTATGCTGCATTCTA - 3'	59.28	82bp
HS3I R	5'- AATAAAAGAAATACAATAAAAC - 3'	57.01	

2 - Reaction Mix (PCR-Premix)

Use a combination of reaction components (PCR-Premix) consisting of:

-DNA polymerase (TAQ) polymerase enzyme

-Hypoxic triphosphate nitrogen bases (dNTPs)

-Regulated solution PCR Buffer

3 - DNA DNA Template

It represents the piece of target DNA to be multiplied using PCR technology.

The mixing process was added 0.5 µL to each tube from the specialized initiator (for both Forward and Reverse), after which 1.5 µL of the target DNA was added to each tube, and after completing the addition process, the tubes were inserted into the Thermo cycler device with caution and care to complete the reaction and using the following program Table (1-4) Prepared for this purpose.

Table (1-4) The program used in the polymerase chain reaction

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	93°C	3 min.	1 Cycle
2-	Denaturation -2	93°C	35sec	35 Cycle
3-	Annealing	59°C*	35sec	
4-	Extension-1	72°C	35sec	
5-	Extension -2	72°C	7 min.	1 Cycle

***Variable by initiator**

After the reaction time expired, PCR Micro tubes containing the double DNA samples were taken out of the machine, 5 microliters were withdrawn from them and carried on the acarose gel at a concentration of 1.5%, which were migrated by the electric relay device for 2-1 hours.

Sodium Test

Principle of action : Sodium and protein are deposited in the serum magnesium uranyl acetate after separation by centrifugation Excess uranyl ions in the floating substance interact with Thioglycolic acid to form a colored complex whose absorption varies inversely with the sodium concentration in the sample Maruna RFL. *et al* . ,1958)) .

Components of solutions:

1. R1 solution: (precipitate) consists of additive uranyl acetate (19 mmol / l) with magnesium acetate (140 mmol / l) in a liter of ethyl alcohol.

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2. R2 solution: (chromatic reagent) consists of ammonium thioglycolates (550 mmol / l) and ammonia (550 mmol / l).

3. CAL:(standard sodium) sodium (+Na) 150 (mmol/L) according to standard reference material 909 b.

How it works:

1. **Sedimentation:** Transfer to centrifugal tubes marked as indicated in the table below.

Pipe Name	Sample	Standard
Sample	**50 µl**	---
Calibration Solution (CAL)	---	**50 µl**
R1 solution	3 mL	3 mL

-Mix the pipes well and leave in portrait position for 5 minutes

- Shake intensively with the Vortex shaking device for 30 seconds and leave it in portrait position for 30 minutes.

- Expel with a centrifuge for 10 minutes at a speed of 8000 r/min.

- Separate the scented material into a separate tube for later use.

2- Spectral chromatography: - Transfer to centrifugal tubes marked according to the indicator in the table below.

Pipe Name	Blank	Sample	Standard
R1 solution	**50 µl**	---	---
Thin solution	---	**50 µl**	**50 µl**
R2 solution	**3 mL**	**3 mL**	**3 mL**

- Mix the pipes well and leave them in a vertical position for 5 minutes at room temperature.

-Read the absorption of the tagged pipes along the405 nm MOG:

3- Accounts:

$$\frac{A_{BK} - A_S}{A_{BK} - A_{STD}} \times C_{STANDARD} = \text{mmol/L (mEq/L)}$$

(ABK) represents the absorption of the empty tube, (AS) represents the absorption of the sample tube, (ASTD) represents the absorption of the standard calibration tube at a wavelength of 405 nm.

4. Reference Values:

Reference values (135-155 mmol/L) are adopted. Values exceeding 300 mmol/L should be diluted in a ratio of 1:1 with distilled water and then multiplied by 2.

Potassium test: Potassium test

Principle of action: Potassium is determined by spectrophotometry through the kinetic coupling inspection system using potassium-based pyruvate kinase. The pyruvate generated is converted into lactate associated with the conversion of NADH to NAD The

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corresponding decrease in light density at 380 nm is proportional to the concentration of potassium in the serum. (Wu *et al* .,2006).

Components of solutions:

1- R1 solution : LDH <50 KU / L with NADH <10 mmol / l then add sodium azide 0. 05% with the addition of stabilizers.

2- R2 solution: consists of pyruvate kinase <50 KU / L with sodium azide 0. 05% and stabilizers.

3- CAL solution : Sodium and potassium standard and consists of sodium (Na +) 160 mmol / l with potassium (K +) 6.0 mmol / l.

How it works:

1- Transfer to centrifugal tubes marked according to the indicator in the table below.

Pipe Name	Calibration	Sample	Detector
Titration Solution **R1**	**1.0 ml**	1.0 ml	1.0 ml
Sample	---	**µl 25**	**---**
Detector	---	---	µl 25

2- Mix, and hug for 5 minutes at 37 ° C.

4- LEDA.F. R2 Detector 250 µL 250 µL 250 µL

5- Mix and cuddle for 1 minute at 37°C and read (A1) at 405 nm.

6- Mix and hug for 3 minutes at 37°C and read (A2) at 405 nm.

3- Accounts:

———————— **x C Calibrator = mmol/L Potassium**

4. Reference Values:

Adults 3.5 to 5.1 mm (13.7-19.9 mg/dL) Samples with concentrations higher than 8.0 mmol/1:1 liter should be diluted with brine and calibrated again.

Results and discussion

It was found through the results of the current study that there was an insignificant decrease of p≤0.05 in the concentration of potassium ion in the blood serum of the study sample members as it reached (4.42 (±0.43) mg/dl) compared to its percentage in the blood serum of the control sample members where it amounted to (5.09(±1.57) mg/dl). The decrease in potassium ion in autistic patients compared to healthy children may be due to the decrease in glutamate in autistic patients compared to healthy children as potassium controls the release of glutamate from the spinal cord and plays a crucial role in maintaining and spreading the potential of action (Ohno *et al*., 2021). While another study indicated a significant rise in the blood serum of the subjects of the study sample compared to the control sample members and this is explained by the fact that potassium is part of the ion pump (K + ATPase) and is a component of the respiratory mitochondrial chain known to be negatively associated with lipid peroxides as a sign of oxidative stress, a mechanism that causes autism spectrum disorder (Guglielmi L. *et al.*, 2015) .

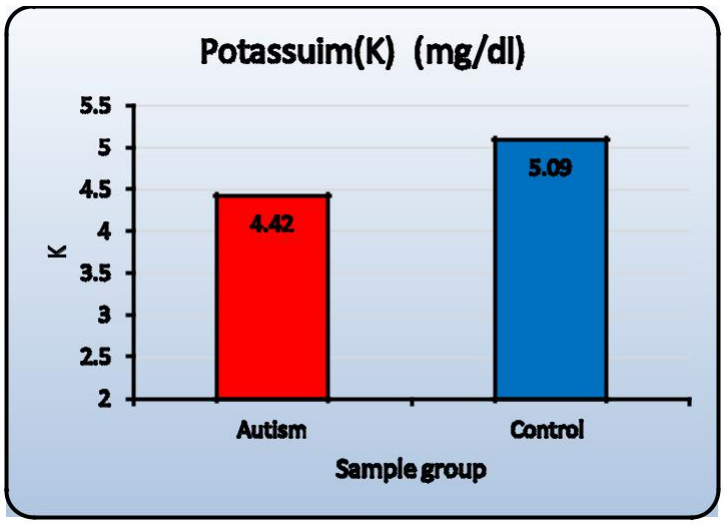

Figure (1-4) Concentration of potassium ion in the blood of study sample subjects (mg/dl)

The results of the current study also indicated a significant difference ≤0.05 in the concentration of sodium ion, where the concentration of E in the blood serum of the study sample individuals (123.67 (± 9.57) mmol /l compared to its concentration in the serum of the control sample individuals was 135.05 (± 9.43) mmol/l). As well as in the control of glutomate action where glutamate-derived GABA vectors exercise their functions by binding to chloride-passive GABA-A receptors and metabolic GABA-B receptors. This explanation could be acceptable because GABAergic transmission through receptors is achieved through various mechanisms such as GABA-A receptor modification with a change in GABA concentration (Deidda G. *et* 2015 *al* .,). Another study contrary to the results of our study conducted on autistic patients found a decrease in the concentration of sodium ion in the blood serum of autistic patients and may be due to the high concentration of extracellular glutamate due to the presence of high concentrations of tumor necrosis factor alpha the risk which causes the occurrence of glutamate toxicity, and this is explained on the basis of the main role of IFN - in inducing neurotoxicity Pdespite the high GABA in plasma of autistic patients, suggests an imbalance in the transmission of inhibitory GABAergic due to the low density of GABAA receptors (*et al*., Kim 2020).

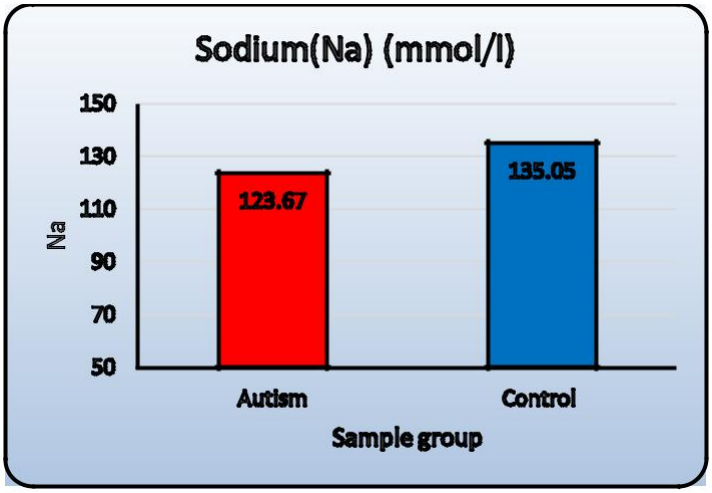

Figure (1-5) **shows** the **concentration** of **sodium ion in** the **serum of the study sample subjects (**mmol/l**)**

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DNA extraction

The current study included the extraction of DNA from the blood samples of children with autism (40) samples as well as from the control samples of (20) samples based on the method of extracting DNA from the total blood that was described in the separation of substances and methods of work, as well as measuring the purity and concentration of the samples (Nano Drop E) The purity and concentration were within ideal limits and suitable for the tetra-primer ARMS-PCRaccessory reaction (2) All samples were deported in the Gel Electrophoresis relay to ensure the success of the extraction process by detecting the presence of DNA by following its movement on the acarose gel at a concentration of (1%).

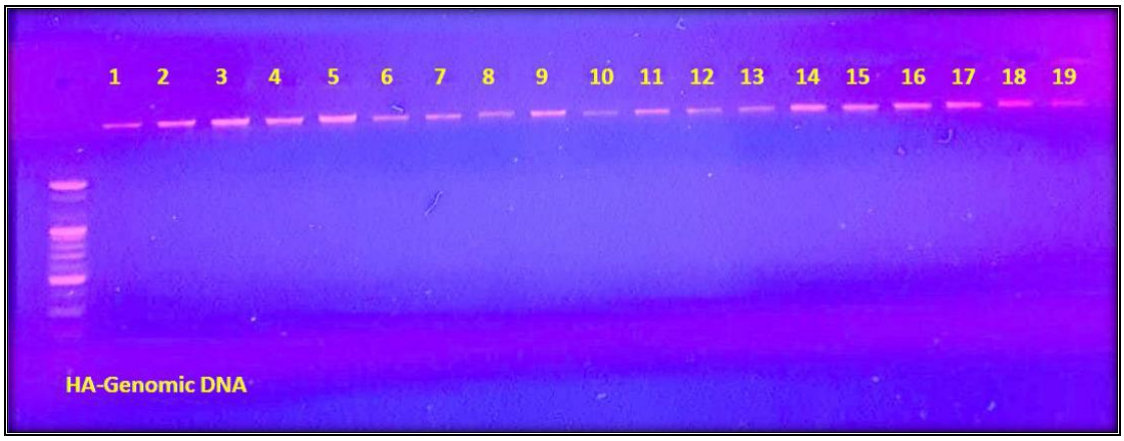

Image (1-1) of **DNA extracted and relayed on acarose gel at a concentration of %** 1 **5 vol. /cm for 1:15 hour**

Results of electrolyte of polymerase chain reaction products Single night polymorphism *rs1048221*

The results of the current study show a table of occurrence of multiplicity in the forms or locations of nitrogen bases (SNPs), where the site ***rs1048221*** showed the occurrence of multiplicity in the base site (A(70bp) in which the number of beams appeared 20 (50%) in the members of the study sample compared to 18 (90) % in the control sample individuals, while the study showed the occurrence of multiple nucleotides form above as it showed two additional forms, C(102) and CA(70+102) and with the number of impressions and frequency of 4 (10%) and 11 (36.36%) respectively compared to the number of times they appeared in the control sample members which amounted to 0 (0%) and 2(10%)

Table 1-5 showing the number of registered genotypes by location of nitrogen bases

	Ge. (70)	Ge. (70+102)	Ge. (102)	SNP	Pronounced like t
Patients	AA=20	CA=11	CC=04	(*rs1048221*)	1
Control	AA=18	CA=02	CC=0		
	Ge. (100)	Ge. (100+87)	Ge. (87)		
Patients	CC=28	GC=08	GG=04	(*rs1189205506*)	2
Control	CC=0	GC=19	GG=01		
	Ge. (82)	Ge. (82+151)	Ge. (151)		
Patients	GG=11	GA=21	AA=06	(*rs1856943620*)	3
Control	GG=0	GA=02	AA=18		

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Table (1-6) showing the genotype frequency and allele of the **BDNF/SNPgene in the subjects of the study and control sample *rs 1048221)***)

SNP:1 (*rs1048221* (Genotype	**Patients No. 40(%)**	**Control No. 20(%)**	**P-value**	**Chi-Square (χ^2)**
AA	20 (50.00%)	18 (90.00%)	0.041	4.15 **
CA	11 (36.36%)	2 (10.00%)	0.072	3.24 **
CC	04(10.00%)	0 (0.00%)	0.403	0.69 NS
Allele	**Frequency**			
A	0.74	0.95	--	--
C	0.26	0.05	--	--
** (P≤0.05) .				

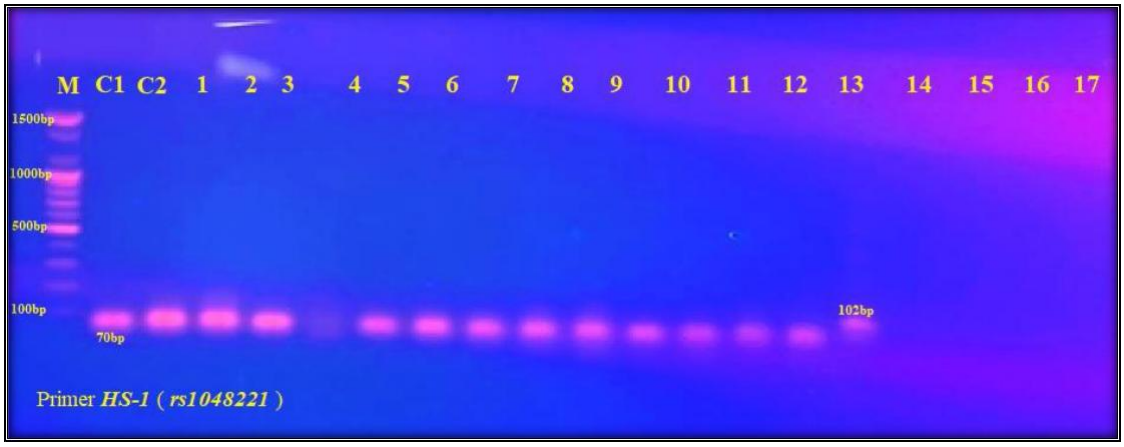

Image (1-2) showing the **output of the electrical migration of single nucleotides at *RS1048221*on the acarose gel** of **some study samples at a concentration of 1.5 5 vol. /cm for 1:15 hour.**

Autism spectrum disorder (ASD) is a neurodevelopmental dysfunction characterized by a lack of social interaction skills, poor communication and repetitive and restrictive behaviors that are thought to be caused by altered neurotransmitter processes, and is considered a brain-derived neurotrophic factor (BDNF) which is a homogeneous protein 27 kDalton and is a member of the neurogrowth factor family that are peptides that are widely expressed throughout the central nervous system and indicate Studies in animals that play an important role in the regulation and growth of neurons, synaptic flexibility, energy balance, and many organisms show heterogeneous forms of factor due to mutations causing pathological symptoms such as overeating, obesity, lack of awareness, poor learning and social behaviors *et al*., 2022 (Althammer).

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Results of electrolyte of polymerase chain reaction products Single RS polymorphism **1189205506**

The results of the current study also showed a table of occurrence of multiple forms or locations of nitrogen bases (SNPs), as the **site rs** showed **1189205506** the occurrence of multiplicity in the base site (C(100bp)), in which the number of beams appeared 28 (70%) in the subjects of the study sample compared to 0(0). % in the control sample individuals, while the study showed the occurrence of multiple nucleotides form above as it showed two additional forms, G(87bp) and CG(100+87bp) and the number of impressions and frequency of 4 (10%) and 8 (20%) respectively compared to the number of impressions in the control sample individuals, which amounted to 19 (95%) and 1(5%)

Table 1-7 shows the genotype frequency and allele of the **BDNF/SNP gene in the subjects of the control and study sample (rs1189205506).**

SNP:2 (*rs1189205506*) Genotype	**Patients No. 40(%)**	**Control No. 20(%)**	**P-value**	**Chi-Square (χ^2)**
CC	28 (70.00%)	0(0.00%)	0.026	4.94 **
GC	08 (20.00%)	19 (95.00%)	0.032	4.56 **
GG	04 (10.00%)	01 (5.00%)	0.016	5.77 **
Allele	**Frequency**			
C	0.90	0.53	--	--
G	0.10	0.47	--	--
** (P≤0.05) .				

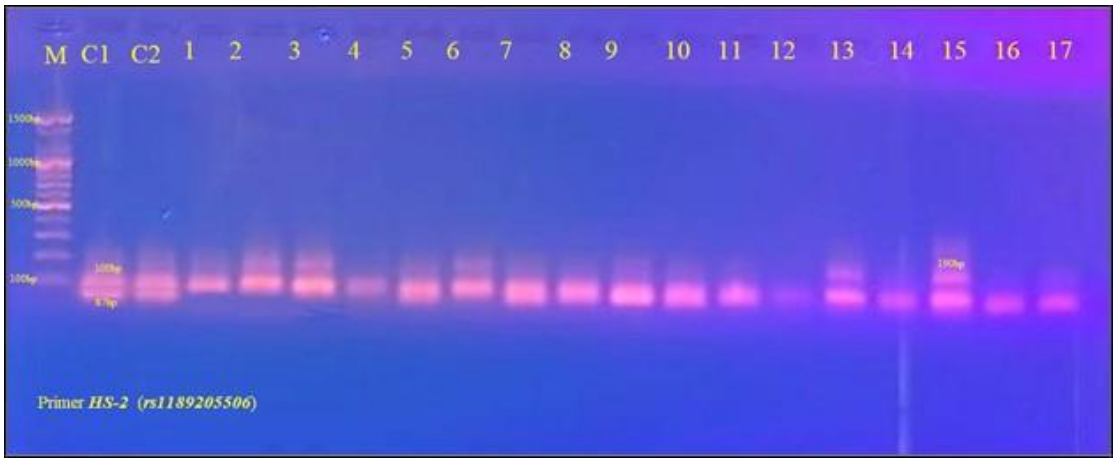

Image (1-3) of the **electrolyte output of single nucleotides at the RS site 1189205506 on the acarose gel** of **some study samples at a concentration of** 1.5 **% 5 vol. /cm for 1:15 hour.**

The imbalance in the glutamate pathway may be a motivation for the use of other glutamate receptors (metabolically oriented), one of the modern mechanisms for producing

an appropriate allegory orientation for autism spectrum disorder, or mGluRs (an amino acid that acts as an excitatory neurotransmitter) that is activated through metabolism and includes a set of protein receptors associated with GPCRs and The phenotype permeability of genetic variants interferes with other disorders such as ADHD disorder that relies heavily on an individual's genes and the identification of NMDARs and AMPARs in the brain of children with autism and that helps develop new AMPA-based therapies to correct deficits in voltage modification of sodium ion channels Na + which is involved in the regulation of glutamate.Some antiepileptics (valproate) may have a negative effect on fetal development iftaken by the mother during pregnancy, as sodium valproate can lead to an abnormal brain or lead tothe development of neurological disorders such as intellectual disability and autism (Meador *et alum). al* .,2013) . The administration of valproic acid to pregnant rats and mice during pregnancy has been shown to lead to autism-like symptoms in the resulting offspring *et al*,.2020 (Tsuji).

Results of electrolyte of polymerase chain reaction products Single night polymorphism RS1856943620

The results of the current study indicated a table of multiplicity in the forms or locations of nitrogen bases (SNPs), as the **site rs** showed **1856943620** the occurrence of multiplicity in the base site (G(82bp)), in which the number of beams appeared 11 (27.5%) in the study sample compared to 18(90). % in the control sample individuals, while the study showed the occurrence of multiple nucleotides form above as it showed two additional forms, A(1 51bp) and GA (82+151bp) and the number of impressions and frequency of 6 (1 5%) and 21 (52.5%) respectively compared to the number of impressions in the control sample individuals, which amounted to 0 (0% and2 (10%)

Table **(1-8**) **showing the genotype frequency** and **allele of the BDNF/SNP gene in control and study sample individuals (*rs1856943620*)**

SNP:3 (*rs1856943620*) Genotype	**Patients No. 40(%)**	**Control No. 20(%)**	**P-value**	**Chi-Square (χ^2)**
GG	11(27.50%)	18 (90.00%)	0.0005	11.71 **
GA	21 (52.50%)	02 (10.00%)	0.45	0.56 NS
AA	06 (15.00%)	0 (00.00%)	0.0008	11.22 **
Allele	**Frequency**			
G	0.66	0.95	--	--
A	0.34	0.05	--	--
** (P≤0.05) .				

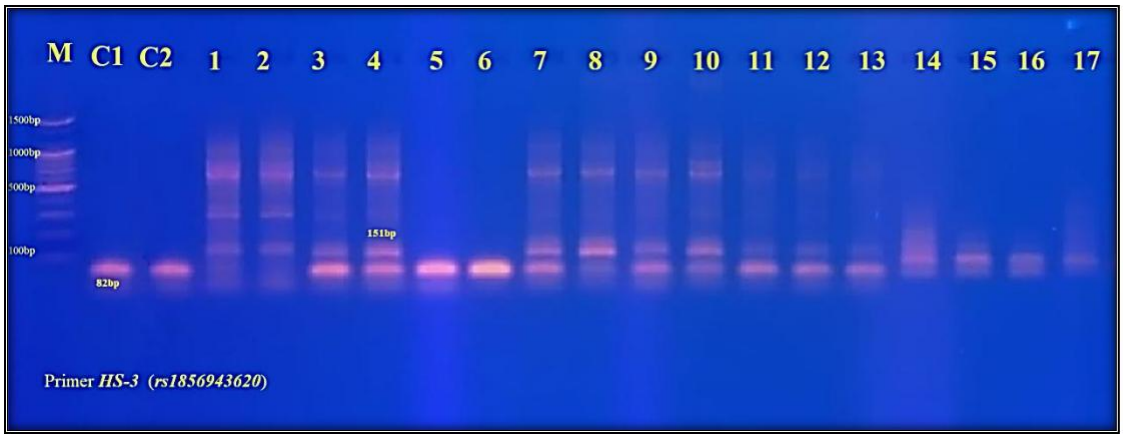

Image (1-4) of the **electrical relay output of single nucleotides at the RS site 1856943620on the acarose gel** of **some study samples at a concentration of 1.5 5 % vol. /cm for 1:15 hour.**

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Studies show that mutations in the Neurexin and Neuroligin families are physiologically linked to autism spectrum disorder (2021 *et al*). , Cuttler) . Mutations in the BDNF gene have multiple roles in bringing about many changes during different stages of neurocognitive development, maturation and sustainability throughout life, and also pointed to an important role for mutations in 5 UTR exons as the BDNF sequences on exons I and II exons are of great importance and They are expressed in areas of the brain important for learning and memory (Gao *et al*., 2022).

Conclusions

1- The state or level of the autism spectrum is affected by low levels of certain ionic elements such as potassium.
2- The multiplicity of genetic forms of the gene responsible for brain-derived nutritional factor is instrumental in developing all spectrums of autism.

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