Amino Acids Profile in Libyan Children with Down's Syndrome

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Introduction:

Down's syndrome (DS):

Down's syndrome was first described by the English physician Langdon down in 1866, but its cause was not known until it was showed that these individuals carried 47 chromosomes being what was designated at this time as chromosome 21.[1,2]

There is a characteristic face with hypoplastic maxilla, flattening of nasal bridge epicanthal folds, an increased incidence of cleft lip, a protruding fissured tongue, low IQ and shortened life expectancy.[3]

Down's syndrome is perhaps the eariest condition associated with mental retardation and the most common genetic cause of developmental disability. The most common of which DS with an overall incidence of 1/900 live births. Worldwide, the prevalence of DS is one in- 700 births, and boys outnumber girls 1.3 to 1.0.[4,5]

The incidence of down's syndrome in Benghazi - Libya is approximately 1.8 per 100 births. There is still not a great deal know about the pathogenesis of D.S.[6]

There are many reports of biochemical deviations in DS but none of these deviant biochemical changes provide an explanation of the basic pathology in the syndrome. The biochemistry of certain metabolic disorders, determined by specific genes, is often fairly well understood, but a more complex situation is encountered in chromosomal disorders like Down's syndrome.[7,8]

Patients and methods:

This study was conducted on 48 children with Down's syndrome selected from the clinic of neurology department in El- Fateh Childern Hospital, and genetic clinic in El-Sabri poly clinic .

48 children with Down's syndrome diagnosed by the clinical features and proved by chromosomal karyotyping, all had 47 chromosome (trisomy 21).

A- Blood sample:

5 ml fasting venous blood sample was collected from each child (patients & control) in plain tubes ,and allowed to clot for 1-2 hours at room temperature. Then the serum was separated by centrifugation at 1000 r.p.m for 20 minutes and stored at 20°C until required for assay .

B- Urine sample:

10 ml urine sample was collected from each child in a container containing few crystals of thymol, and stored at deep freezer untile analyzed.

Serum and urine amino acid chromatography screening by thin layer chromatography (TLC).[9,10]

Principle:

Serum or urine is chromatographed on precoated cellulose thin layer plates. The separated amino acids are made visible by reaction with ninhydrin. Elevated cocentrations with the band of a normal control on the chromatogram.

Reagents:

The following reagents were obtained from BDH-chemicals LTD. England .

- chromatographic solvent:

a- n. butanol : acetic acid (1:1)

b- Glacial acetic acid: water (1:2)

c- Ninhydrin stain: 7g ninhydrin was dissolved in 50 ml of solvent A, mixed vigorously to dissolve.

d- cellulose thin layer plate (10X20 cm)

- standard amino acids : 5mM/L of each was prepared and neutralized before use by adding 0.1 M NaOH.

The ammo acids used as standard were:

Aspartic Acid, threonine, aspargine, serine, glutamic acid, glutamine, proline, glycine, alanine, citruline, GABA, valine, methionine, isoleucine Jeucine, tyrosine, phenylalanine, orinthine, lysine, histidine, tryptophan, arginine and taurine.

* Mark the plate lightly 1 cm from the bottom to demarcate sample application lines and mark a finishing line (solvent front) 8.5 cm from the bottom.

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* Apply 2 μ.1 plasma or 5 μl urine along application line .
* for the first development, place the plates into chromatography tank number (1) and allow solvent to migrate to finish line.
* for the second development place chromatograms in chromatography tank number (2), containing 56 ml solvent (A), 24 ml solvent (B) and 6 ml ninhydrin reagent, and allow the solvent to migrate to the finish line.
* remove the plate and put it in oven (100°C) for 5 minutes.
* examine the ninhydrin-development chromatogram plates within 1 hour after color development .Record the results by comparing the bands.

Each amino acid can be identified by its relative flow (RF), this is obtained by putting up standard markers.

C- quantitative serum amino acids determination:

Concentration of amino acids were determined by ion exchange column chromatography in serum from fasting blood. Serum was deproteinized by 5% sulphosalicylic acid (1:1 V/V).[11]

The technique for amino acids analysis has been the standard technique using analyzer biotronic LC 5000 (Eppendorf-Biotronik, Humborg, Germany).[12]

Results:

Amino acids profile

A. serum amino acids:

Statistical analysis of the result concerned amino acids revealed that, there is:

1- amino acids level with significant difference from control, it was found that:

D.S had a significant lower mean serum levels of serine (28.89 ± 15.03), glutamine (27.46± 19.71), histidine (19.59 ± 8.15) and arginine (24.09 ±19.10) than the corresponding control levels {(39.06 ± 13.34), (37.42 ± 17.85), (24.16±8.23) and (36.65±13.49)} respectively. Table l, Fig I.

Table 1 : Mean serum value of amino acids that show a statistically significant different levels among the studied group:

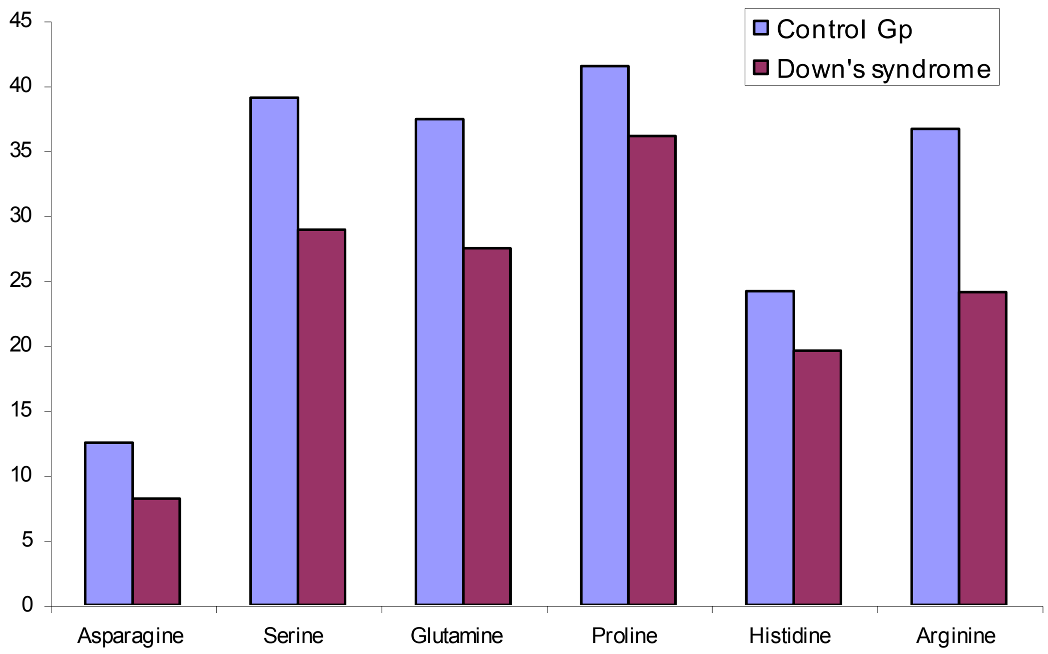
Group / Ammo acids	Control group (n=37)	Group I Down's syndrome (n=48)
serine		
Range	24.5-95.8	1.1-77.2
Mean± SD	36.06±13.34	28.89±15.03
P		<0.01 *
Glutamine		
Range	3.3-90.0	4.2-59.4
Mean± SD	37.42±17.85	27.46±19.71
P		<0.05 *
Histidine		
Range	3.7-56.4	4.0-52.6
Mean± SD	24.16±8.23	19.59±8.15
P		<0.05 *
Arginine		
Range	8.9-66.9	1.1-82.6
Mean± SD	36.65±13.49	24.09±19.10
P		<0.05 *

* P <0.01 and <0.05 : significantly different from control
(n) : indicates the number of patients.

2- Amino acids level with insignificant difference from control:

No significant statistical difference was found in the mean values of aspartic acid, threonine, glutamic acid, glycine, alanine, citrulline, GABA, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, orinithine, lysine and tryptophan levels between the studied group and control group.

Fig. (1) The mean values of serum amino acids that show a statistically significant different levels among the studied group.



B-Urine amino acids:

The following amino acids were detected in significantly higher percentage of children with D.S when compared to their matching controls asparagines (30.9% vs 8%), serine (76.4% vs 30%) glutamic acid (78.2% vs 20%), methionine (74.6% vs 40%), isoleucine (47.3% vs 24%), leucine (47.3% vs 8%), tyrosine (45.5% vs 6%), phenylalanine (47.3% vs 8.0%), histidine (78.2% vs 58%) and tryptophan (21.8% vs 4%), while the following amino acids were detected in significantly lower percentage of childern with D.S compared to their matching control, glutamine (14.6% vs 52%), orinthine (49.1% vs 72%), lysine (49.1% vs 72%), arginine (1.8% vs 14%) and taurine (10.9% vs 38.0%) .Table 2, Fig. 2.

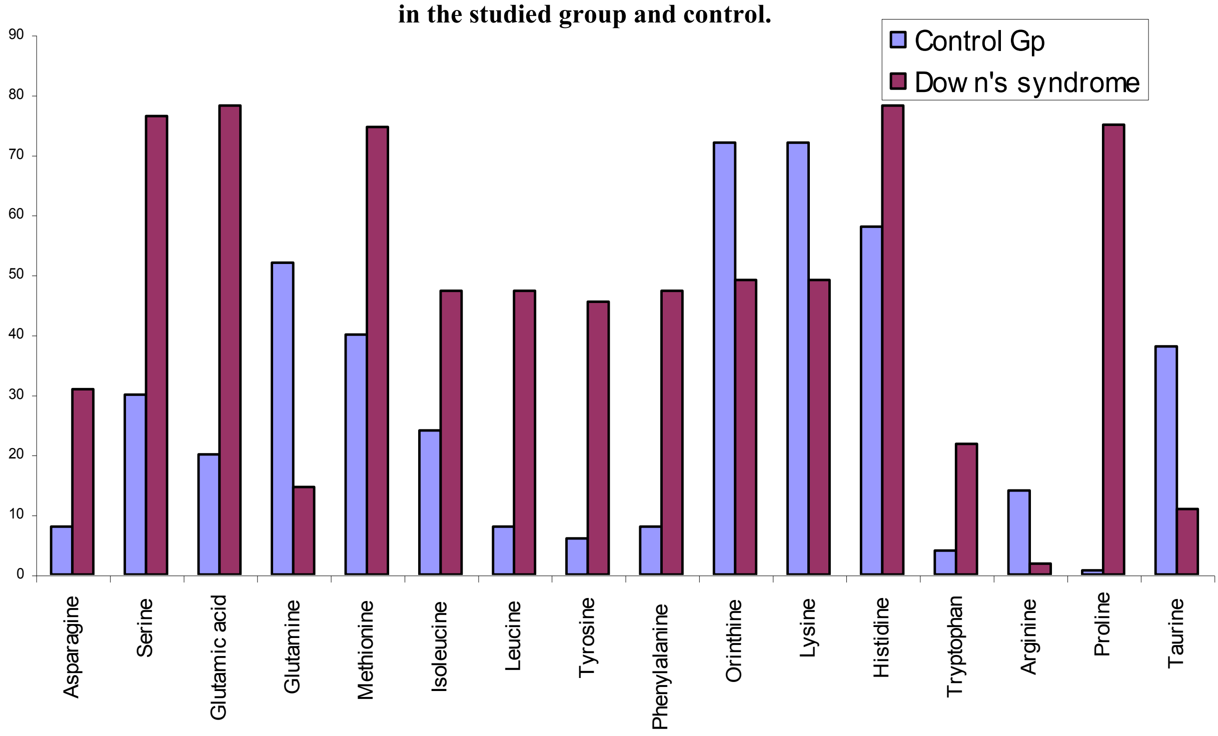
Table 2: amino acids qualitatively estimated in the urine of D.S children compared to control.

Group / Ammo acids	Control group (n=50) Detected in	Group I Down's syndrome (n=55) Detected in
Asparagine		
No. of cases	4 (8.0%)	17 (30.9%)
X^2		7.22
P		0.001*
Saerine		
No. of cases	15 (30.0%)	42 (76.4%)
X^2		20.85
P		0.001*
Glutamic acid		
No. of cases	10 (20.0%)	43 (78.2%)
X^2		33.17
P		0.001*

Group / Ammo acids	Control group (n=50) Detected in	Group I Down's syndrome (n=55) Detected in
Glutamine		
No. of cases	26 (52.0%)	8 (14.6%)
X^2		15.11
P		0.001*
Methionine		
No. of cases	20 (40.0%)	41 (74.6%)
X^2		11.45
P		0.001*
Isoleucine		
No. of cases	12 (24.0%)	26 (47.3%)
X^2		5.1
P		0.02*
Leucine		
No. of cases	4 (8.0%)	26 (47.3%)
X^2		17.91
P		0.001*
Tyrosine		
No. of cases	3 (6.0%)	25 (45.5%)
X^2		18.87
P		0.001*
Phenylalanine		
No. of cases	4 (8.0%)	26 (47.3%)
X^2		17.91
P		0.001*
Orinthine		
No. of cases	36 (72.0%)	27 (49.1%)
X^2		4.81
P		0.03*
Lysine		
No. of cases	36 (72.0%)	27 (49.1%)
X^2		4.81
P		0.03*
Histidine		
No. of cases	29 (58.0%)	43 (78.2%)
X^2		4.06
P		0.04*
Tryptophan		
No. of cases	2 (4.0%)	12 (21.8%)
X^2		5.73
P		0.01*
Arginine		
No. of cases	7 (14.0%)	1 (1.8%)
X^2		3.93
P		0.04*
Taurine		
No. of cases	19 (38.0%)	6 (10.9%)
X^2		9.15
P		0.001*

X^2=Chi square test P < 0.05 is considered significant

Fig. 2: The significant percentage of urinary amino acids detected in the studied group and control.



Discussion:

Down's syndrome is the most common genetic disease, in which brain is associated with a decreased neuronal number and abnormal neuronal differentiation. The cause of neurodegenerative process in Down's syndrome is unknown.[13]

Interest in the detection of amino acids in the urine has been stimulated by the development of chromatographic techniques.[14,15] The detection of aminoaciduria in D.S distrubed children offers a new biochemical approach in the study of the basic disturbance and may even suggest genetic implicates.[16,17]

In the present study, the mean value of serum amino acid levels in a group of Libyan children with Down's syndrome showed a significantly low levels of serine, glutamine, histidine and arginine compared to controls.

The most interesting finding in this work is the low level of the mean value of serum serine in patients with Down's syndrome compared with controls. This is because serine may have a role in mental retardation (D.S). Similar findings were reported by Libyan workers in (1993),[18] and by Lejeune, et al in (1992)[19] which was proved also by other workers in different parts of the world.[20,21] Low serine level is considered a specific and characteristic amino acid's abnormality in patients with Down's syndrome, and not in patients with other causes of mental retardation.[19,20]

The decreased serine level in the sera of patients with Down's syndrome could be of substantial evidence that there is a defect in serine trans-hydroxymethylase, converting glycine to serine in the brain.[22] Besides, it has been stated that glycine (one of the putative neurotransmitters of the brain) has binding sites in the brain stem and spinal cord.[23]

In additions, the defect in glycine metabolism was explaned to be due to absence of glycine oxidase,[24] in which serine could be synthesized from glycine. Serine being a precursor of choline, acetyle choline and cephalins of brain and nervous system, seems to be an important amino acid in the structural lipids in the brain.[18]

The relation between amino acids metabolism and mental retardation has attracted the attention of many authors. Thus, a significant decrease in plasma homocysteine level in down's syndrome was observed by some authors.[25,26] An increased serum level of glycine,[18] lysine[20] and leucine, phenylalanine, isoleucine, cystine, were found in patients with Down's syndrome,[27] Tryptophan and tyrosine were decreased in patients with Down's syndrome compared with the matching controls.[27]

The study of Lejeune and his colleagues in (1992),[19] showed that the concentration of serum cysteine and lysine were high in patients with down's syndrome, and the concentration of serum serine was low when compared with controls. Our findings confirmed Lejeune's results only regarding the decreased serum concentration of serine in down's syndrome.
Lejeune and his colleagues[19] suggested the presence of "enzyme imbalances" in Down's syndrome, for example superoxide dismutase, cystathionine synthetase B, and other enzymes of purine metabolism, which were affected by genes on chromosome 21. Lejuene's aims were to link the metabolic consequences of additional chromosomal material with pathophysiological basis of mental ratardation.
His theory was that the enzyme imbalance may be related to the primary chromosomal abnormality and that intellectual importance could perhaps follow dietary correction of these biochemical imbalances.[19,20]
Increased serum asparagine level in children with Down's syndrome was previously reported.[28,29]
A low serum glutamine level in children with Down's syndrome was reported.[29,30]
Some other workers found a significant deficit of glutamate and GABA in the Down's syndrome children when compared to matched control.[31]
The low level of serum glutamine in Down's syndrome was discovered as early as 1949.[32] Since that time, many workers evaluated the effect of glutamic acid treatment in the intelligence of patients with down's syndrome.
Also, according to Harrison, the amino acids normally found in the urine are glutamic acid, serine, glycine, threonine, alanine, glutamine, tyrosine and aspartic acid.[33]
While in another study, the urinary amino acids demonstrated in paper chromatography and in additon to those of Harrison are arginine, cystine, ornithine, histidine, leucine, isoleucine, lysine, metheonine, phenylalanine, taurine, trypytophan and valine.[34]
Reduced amount of urinary metabolites of tryptophan found in Down's syndrome was explained by diminished or delayed intestinal absorption,[35] contrasting with these observations, no difference between Down's syndrome persons and controls were observed in the excretion of urinary tryptophan metabolites.[36]
On the other hand, in the present study, tryptophan showed high excretion in Down's syndrome as compared to controls .
In this work, histidine, aspargine, serine, glutamic acid, methionine, tyrosine, isoleusine ,leusine, tryptophan and phenylalnine were found to be excreted in a significantly increased numbers of children with Down's syndrome, while lysine, ornithine, arginine, glutamine and taurine were excreted in a significantly decreased number of children from the same group as compared with control group.
In addition , increased excretion of serine, tyrosine, histidine, arginine, ornithine, taurine, phenylalanine and aspargine in a group of patients with Down's syndrome was also reported . These results are in agreement with ours.
No inherited disorders of taurine metabolism has yet been described in man, although altered excretion of taurine has been reported in several physiological and pathological conditions. Hereditary mental depression with taurine deficiency has been reported.
Earlier studies suggest that there is a lower limit of normal taurine excretion in Down's syndrome compared to control. This is strongly in agreement with the findings of the present study.[31]

Recommendation and preventive services:
Primary prevention depends largely on genetic counseling, early detection of fetal abnormalities during pregnancy, and safe childbirth. Secondary prevention aims to prevent the progression of disability by other medical or psychological means. The latter includes enriching education and early attempts to reduce behavioral problems.
1- Genetic counseling: this begins with assessment of the risk of an abnormal child being born. Such an assessment is based on a study of the family history and knowledge of the genetics of conditions that give rise to mental defect. The parents are then given an explanation of the risks and are encouraged to discuss them. Most parents seek advice only after a first abnormal child has been born.
2- Prenatal care: this begins before conception by providing immunization against rubella. Some causes of mental retardation can now be diagnosed in utero. Amniocentesis, fetoscopy and ultrasound scanning of the fetus can reveal chromosomal abnormalities, most open neural tube defects, and about 60 percent of inborn errors of metabolism

3- Postnatal prevention: in Britain, all infants are routinely tested for PKU, and a routine test for hypothyroidism and galcatosaemia is becoming increasingly common Improved methods of care for premature and low birth weight infants can prevent mental retardation in some infants who previously would have suffered brain damage.

Research is a major key to progress in prevention, diagnosis, treatment, education and care in the field of mental retardation .

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