MARRAKECH
جامعة القاضي عياض
UNIVERSITÉ CADI AYYAD

كليـــــــة الطب
والصيدلة - مراكش
FACULTÉ DE MÉDECINE
ET DE PHARMACIE - MARRAKECH

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# INVOLVEMENT OF HEXOSYLCERAMIDES IN NEURODEGENERATIVE DISEASES: RETROSPECTIVE STUDY .

## THESIS

PRESENTED AND PUBLICLY DEFENDED ON 11 / 11 / 2024

BY

**Mrs. HAJAR MCHACHTI ABAKHTI**

BORN ON September 14th, 1996, IN MARRAKECH

## TO OBTAIN THE DEGREE OF DOCTOR OF MEDICINE

## KEYWORDS

Glucosylceramides – Galactosylceramides – Hexosylceramides – Neurodegenerative diseases – Thin-Layer Chromatography

## JURY

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بسم الله الرحمن الرحيم
قالوا سبحانك لا علم لنا الا ما علمتنا انك انت العليم الحكيم
صدق الله العظيم

# *HIPPOCRATIC OATH*

*As a member of the medical profession:*

*I solemnly pledge to dedicate my life to the service of humanity;*

*The health and well-being of my patient will be my first consideration;*

*I will respect the autonomy and dignity of my patient;*

*I will maintain the utmost respect for human life;*

*I will not permit considerations of age, disease or disability, creed, ethnic origin, gender, nationality, political affiliation, race, sexual orientation, social standing or any other factor to intervene between my duty and my patient;*

*I will respect the secrets that are confided in me, even after the patient has died;*

*I will practice my profession with conscience and dignity and in accordance with good medical practice;*

*I will foster the honor and noble traditions of the medical profession;*

*I will give to my teachers, colleagues, and students the respect and gratitude that is their due;*

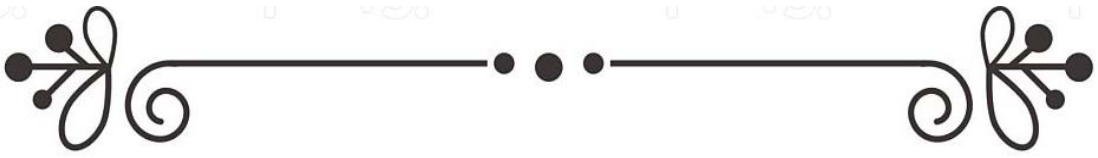
*I will share my medical knowledge for the benefit of the patient and the advancement of healthcare;*

*I will attend to my own health, well-being, and abilities in order to provide care of the highest standard;*

*I will not use my medical knowledge to violate human rights and civil liberties, even under threat;*

*I make these promises solemnly, freely, and upon my honor.*

***Geneva Declaration, 1948***



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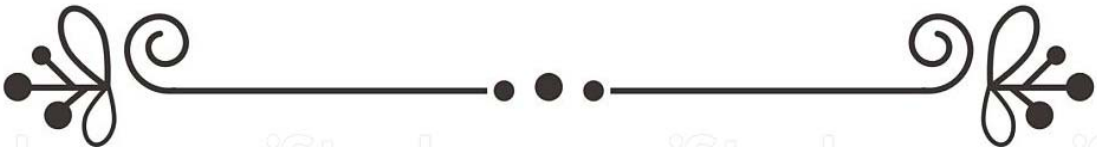

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| 267 | AHMANNA Hussein-choukri | Pr Ass | Radiologie |
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| 270 | FOURA Salma | Pr Ass | Chirurgie pédiatrique |

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| 325 | RAITEB Mohammed | Pr Ass | Maladies infectieuses |
| 326 | DREF Maria | Pr Ass | Anatomie pathologique |
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| 342 | ATBIB Yassine | Pr Ass | Pharmacie clinique |
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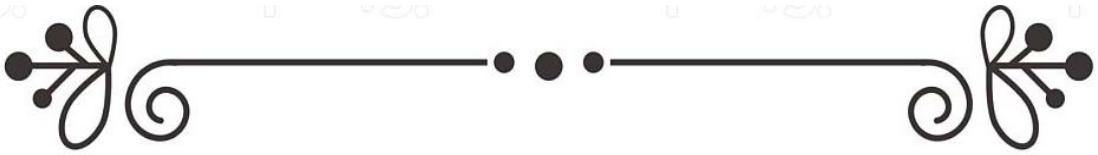
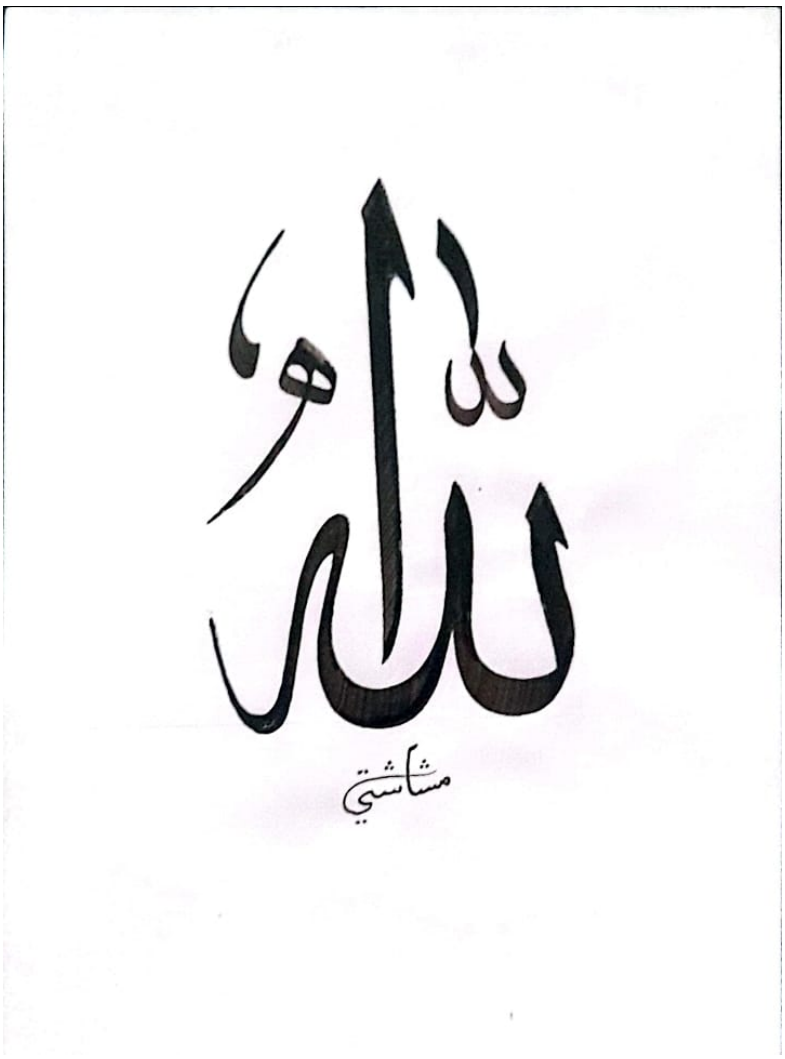


# *DEDICATIONS*

*I dedicate this thesis to ...*




الحمد لله رب العالمين، الذي منّ علينا بنعمة الإسلام، وهدانا إلى صراط المستقيم. أشكرك يا رب على التوفيق الذي منّت به عليّ في إنجاز رسالتي. أسألك يا رب أن تجعل هذا العمل خالصاً لوجهك الكريم، وأن تنفع به المسلمين، وأن تجعله سبباً لزيادة علمي وعملي الصالح. أسألك يا رب أن توفقني لما تحب وترضى، وأن تجعلني من عبادك الصالحين

*Praise be to Allah the Almighty, Lord of all the Worlds, Who has bestowed upon us the blessing of Islam and guided us to the Straight Path. I thank You, O Lord, for the success You have granted me in completing my thesis. I ask You, O Lord, to make this work pure for Your sake, and to benefit your righteous servants with it, and to make it a means of increasing my knowledge and righteous deeds. I ask You, O Lord, to guide me to what You love and approve, and to make me one of Your righteous servants.*

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***My little Brother: Ilyasse Mchachti Abakhti***

*The constant source of fun, adventure, and inspiration in my life. Your unwavering encouragement and belief in me have been instrumental in my journey. I hope I have made you proud, and I can't wait to witness your continued growth and success. You're a true inspiration, a constant reminder that life is meant to be lived to the fullest. I'm lucky to have you as my brother, and I cherish our bond. As you continue to pursue your dreams, know that I'll always be there to cheer you on. I can't wait to see succeed and grow into the good man you're thriving to become.*

***In memory of Mima, my maternal grandmother***

*The most tender and caring woman I have ever known. You always put the needs of others before your own and showered me with love and affection. I cherish the memories of you and your gentle soul. Though you are no longer with us, your spirit lives on in my heart. I wish you could be here to witness this milestone and share in my joy. I am certain you would be incredibly proud of me. I miss you so much.*

***In memory of my maternal grandfather and my paternal grandparents***

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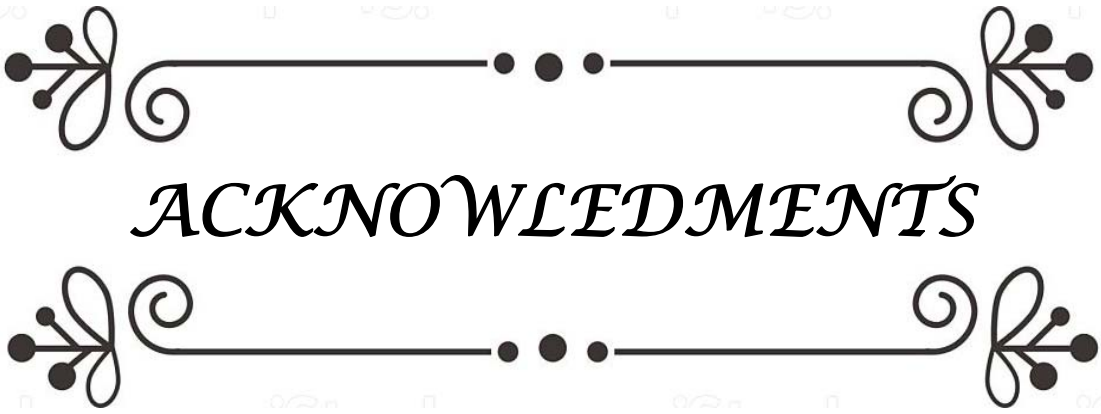

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*Head of the Internal Medicine Department*

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*Professor of Bio-organic coordination chemistry*

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*Professor of Internal Medicine*

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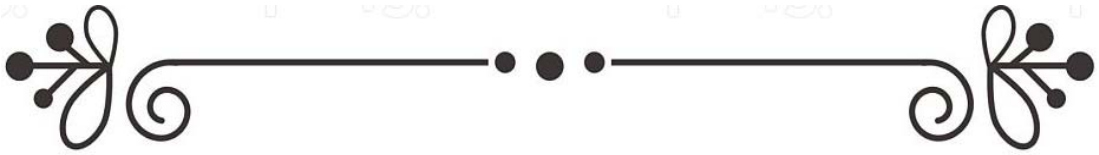
# *ABBREVIATIONS*

# List of abbreviations

| | |
|---|---|
| **NDD** | : Neurodegenerative disease |
| **DBD** | : Degenerative brain disorders |
| **HexCer** | : Hexosylceramides |
| **GlcCer** | : Glucosylceramides |
| **GalCer** | : Galactosylceramides |
| **SphL** | : Sphingolipids |
| **TLC** | : Thin-layer chromatography |
| **GCS** | : Glucosylceramide synthase |
| **GCase** | : Glucosylceramidase |
| **GALC** | : Galactosylceramidase |
| **CGT** | : Galactosylceramide synthase/ceramide galactosyltransferase |
| **ASM** | : Sphingomyelinase |
| **ARSA** | : Arylsulfatase A |
| **PPT1** | : Palmitoyl protein thioesterase |
| **TPP1** | : Tripeptidylepeptidase |
| **HEX-A** | : Hexosaminidase-A |
| **Beta HEX-A** | : Beta-hexosaminidase |
| **CNS** | : Central nervous system |
| **FAPP2** | : 4-phosphate adaptor protein-2 |
| **ER** | : Endoplasmic reticulum |
| **CBC** | : Complete blood count |
| **MRI** | : Magnetic resonance imagery |
| **EEG** | : Electroencephalogram |
| **VEP** | : Visual provoked potential |
| **CSC** | : Corticosubcortical atrophy |
| **WMSA** | : White matter signal abnormality |
| **GD** | : Gaucher disease |
| **KD** | : Krabbe disease |
| **MLD** | : Metachromatic Leukodystrophy |
| **NCL** | : Neuronal ceroid lipofuscinosis |
| **LSD** | : Lysosomal storage diseases |
| **PM** | : Psychomotor |
| **HMG** | : Hepatomegaly |
| **SMG** | : Splenomegaly |



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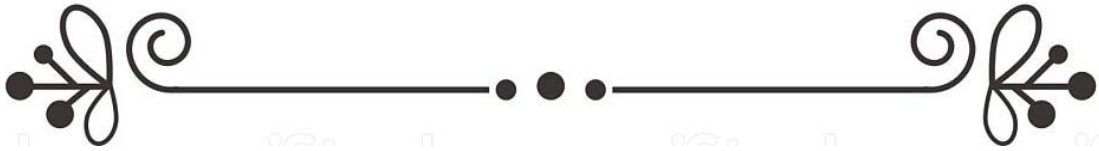

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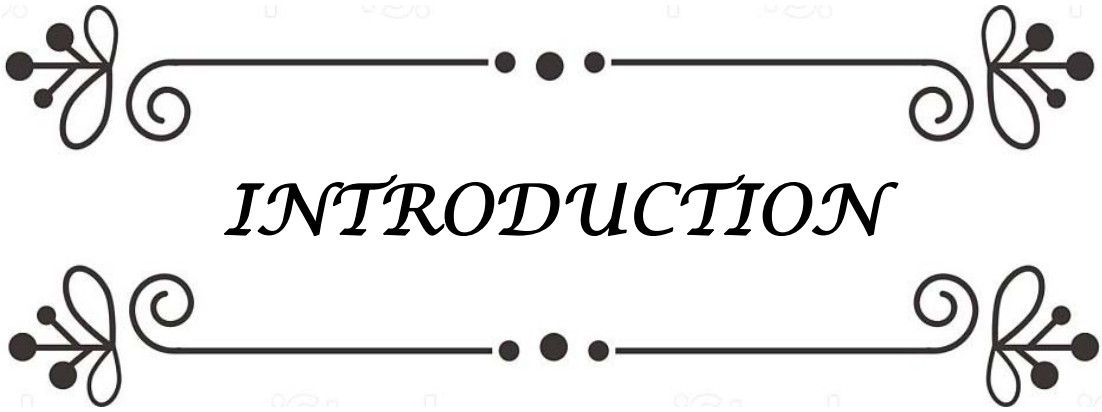


# *INTRODUCTION*

Neurodegenerative diseases (NDDs) represent a large group of neurological disorders with heterogeneous clinical and pathological expressions affecting specific subsets of neurons in specific functional anatomic systems; they arise for unknown reasons and progress in a relentless manner. [1]

NDDs are characterized by progressive loss of selectively vulnerable populations of neurons, they can be classified according to primary clinical features, anatomic distribution of neurodegeneration or principal molecular abnormality. [2]

Over the past decade, the perception of Hexosylceramides has changed. Studies suggest that alterations in their metabolism, may contribute to the pathogenesis of neurodegenerative diseases.

It took a long time to understand that these lipids are not only structural membrane molecules with a stiffening role but they are functional molecules fundamental for cell fate [3] and play important roles in neuronal functions by regulating rates of neuronal growth and differentiation and death of central nervous system (CNS) cells.

The mechanisms that underlie the functions of neuronal and glial cells are not completely clarified and many studies highlight that these lipids are some of the main actors.[4]

Understanding the molecular mechanisms underlying the involvement of Hexosylceramides in neurodegenerative diseases is an active area of research, but despite that, further studies are still needed to better elucidate the specificity of GlcCer and GalCer in the development and/or progression of neurodegenerative disorders.

In this study, our objective is to highlight on:

- The association between abnormal urinary excretion of Glucosylceramides and Galactosylceramides, and the neurodegeneration in patients suspected with inherited metabolic diseases.
- Their potential implication in the evolution and prognosis of those diseases.

# *MATERIAL AND METHODS*

# I. Study presentation

Our research is a retrospective study spread over a period of **5 years**, ranging from December 2018 to December 2023, involving 64 **cases** of patients exhibiting symptoms of neurological degeneration or suspected of having/developing a neurodegenerative disease.

This study took place in the metabolic platform at the Faculty of Medicine and Pharmacy of Marrakech.

# II. Material (patients, inclusion and exclusion criteria)

Our study involves patients from the pediatric, neurology and internal medicine departments in Mohammed VI University Hospital of Marrakech and the metabolic platform at the Marrakech faculty of Medicine and Pharmacy.

- Inclusion criteria:
  - → Patients who suffer from psychomotor delay/regression, visceral or neurological disorders, and/or any other symptom suggestive of neurological degeneration or an occurring degenerative disease,
  - → Patients whose thin-layer chromatography revealed the presence of Hexosylceramides (Glucosylceramides and Galactosylceramides).

- Exclusion criteria:
  - → Patients with incomplete medical records,
  - → Patients with no neurological symptoms.

A total of **47 patients** (25 female and 22 male) satisfied the inclusion criteria and were included in our study. Figure 1 shows the flow chart of patient selection:

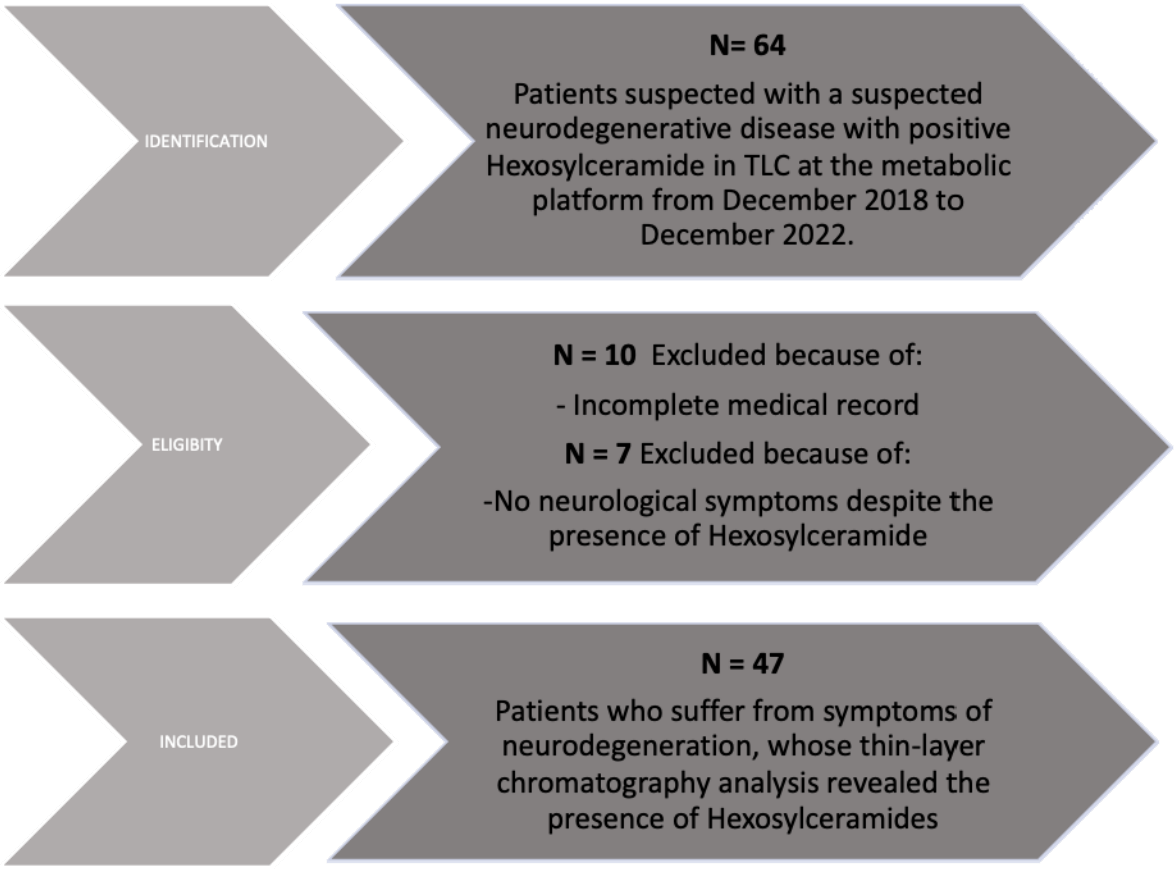



**Figure 1** : Flowchart of the selection process of the patients in the study.

# III. Methods

## 1. Data collection

To carry out this work, we consulted:

- Registers for recording documents;
- Admission forms
- Reports.

We established an operating sheet (see appendix) to collect the epidemiological, clinical and paraclinical data.

## 2. Statistical analysis of data

The data regarding the patients' history, basic clinical examination, laboratory investigations, imaging results, and outcome measures were coded and entered using Microsoft Excel Software.

## 3. Thin-Layer Chromatography

### 3.1. History

In 1855. ***F.F. Runge*** produced colored precipitations by letting solutions of inorganic compounds migrate against each other on paper, producing pretty pictures. Today one would call this 'Chrom art' (on TLC plates); contemporaries called them 'professor's blots' (on paper).

Of course, this was mainly an important historic event for paper chromatography, but it also influenced the beginning of TLC [5] .

**TLC** actually began with the Dutch biologist, ***Beyerinck***, in 1889 [6], when he allowed a drop of a mixture of hydrochloric and sulfuric acids diffuse through a thin layer of gelatin. The hydrochloric acid traveled faster than the sulfuric acid and formed a ring around the sulfuric acid. The hydrochloric acid zone was made visible by brushing on a solution of silver nitrate, and the sulfuric acid zone was made visible with barium chloride.

Nine years later in 1898, ***Wijsman*** showed that malt diastase contains two enzymes by allowing the mixture to diffuse through a gelatin layer containing starch and fluorescent bacteria. In the first use of fluorescence as a detection method, a separated, fluorescent beta-amylose band was detected with 40 pg sensitivity.

In 1938, *Izmailov* and *Schraiber* [7] analyzed plant tinctures by placing a drop of sample solution on a horizontal 2 mm layer of aluminum oxide without binder on a glass microscope slide. This was the first use of thin-layer chromatogram development with mobile phase. The technique was called 'spread layer chromatography' or 'spot chromatography'. Of course, this is different from the practice of TLC today; how-ever, they described the basic principle underlying the TLC process.

Since 1938 progress of thin-layer chromatography towards high-performance involved use of many different techniques.

### 3.2. Definition:

**Thin-Layer Chromatography** (**TLC**) is a quick, sensitive, and inexpensive technique used to determine the number of components in a mixture, verify the identity and purity of a compound, monitor the progress of a reaction, determine the solvent composition for preparative separations, and analyze the fractions obtained from column chromatography [8] .

TLC is applied in many fields, including environmental, clinical, forensic, pharmaceutical… Applications of TLC to the analysis of a variety of compounds, including lipids, vitamins, amino-acids and other substances [8].

There are several distinct advantages to thin-layer chromatography: [9]

- *High sample throughput*
- *Low cost*
- *The possibility to analyze several samples and standards simultaneously*
- *Minimal sample preparation*
- *Plates can be stored for later identification and quantification*

### 3.3. Principles of TLC:

TLC is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, this layer of adsorbent is known as the ***stationary phase***. After the sample has been applied on the plate, a solvent or solvent mixture is drawn up the plate via capillary action, it's called the ***mobile phase***.

Because different analytes ascend the TLC plate at different rates, separation is achieved. It is thus based on the principle of adsorption chromatography or partition chromatography or combination of both, depending on adsorbent, its treatment and nature of solvents employed.

The components with more affinity towards stationary phase travel slower. Components with less affinity towards stationary phase travels faster. Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate.

### 3.4. Components of TLC:

TLC system components consist of (Figure 2):

1. **TLC plates,** preferably ready made with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particle size.

2. **TLC chamber**: This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.

3. **Mobile phase**: This comprises of a solvent or solvent mixture. The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.

4. **A filter paper**: This is moistened in the mobile phase, to be placed inside the chamber. This helps develop a uniform rise in a mobile phase over the length of the stationary phase.

5. **Preparation of Thin Layer**: A continuous layer coated manually or with the use of applicator. The thickness of layer is 200um. Spreading Activation of plates at 110oC. Slurry of coating material is applied by: Pouring, Dipping, Spraying and Spreading.

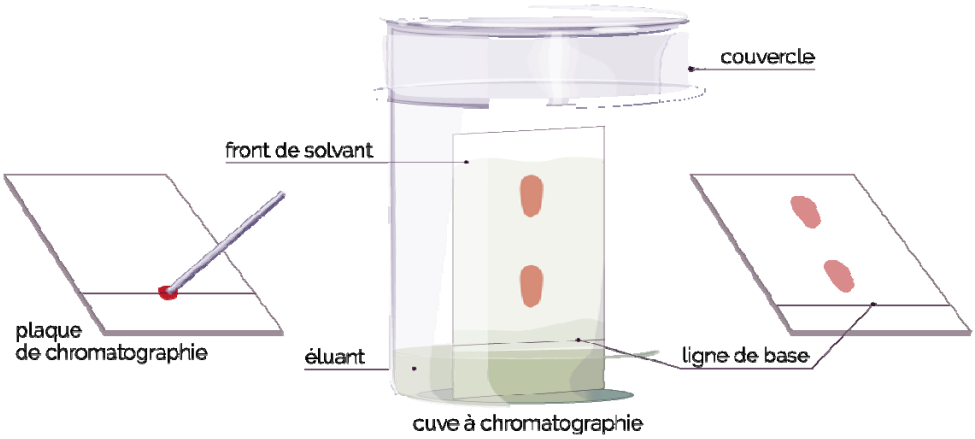



**Figure 2**: Components of Thin Layer Chromatography

### 3.5. Retention Factor (Rf) Value:

The behavior of a compound on a TLC is usually described in terms of its relative mobility or Rf value. Rf or Retention factor is a unique value for each compound under the same conditions.

$$Rf = \frac{\text{distance travelled by the component}}{\text{distance travelled by the solvent}}$$

**Figure 3**: Formula to calculate the Retention factor (Rf)

The Rf for a compound is a constant from one experiment to the next only if the chromatography conditions below are also constant:

- solvent system
- adsorbent
- thickness of the adsorbent
- amount of material spotted
- temperature

Since these factors are difficult to keep constant from experiment to experiment, relative Rf values are generally considered [10].

## 4. Thin-Layer Chromatography in our study

### 4.1. Reagents and solvents:

In our study, TLC analysis and lipid extraction were performed using for extract solubilization and as running solvents: **Chloroform** (≥99.5% CAS 67-66-3, Supelco, Sigma-Aldrich, Saint-Louis, MO, USA), **methanol** (≥99.5% CAS 67-56-1, Supelco, Sigma-Aldrich, Saint-Louis, MO, USA) and **distilled water** (GFL 2004 Water Still for single distillation, Lauda, Burgwedel, Germany)

TLC staining solutions were prepared using as reagents:

- **Orcinol**: for detection of glycosides/glycolipids (ex: HexCer). Reagent: dissolve 0.1g orcinol in 40.7ml conc. HCl, add 1ml 1% ferric (111) chloride, and dilute to 10ml Spray and heat at 80°C for 90 minutes. Results: Glycolipids produce violet spots.

For all analysis and extraction procedures, **glacial acetic acid** was purchased from Loba Chemie PVT, Mumbai, India. Analytical **Galactosylceramides** and **glucosylceramides** were purchased from Sigma-Aldrich

**Ammonium formate** for mass spectrometry was obtained from Sigma-Aldrich. The extracts were prepared using **chloroform** and **methanol**. [11]

### 4.2. Clinical samples:

First-void morning urine samples (10-20 mL) were collected from each patient for the characterization of Hexosylceramides.

The algorithm for patient sample analysis is shown in Figure 4. Biochemical analyses were confirmed using enzymatic assays depending on the suspected condition, and performed using 60-70 µL of whole blood spotted on Whatman filter paper.

### 4.3. TLC analysis of untreated urine samples:

First-void morning urine was applied to glass silica gel coated TLC plates. The volume of urine used is calibrated to meet a 5-µg creatinine content to enable different dilutions of urine to be compared [12].

The following formula was used for calculation:

**Volume of urine (µL) = 5 / (0·113 x mmol creatinine / L).**

After each sample deposit, the plate is dried under a stream of hot air, TLC plates were eluted with a solvent system composed of n-butanol/ glacial acetic acid/water. After two successive elution [13], the plates were stained with an 0.2% orcinol solution in a 98% sulfuric acid/methanol mixture to detect oligosaccharides [14]

The TLC plate was incubated at 100 °C during 10 min after spraying before observation.

### 4.4. Extraction of urinary lipids:

The extraction method for non-polar urinary lipids including Glucosylceramides and Galactosylceramides was adapted from Folch et al. [15] with slight modifications (Figure 4). Briefly, 15 µL of glacial acetic acid were added to 10 mL of freshly collected morning urine and the sample was left overnight at 4 °C, since the acidification reduces the solubility of these lipids [16].

A 2-mL volume of distilled water was added to the filtrate and the mixture was stirred for 30min at room temperature before centrifugation at 2000 rpm for 30 min. The lower phase,

containing the lipid extract, was evaporated to dryness under a stream of nitrogen gas and stored at – 20 °C until use.

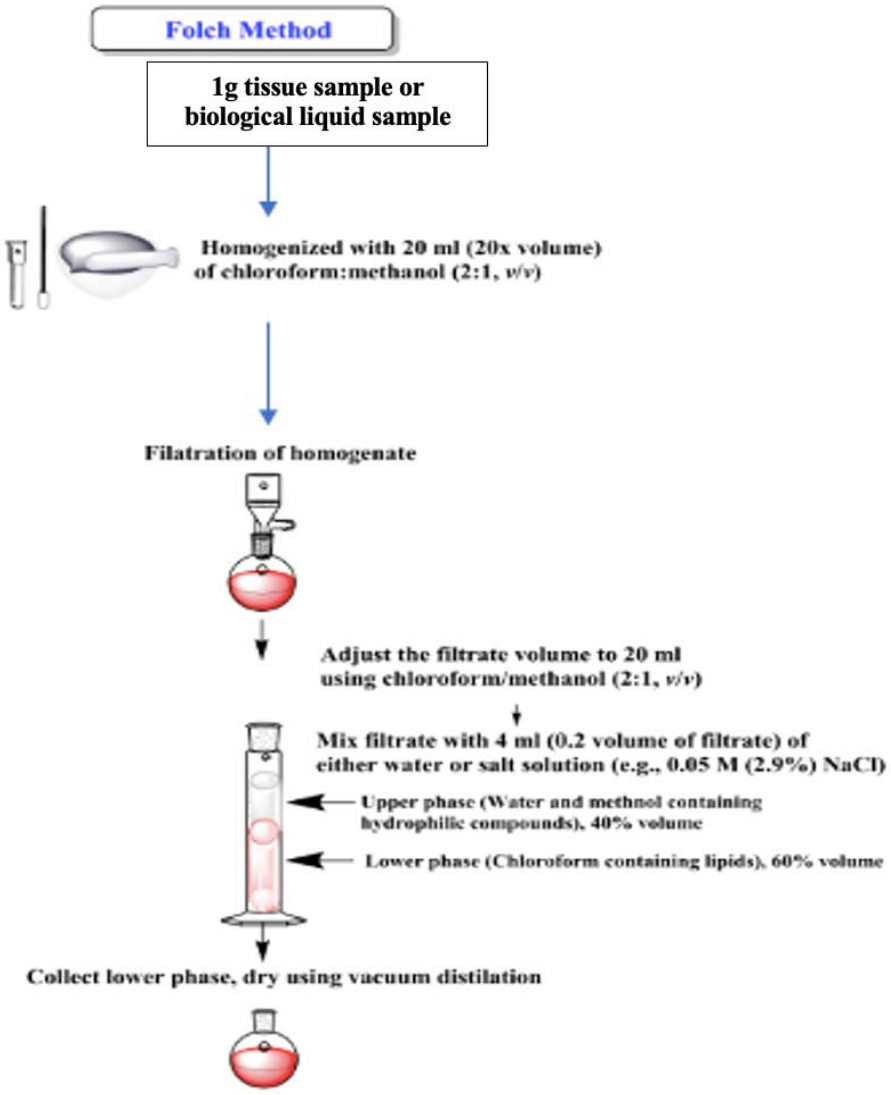



**Figure 4**: Flow chart of the algorithm used in Folch method. After centrifugation during 10 min at 2500 rpm, the pellet was collected and extracted by 5 mL of 2:1 (v/v) chloroform/methanol mixture. After sonication (Branson® sonicator, 6 ×30 s), 1 mL of distilled water was added, and the resulting solution submitted to magnetic stirring for 2 h and filtered through a sintered glass funnel of medium porosity.

### 4.5. TLC analysis of urinary lipid extracts:

The extract was dissolved in (0.1-0.2) mL of a chloroform: methanol 2:1 (v/v) mixture, and half of the solution was spotted on the Aluminum silica gel coated TLC plate along with commercial standards Galactosylceramides (**GalCer**) and Glucosylceramides (**GlcCer**).

The running solvent was a mixture of chloroform/methanol/ water (72:28:3.5, v/v/v). After drying, the lipids were revealed by spraying the following solutions, orcinol 0.2% in 98% sulfuric acid/methanol 13:87 (v/v) to detect glycolipids after heating [14].

For our Cohort, the lipid extracts are separated by one-dimensional thin-layer chromatography (1D-TLC),

While 1D- TLC is somewhat less sensitive, multiple samples can be analyzed simultaneously on a single plate and in a shorter time than in 2-D TLC. This aligns more with our main objective: a rapid diagnosis orientation.

In order to capitalize on the information obtained by TLC and avoid errors, a preparative TLC was performed for each band visualized on our plates, at least once. Once the bands have been identified, they are removed by scraping the band with a spatula, the scraping powder obtained is transferred to a tube, the solvent is added and the adsorbent is separated by filtration.

Subsequently, a combination of all the data at our disposal: the retention factor (Rf) of the biomarker (the band characterized by the preparative TLC), the patient's symptoms, and the pure Rf standards, allows us to associate a biomarker to a band revealed on our TLC plate.

## IV. Ethical considerations

The ethical rules regarding the respect of anonymity, confidentiality and the protection of patient data were followed during the completion of this work.

# *RESULTS*

# I. Baseline characteristics:

## 1. Age at admission

The mean age at the time of admission for the entire study was 5,22 years, ranging from 27 days to 25 years. The age group most affected was the 3-10 age range, with a frequency of 38.3%. (TableI, Figure5)

Table I : Distribution by patients' ages

| Age | < 1 year | 1 to 3 years | 3 to 10 years | 10 to 16 years | > 16 years |
|---|---|---|---|---|---|
| Number (%) | 7 (14.9%) | 14 (29.8%) | 18 (38.3%) | 5 (10.6%) | 3 (6.4%) |

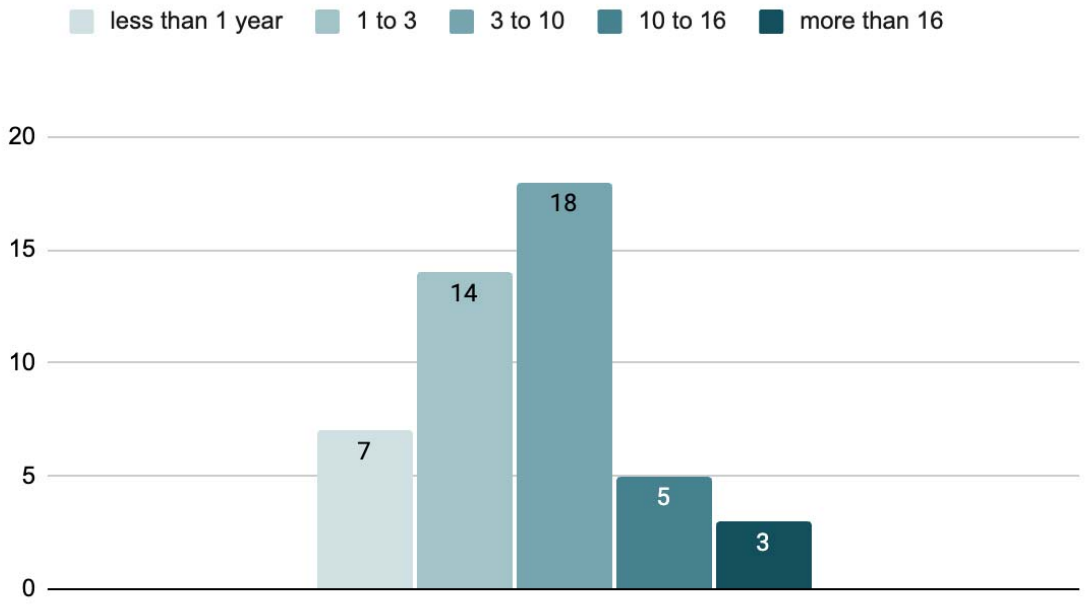



Figure 5 : Distribution of studied population by age at the time of admission.

## 2. Gender

47 patients were identified of whom were 22 male (46.8%) and 25 females (53.2%). Analysis of distribution based on age and gender shows slight female predominance with a sex male to female ratio of 0.85:1. (Figure 6)

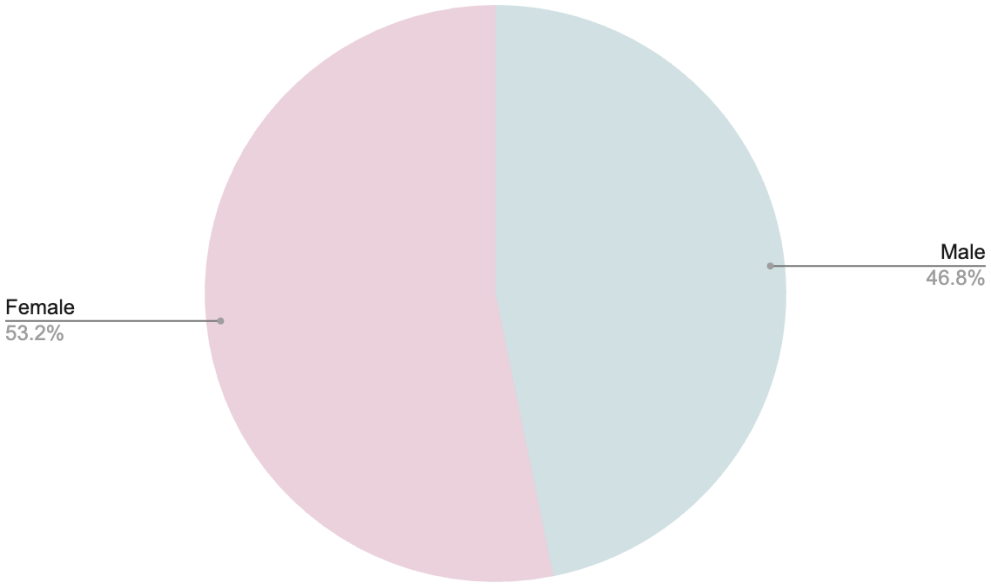



**Figure 6** : Distribution of studied population by gender.

## 3. Habitat

Our study included 47 patients distributed between rural and urban areas. A slight predominance of is noted in patients located in the rural area (51.1%) (Figure 7)

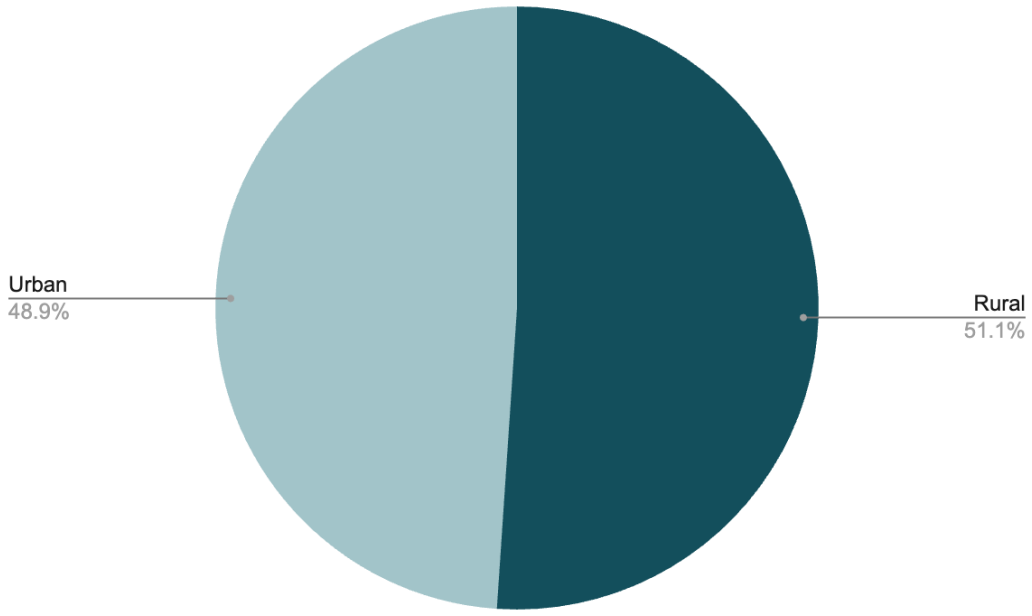



**Figure 7 :** Distribution of studied population by living environment.

# II. Medical history

## 1. Personal

The table 2 presents the personal past medical history of our serie (TableII, Figure 8):

Table II : Personal past medical history.

| Past medical history | Number of cases |
|---|---|
| No history | 38 (80.9%) |
| Cholestatic jaundice | 4 (8.5%) |
| Liver cirrhosis | 1 (2.1%) |
| Perinatal asphyxia | 2 (4.25%) |
| Intrauterine growth retardation | 2 (4.25%) |

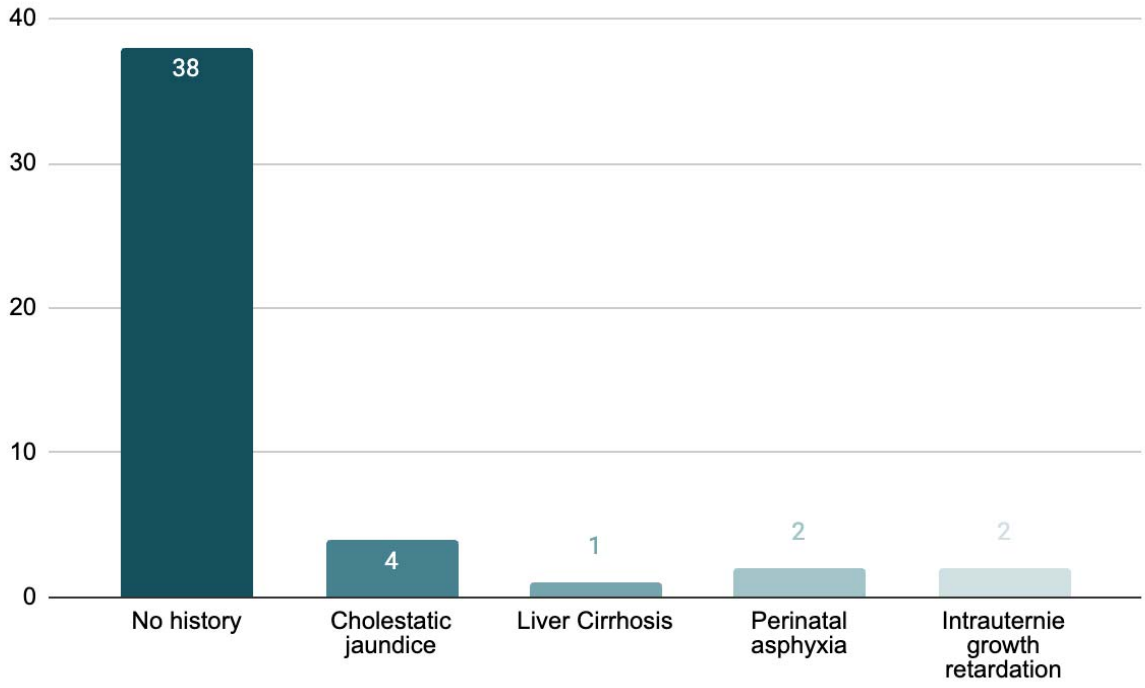



**Figure 8** : Chart of our patients' personal past medical history.

## 2. Familial

Out of the 47 patients, 28 patients (59.6%) were born to first (46.8%), second (8.5%) and third-degree (6.4%) consanguineous parents. 15 (31.9%) patients with no medical history, 10 (21.3%) patients with history of death among siblings and 10 (21.3%) patients had similar cases in the family. (Figure 9)

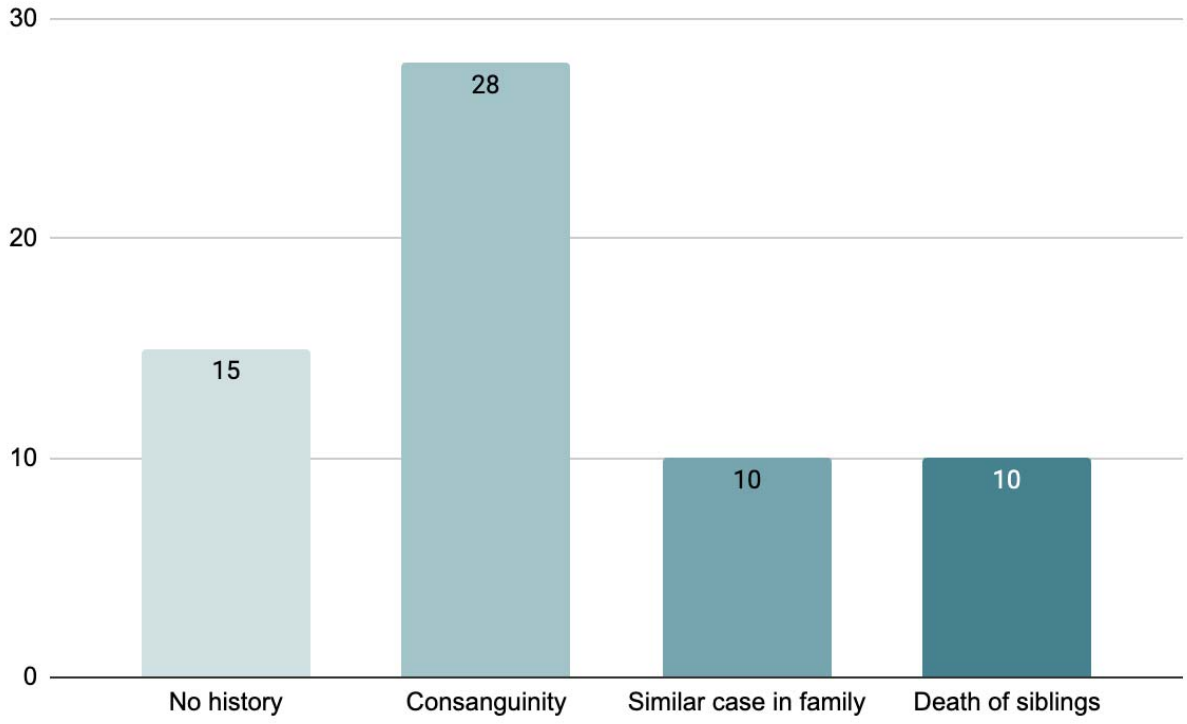



**Figure 9** : Chart of familial past medical history.

# III. Clinical data

## 1. Age at onset of symptoms

The average age at onset of symptoms was 2.63 years with a maximum of 18 years and a minimum of 3 days of life. We grouped our patients by age into neonatal, early infantile, late infantile, juvenile and adult. The predominant age group was early infantile with a percentage of 40.4% (TableIII, Figure 10)

Table III : Distribution by patients' ages at onset of symptoms.

| Age at onset of symptoms | Neonatal ≤ 1 month | Early infantile 1 month to 1 year | Late infantile 1 to 4 years | Juvenile 5 to 15 years | Adult ≥ 16 years | Total |
|---|---|---|---|---|---|---|
| **Number (%)** | 3 (6.5%) | 19 (40.4%) | 16 (34%) | 8 (17%) | 1 (2.1%) | **47 (100%)** |

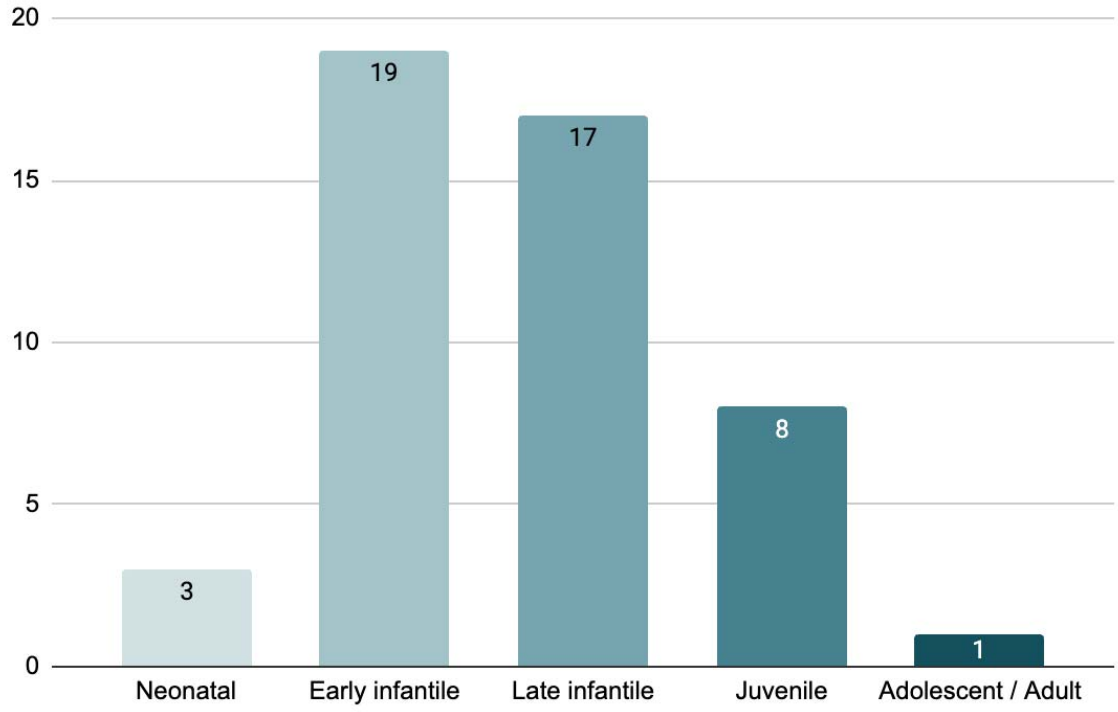



Figure 10 : Chart of age at onset of symptoms

## 2. Clinical manifestations:

Onset symptoms are symptoms that mark the beginning or onset of a disease or illness.

The chief complaints in our series were as follows:

- Psychomotor regression: This was the primary symptom observed in 21 patients (44.7%).
- Psychomotor delay, observed in 20 patients (42.5%) followed by seizures in 18 patients (38.3 %)

The clinical manifestations deduced from thorough physical examination are presented in the table (TableIV) and chart (Figure 11) below:

Table IV : Table of clinical manifestations.

| Symptoms | | Number | Percentage |
|---|---|---|---|
| Psychomotor regression | | 21 | 44.7% |
| Psychomotor delay | | 20 | 42.5% |
| Seizures | | 18 | 38.3 % |
| Hepatomegaly | | 9 | 19.1 % |
| Delayed growth | | 9 | 19.1 % |
| Facial dysmorphia | | 8 | 17 % |
| Splenomegaly | | 5 | 10.6 % |
| Abnormal movements | | 9 | 19.1 % |
| Ocular impairment | Nystagmus | 10 | 21.2 % |
| | Decreased visual acuity/Blindness | 2 | 4.25 % |
| Hypotonia | | 7 | 14.9 % |
| Ataxia | | 4 | 8.5 % |
| Extrapyramidal symptoms | | 3 | 6.4 % |

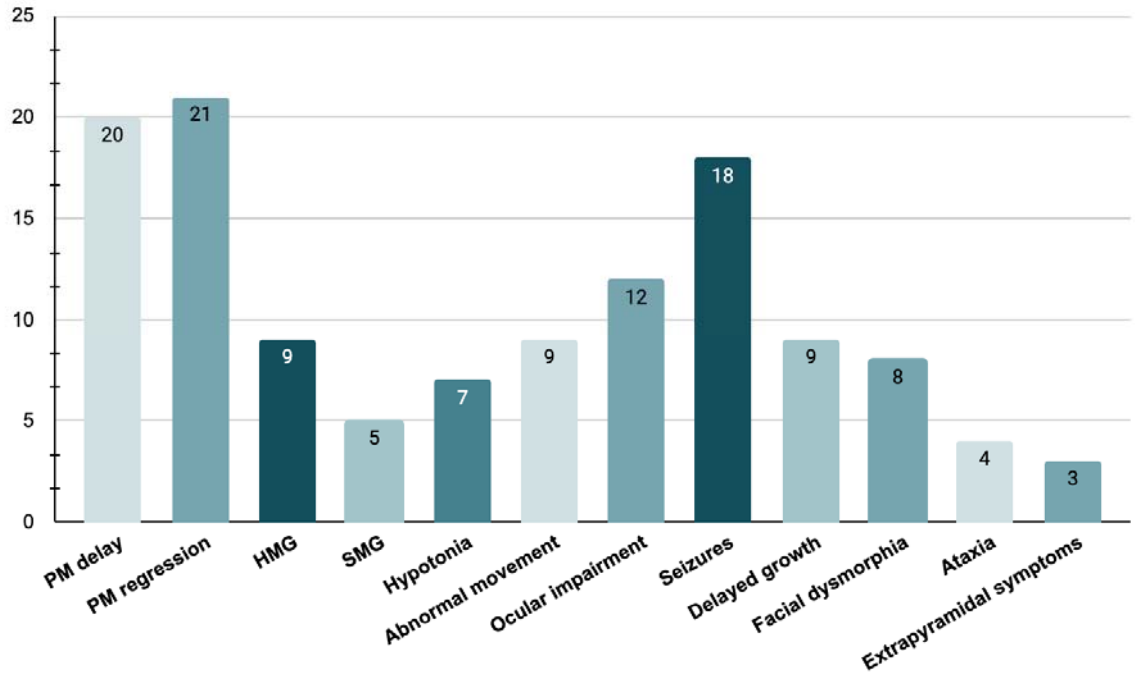



**Figure 11** : Chart of clinical manifestations (PM= Psychomotor, HMG= Hepatomegaly, SMG= Splenomegaly).

# IV. Paraclinical data

## 1. General tests:

### 1.1. Biology:

❖ **Complete blood count:**

CBC came back normal for 3 patients while 5 (10.4%) patients had isolated anemia and 1 patient's CBC showed pancytopenia. We didn't have data for the rest of the patients.

❖ **Liver function:**

11 patients underwent liver function test, with 3 normal and 8 showing hepatocellular insufficiency (16.6%).

❖ **Hemostasis blood test**

14 patients had hemostasis blood test in which 8 patients had normal results and 6 patients had coagulation abnormalities with Prothrombin Ration < 70% (12.5%).

### 1.2. Radiology and other:

❖ **Brain MRI:**

Brain MRI was performed in 31 (65.9%) cases. 11 (23.4%) patients had normal Brain MRI results, while 20 (42.5%) patients showed different abnormalities. The table below (TableV) presents the results of the MRIs and figures (Figure12, 13, 14) showed examples of Brain MRIs with the abnormalities found in our study.

Table V : Table of the results of the brain MRIs. (WMSA= White matter signal abnormality, CSC= Corticosubcortical atrophy)

| Brain MRIs results | Number of results |
|---|---|
| No MRI | 16 |
| No abnormalities | 11 |
| CSC/Cerebral atrophy only | 8 |
| WMSA (Leukodystrophy) only | 7 |
| WMSA + CSC Atrophy | 2 |
| Bi-pallidal and bulboprotuberontial lesions | 1 |
| WMSA + CSC ATROPHY + Dysmyelinating disease | 1 |
| Dysmyelinating plaques | 1 |

Table VI : Table of MRI results and patients' diagnosis (MLD= Metachromatic leukodystrophy, RR= Relapsing-Remitting)

| | No brain MRI (n=17) | Normal brain MRI (n=11) | Abnormal brain MRI (n=20) |
|---|---|---|---|
| **No diagnosis** | 12 patients | 8 patients | 12 patients |
| **Confirmed diagnosis** | 1- Gaucher disease<br>2- Gaucher disease<br>3- Niemann-Pick A disease<br>4- Niemann-Pick A disease<br>5- Ornithine transcarbamylase deficiency | 1- Menkes disease<br>2- Tyrosinemia type 1<br>3- Krabbe disease | 1- MLD<br>2- MLD<br>3- RR Multiple sclerosis<br>4- Free sialic acid disease<br>5- Profound Biotinidase deficiency<br>6- Neurowilson<br>7- Tay-Sach disease<br>8- Neuronal ceroid lipofuscinosis1 |

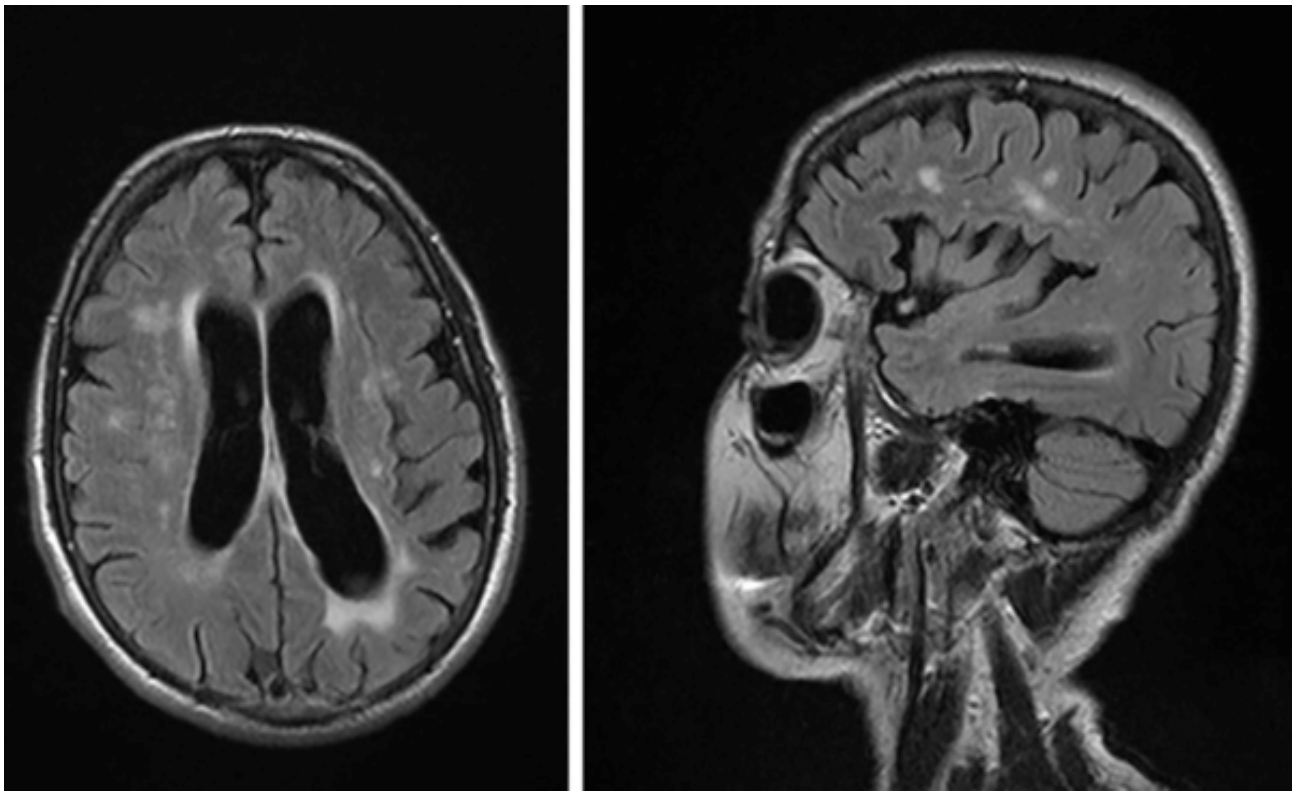


Figure 12: Sagittal and Axial FLAIR MRI sequence showing corticosubcortical atrophy.

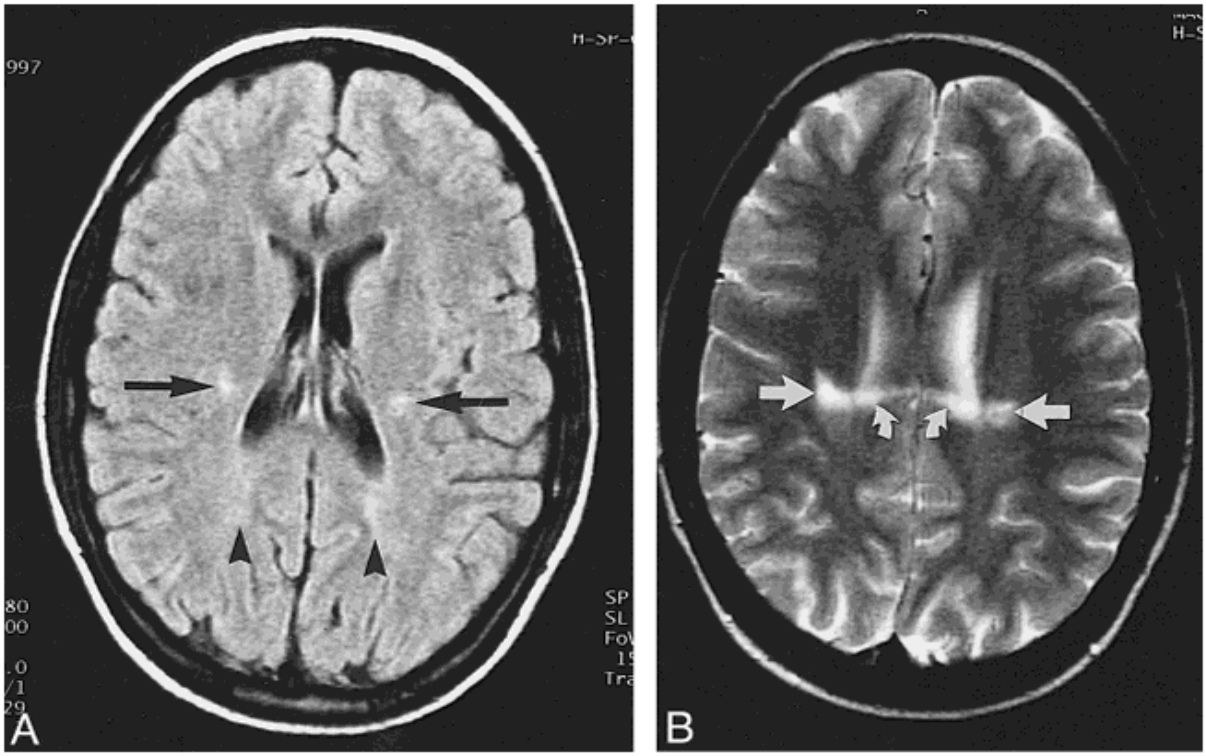



**Figure 13** : Axial MRI showing white matter signal abnormality

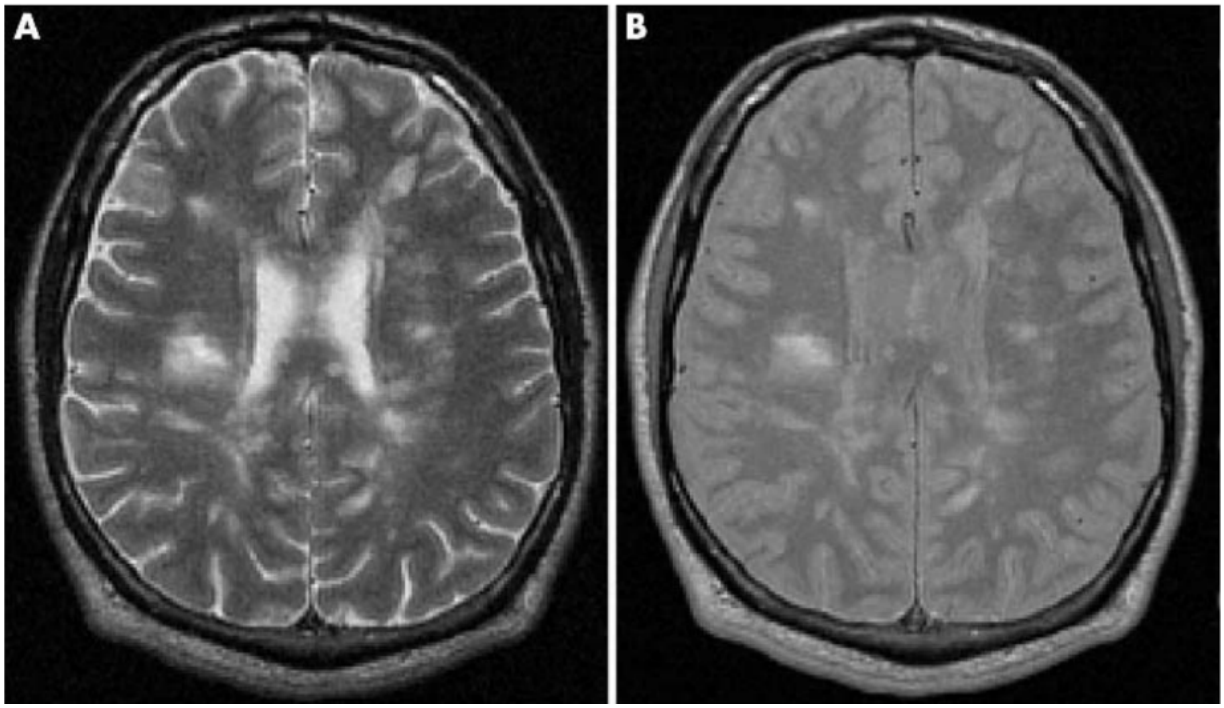



**Figure 14** : Axial MRI of a patient with Relapsing-Remitting Multiple sclerosis showing multiple dysmyelinating lesions.

❖ **Brain scan:**

Brain scans were performed in 5 patients. 4 (P1, P2, P3, P4) patients had normal scans while the 5th patient's (P5) scan showed corticosubcortical atrophy. The table (TableVII) below present the results of the Brain scans and the clinical features corresponding to each patient.

**Table VII : Table of the results of the brain scans.**

| Scan results | Patients | Age, gender | Symptoms | Other tests |
|---|---|---|---|---|
| Normal | P1 | 1, ♀ | Psychomotor regression, hypotonia, seizures, delayed growth | - Brain MRI: Cortical dysplasia |
| | P2 | 3, ♂ | Psychomotor delay, seizures, abnormal movements | - EEG: Normal |
| | P3 | 1.8, ♂ | Psychomotor regression, seizures | - |
| | P4 | 25, ♀ | Abnormal movements, seizures | - Brain MRI: White matter signal abnormalities, Corticosubcortical atrophy, dysmyelinating disease |
| Abnormal | P5 | 3, ♂ | Psychomotor regression, hypotonia, seizures, ocular impairment | - Brain MRI : Corticosubcortical atrophy<br>- Enzymatic activity: Palmitoyl protein thioesterase (PPT1) deficiency |

❖ **Electroencephalogram (EEG):**

Of our 47 patients, 16 (33.3%) presented with seizures, and 7 (14.6%) underwent EEG testing. Among these, 4 EEGs revealed normal activity, while 3 showed generalized paroxysmal discharges, suggestive of epilepsy. Table VIII below summarizes the data of the patients who had EEGs performed in our study.

Table VIII : Table of patients' data who underwent EEG testing in our study (PM= Psychomotor, WMSA= White matter signal abnormality, CSC= Corticosubcortical.

| | EEG RESULTS | | | | | | |
|---|---|---|---|---|---|---|---|
| | Normal EEGs | | | | Abnormal EEG | | |
| **Patients** | **P1** | **P2** | **P3** | **P4** | **P5** | **P6** | **P7** |
| **Age (Age at onset)** | 3 years (0.5) | 3 years (1.5) | 4 years (1) | 2 years (0) | 1 year (1) | 2 years (0.6) | 7 years (2) |
| **Gender** | Female | Male | Male | Female | Female | Male | Female |
| **Clinical manifestations** | - PM delay<br>- PM regression<br>- Ocular impairment<br>- Seizures<br>- Delayed growth | - PM delay<br>- Ab. Movement<br>- Seizures<br>- Delayed growth | - Seizures<br>- Ataxia | - Seizures | - PM delay<br>- Seizures<br>- Ataxia | - PM delay<br>- PM regression<br>- Seizures | - PM regression<br>- Abnormal movement<br>- Seizures<br>- Extrapyramidal symptoms |
| **Brain MRI/ Brain scan results** | Cerebral Atrophy+ WMSA | Normal | WMSA | Normal | CSC Atrophy | Normal | CSC Atrophy |

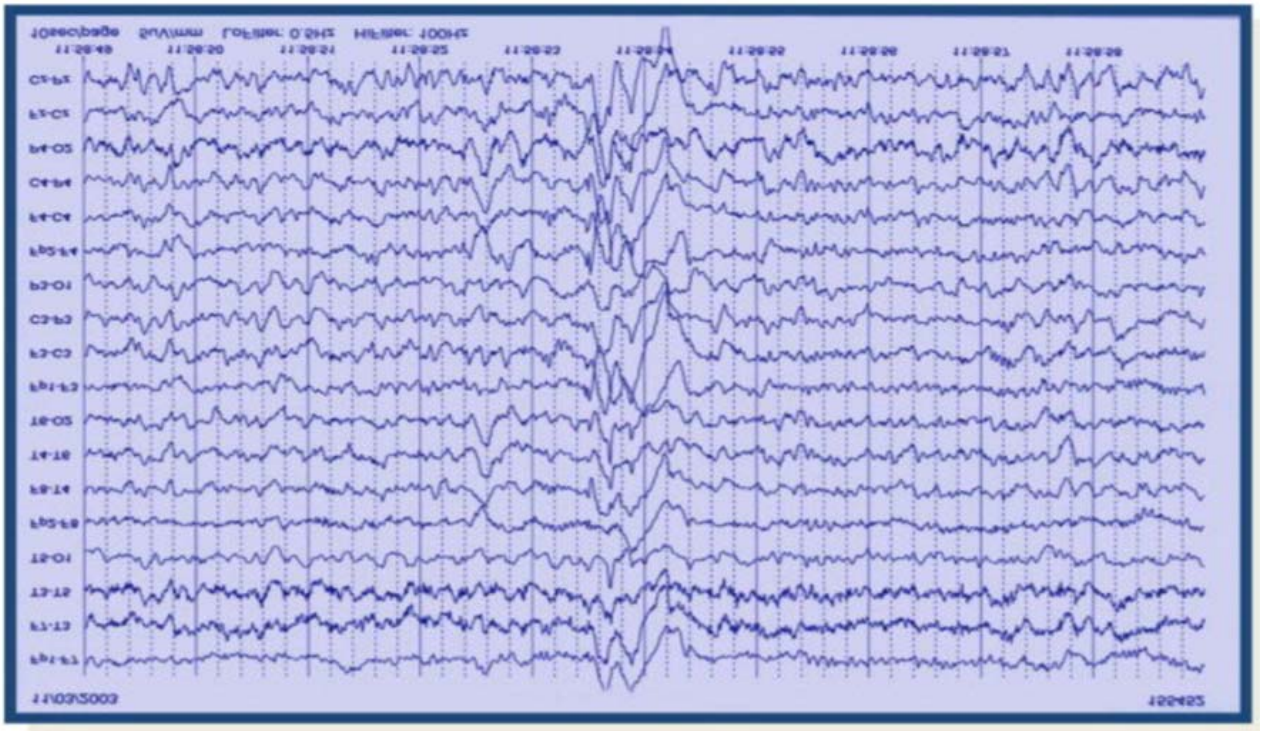


**Figure 15** : EEG showing frequent paroxysmal generalized bursts of low amplitude spike-and-wave discharges

❖ **Visual evoked potential (VEP):**

In our study, only one patient underwent VEP testing due to suspected retrobulbar optic neuritis flare-up following a sudden decrease in visual acuity in the left eye. The patient is a 14-year-old male diagnosed with Relapsing-Remitting Multiple sclerosis at the age of 10, his brain MRI revealed several dysmyelinating lesions.

The VEP showed a prolonged P100 latency which confirmed the left-sided demyelinating optic neuropathy.

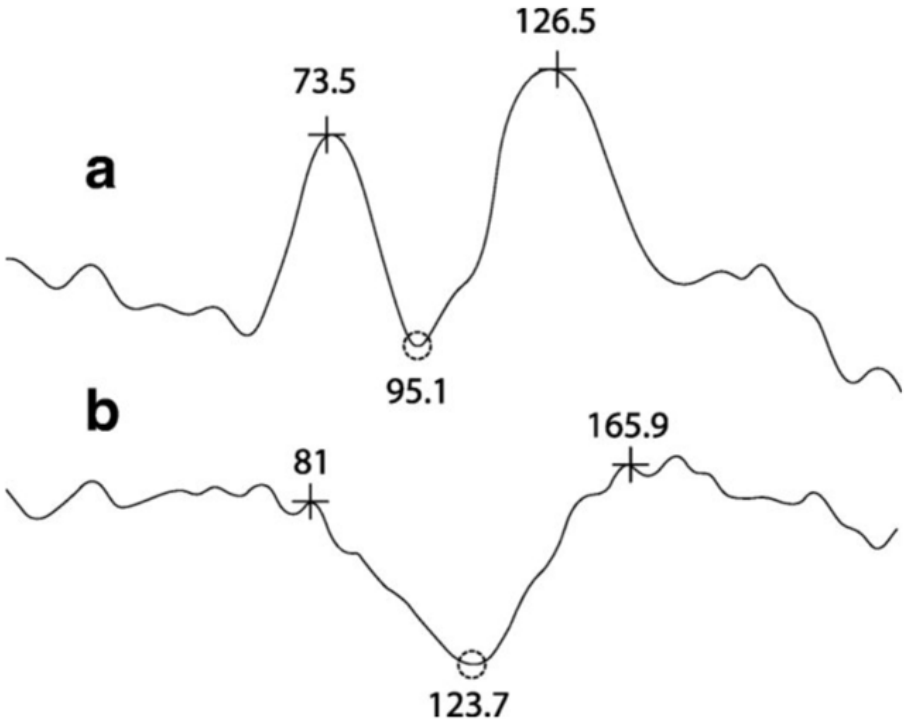



**Figure 16** : (a) Normal and (b) abnormal prolonged VEP P100 latency.

## 2. Specialized tests:

The specialized tests used to help diagnose the patients in our study are:

- Enzymatic dosage using Tandem mass spectrometry from Dried Blood Spot (DBS),
- Biomarkers essays such as Lyso-GL1for Gaucher disease and Lyso-Sphingomyelin for Niemann-Pick disease using HPLC/MS.
- Thin-Layer Chromatography (TLC).

### 2.1. Enzymatic and biomarkers dosage:

The enzymatic dosages included are reported on the table below:

Table IX : The enzymatic activity tests used in our study.

| Disease | Enzymatic test |
|---|---|
| Gaucher disease | Glucocerebrosidase (GCase) |
| Krabbe disease | Galactocerebrosidase (GALC) |
| Niemann-Pick disease | Sphingomyelinase (ASM) |
| Metachromatic leukodystrophy | Arylsulfatase A (ARSA) |
| Neuronal ceroid lipofuscinosis 1 | Palmitoyl protein thioesterase (PPT1) |
| Neuronal ceroid lipofuscinosis 2 | Tripeptidylepeptidase (TPP1) |
| Tay-Sach disease | Hexosaminidase-A (HEX-A) |
| Sandhoff disease | Beta-hexosaminidases A (Beta HEX-A) |

Out of 47 patients in our study:

- 25 patients (53.1%) didn't get tested for enzymatic activity or biomarkers.
- 11(23.4%) patients were tested for Krabbe disease, Metachromatic leukodystrophy (MLD), Neuronal ceroid lipofuscinosis 1&2 (NCL1, NCL2), Tay-Sach disease and Sandhoff disease. The tests came back normal for these diseases. Among these 11 patients, 3 were also tested negative for both Gaucher disease and Niemann-Pick disease, 1 patient tested negative for Gaucher disease and 1 other patient tested negative for Niemann-Pick disease.
- 1 (2.1%) patient was suspected with Niemann-Pick disease and tested negative for this disease and 1 (2.1%) other patient was tested for both Gaucher and Niemann-Pick disease but the enzymatic essay was normal for both diseases.
- Enzymatic essay in 1 (2.1%) patient showed GALC deficiency which confirmed Krabbe disease.
- A diagnosis of Gaucher disease was established in 2 (5.16%) patients following the identification of low glucocerebrosidase levels.
- 2 patients (4.2%) were diagnosed with Niemann-Pick A disease after enzymatic dosage revealed deficiency in Sphingomyelinase in both patients.

- ❖ 2 patients (4.2%) were diagnosed with Metachromatic Leukodystrophy after enzymatic dosage revealed low Arylsulfatase A activity in both patients.
- ❖ Tay-Sachs disease and Neuronal ceroid lipofuscinosis1 was identified in 2 different patients (4.2%) upon finding low Palmitoyl protein thioesterase (PPT1) and Hexosaminidase-A activity respectively.

Regarding the biomarker's dosage, 4 patients suspected of Gaucher disease had normal Lyso-GL1 levels. Additionally, 1 patient suspected of Niemann-Pick disease had normal Lyso-Sphingomyelin levels.

### 2.2. Chromatography:

Thin-Layer Chromatography was performed on all 47 patients (100%), the TLCs revealed the presence of Hexosylceramides (Glucosylceramides and/or Galactosylceramides) in all patients. (Figure 17)

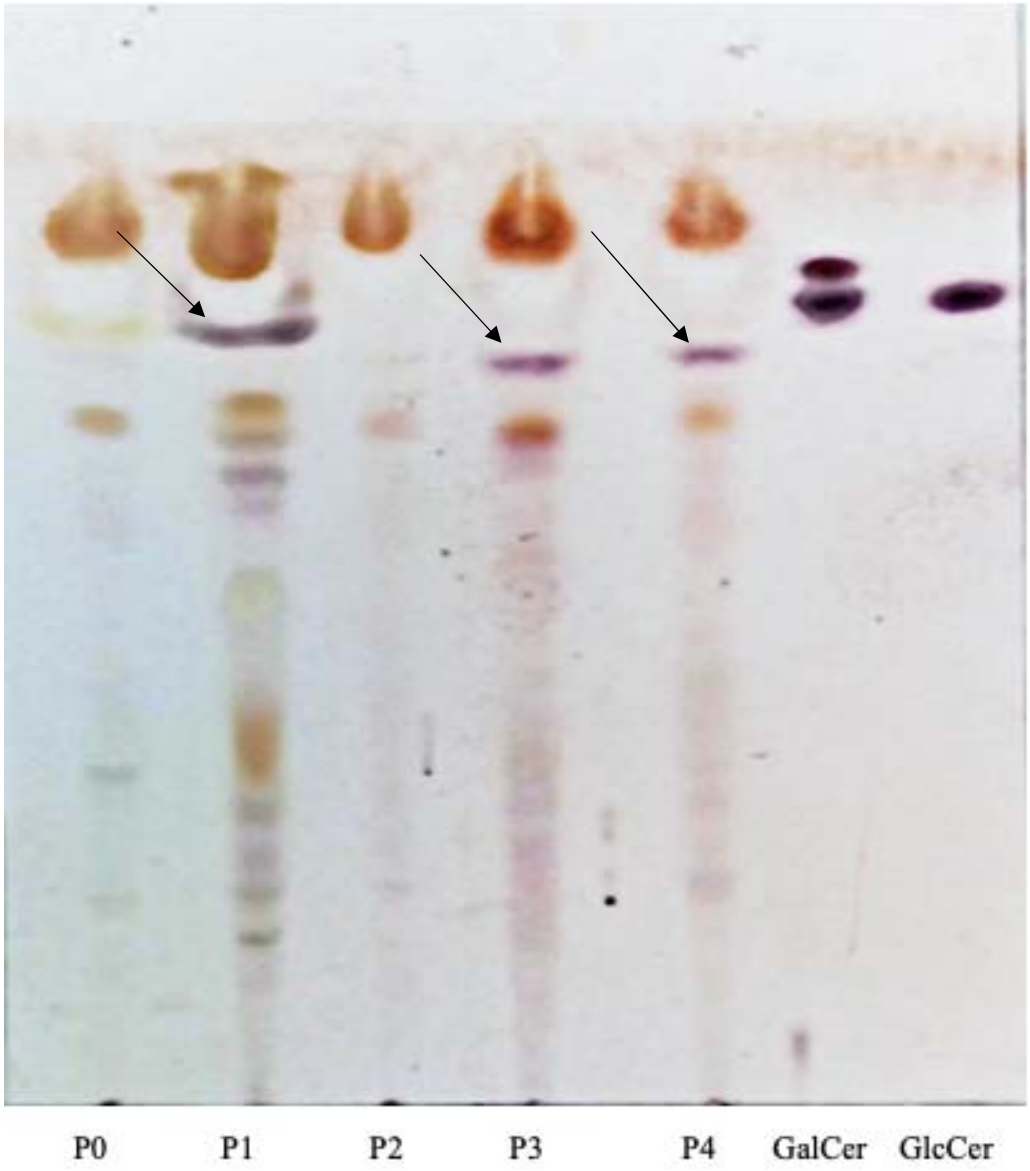



**Figure 17 :** Thin layer chromatography (TLC) plates of urinary lipid extract showing the Hexosylceramides excretion from patients comparatively to commercial standards. Standards correspond to GalCer: Galactosylceramides (Rf 0.79-0.82) and GlcCer: Glucosylceramides (Rf 0.77). Patients correspond to P1: Patient with Gaucher disease, P3: Patient with psychomotor delay and abnormal movements whose brain MRI showed white matter signal abnormality and P4: Patient with Profound biotinidase deficiency. The amount of spotted extract was equivalent to 5mg of creatinine. Migration solvent: chloroform/methanol/water. Lipid fractions were visualized using orcinol. Rf: ratio to the front.

In our study, we distinguished 2 clinical forms:

- Form I: with only neurological symptoms (Psychomotor regression, psychomotor delay, seizures, ocular impairment...). 37 (78.7%) patients were concerned with the 1st form features
- Form II: with neurological and visceral symptoms (Hepatomegaly and splenomegaly). Overall, 10 (21.3%) patients were presented with the 2nd form's features.

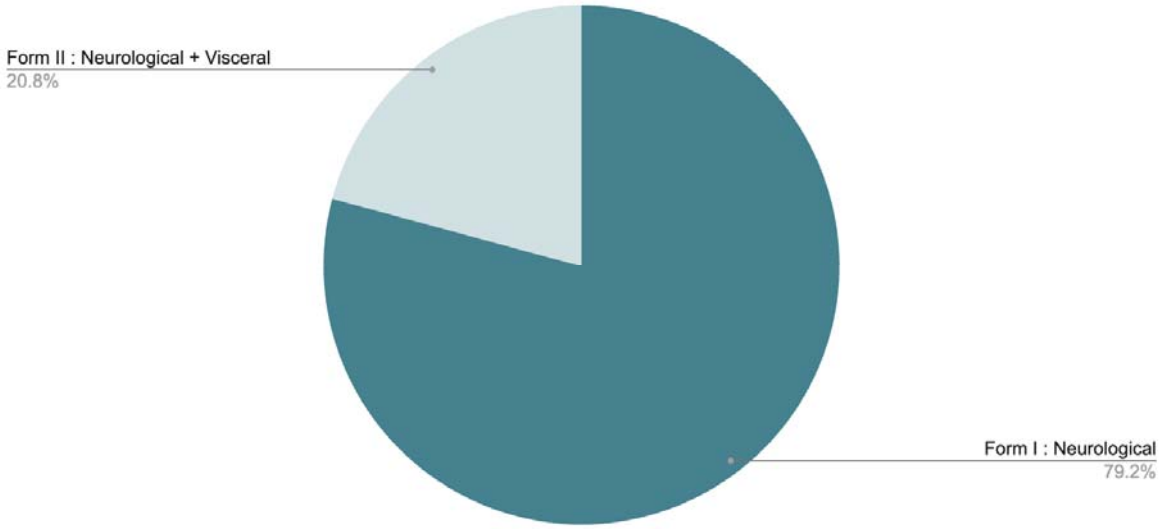



**Figure 18 :** Pie chart of the 2 clinical forms found in our study.

To simplify the analysis of the collected data, we have summarized the information of our patients in the table below.

# V. Diagnosis:

We were able to diagnose 16 patients (42.1%). Analysis and diagnosis information for the patients enrolled in the study; are shown in the following table (TableXI):

Table X : Summary of the clinical and paraclinical data of the patients classified according to the clinical form (HCI= Hepatocellular insufficiency)

| | | Form I (n=37) | Form II (n=10) |
|---|---|---|---|
| Age (average age) | | 27 days-25 years (5.4 years) | 6 months-11 years (4.5 years) |
| Gender | Female | 19 (51.4%) | 6 (60%) |
| | Male | 18 (48.6%) | 4 (40%) |
| Medical history | Personal history | - No history (86.5%)<br>- Perinatal asphyxia (5.4%)<br>- Intrauterine growth retardation (5.4%)<br>- Cholestatic jaundice (2.7%)<br>- Liver cirrhosis (0%) | - No history (60%)<br>- Cholestatic jaundice (30%)<br>- Liver cirrhosis (10%)<br>- Perinatal asphyxia (0%)<br>- Intrauterine growth retardation (0%) |
| | Familial history | - No history (32.4%)<br>- Consanguinity (59.5%)<br>- Death of siblings (24.3%)<br>- Similar case in family (24.3%) | - No history (30%)<br>- Consanguinity (60%)<br>- Similar case in family (10%)<br>- Death of siblings (10%) |
| Age at onset of symptoms (average age) | | 1 day -18 years (2.86 years) | 3 days-5 years (1.79 years) |
| Revelation mode | | - Psychomotor regression (48.6%)<br>- Psychomotor delay (45.9%)<br>- Seizures (45.9%)<br>- Ocular impairment (24.3%)<br>- Abnormal movement (18.9%)<br>- Hypotonia (16.2%)<br>- Ataxia (8.1%)<br>- Extrapyramidal symptoms (7.9%)<br>- Delayed growth (16.2%)<br>- Facial dysmorphia (18.9%) | - Hepatomegaly (90%)<br>- Splenomegaly (50%)<br>- Psychomotor regression (30%)<br>- Psychomotor delay (30%)<br>- Seizures (10%)<br>- Ocular impairment (30%)<br>- Abnormal movement (20%)<br>- Hypotonia (10%)<br>- Ataxia (10%)<br>- Delayed growth (30%)<br>- Facial dysmorphia (10%) |

Table X : Summary of the clinical and paraclinical data of the patients classified according to the clinical form (HCI= Hepatocellular insufficiency) (suite…)

| | | | Form I (n=37) | Form II (n=10) |
|---|---|---|---|---|
| Tests | General tests | Biology | - *CBC*: Normal (7.9%)<br>- *Liver function*: HCI (13.1%), Normal (7.9%)<br>- *Hemostasis test*: Low TP (7.9%) | - *CBC*: Pancytopenia (10%), Anemia (10%)<br>- *Liver function*: HCI (30%)<br>- *Hemostasis test*: Low TP (30%), Normal (10%) |
| | | Radiology | - *Brain MRI* : Normal (21.6%), Abnormal MRI (54%) (Table6), No MRI (24.4%)<br>- *Brain scan*: Normal (10.8%), Corticosubcortical atrophy (2.7%) | - *Brain MRI*: Normal (30%), No MRI (70%) |
| | | Other | - **EEG**: Normal (10.5%), Generalized paroxysmal<br>- discharges (8.1%)<br>- **VEP**: Demyelinating optic neuropathy (2.7%) | - |
| | Specialized tests | Enzymatic Dosage | - No enzymatic dosage (62.1%)<br>- Normal GALC, ARSA, PPT1, TPP1, HEX-A, Beta HEX-A enzymatic activity for (13.5%)<br>- Normal GCase and ASM activity (5.4%)<br>- Normal GALC, ARSA, PPT1, TPP1, HEX-A, Beta HEX-A, GCase and ASM enzymatic activity (2.7%)<br>- Normal GCase and ASM activity (2.7%)<br>- Normal ASM activity (2.7%)<br>- ARSA deficiency (5.4%)<br>- GALC deficiency (2.7%)<br>- PPT1 deficiency (2.7%)<br>- HEX-A deficiency (2.7%) | - No enzymatic dosage (30%)<br>- Normal GALC, ARSA, PPT1, TPP1, HEX-A, Beta HEX-A enzymatic activity for (10%)<br>- Normal GCase and ASM activity (10%)<br>- Normal ASM activity (10%)<br>- GCase deficiency (20%)<br>- ASM deficiency (20%) |

Table XI : Clinical data of patients with diagnosis

| Clinical form | Diagnosis | Medical history | Age (year), gender of patients | Age at onset (years) | Clinical features | Radiology results | Biology test results | Means of diagnosis | Prognosis and treatment |
|---|---|---|---|---|---|---|---|---|---|
| **Form I (n=9)** | **Remitting-Relapsing Multiple Sclerosis** | No medical history | 14, ♂ | 10 | Ocular impairment: brutal decrease in visual acuity | -**Brain MRI**: Dysmyelinating lesions<br>-**VEP**: demyelinating optic neuropathy | ∅ | Brain MRI | Improvement during treatment: GYLENYA 0.5mg/day orally |
| | **Tay-Sach disease** | 2nd degree consanguinity | 1, ♂ | 0.75 | Psychomotor regression, Hypotonia, Ocular impairment: loss of vision | **Brain MRI:** Corticosubcortical atrophy | - HEX-A deficiency | Enzymatique dosage of HEX-A, | Treatment: Depakine: 30mg/kg/day) Life expectancy is 5 years |

Table XI : Clinical data of patients with diagnosis (suite…)

| Clinical form | Diagnosis | Medical history | Age (year), gender of patients | Age at onset (years) | Clinical features | Radiology results | Biology test results | Means of diagnosis | Prognosis and treatment |
|---|---|---|---|---|---|---|---|---|---|
| | **Metachromatic leukodystrophy** | 1st degree consanguinity | 2.5, ♀ | 1.5 | Psychomotor regression, seizures | **Brain MRI**: White matter signal abnormality | - ARSA deficiency | Enzymatique dosage ARSA, | Patient passed at 5 years. |
| | **(n=2)** | No history | 2.75, ♂ | 2.5 | Psychomotor regression, seizures | **Brain MRI**: (02/2023) Delayed myelination (supratentorial) **Brain MRI**: (07/2024) White matter signal abnormality (supra and infratentorial) | - ARSA deficiency | Enzymatique dosage ARSA | Worsening of the psychomotor regression, seizures are manageable with Depakine: 30mg/kg/day). Life expectancy 4-16 after age at onset. |

Table XI : Clinical data of patients with diagnosis (suite…)

| Clinical form | Diagnosis | Medical history | Age (year), gender of patients | Age at onset (years) | Clinical features | Radiology results | Biology test results | Means of diagnosis | Prognosis and treatment |
|---|---|---|---|---|---|---|---|---|---|
| | **Krabbe disease** | - 2nd degree consanguinity<br>- Death of sibling | 0.5, ♀ | 0.5 | Psychomotor regression, Hypotonia | Normal Brain MRI | - GALC deficiency | Enzymatic dosage of GALC | Patient passed at 7 months away during the diagnostic process |
| | **Neuronal ceroid lipofuscinosis 1** | - 1st degree consanguinity<br>- Death of sibling | 3, ♂ | 0.33 | Psychomotor regression, hypotonia, seizures, ocular impairment: nystagmus | **Brain MRI:** Corticosubcortical atrophy<br>**Brain scan:** Corticosubcortical atrophy | - PTT1 deficiency | Enzymatique dosage PTT1 | Worsening of neurological deterioration, life expectancy is 12 years of age.<br>Depakine 30mg/kg/day |

Table XI : Clinical data of patients with diagnosis (suite…)

| Clinical form | Diagnosis | Medical history | Age (year), gender of patients | Age at onset (years) | Clinical features | Radiology results | Biology test results | Means of diagnosis | Prognosis and treatment |
|---|---|---|---|---|---|---|---|---|---|
| | **Profound biotinidase deficiency** | - 1st degree consanguinity<br>- Similar case in family | 24, ♂ | 18 | Psychomotor regression, seizures | **Brain MRI**: White matter signal abnormality, dysmyelinating disease | - **Urine organic acids screening:** elevated 3-hydroxyisovaleric acid x33/control | Urine organic acids screening (GC/MS) | Slight improvement on Biotin supplements |
| | **Neurowilson** | 1st degree consanguinity | 6, ♂ | 3 | Psychomotor regression, Ataxia, Extrapyramidal symptoms, Ocular impairment: Nystagmus, Delayed growth | **Brain MRI**: Bi-pallidal and bulboprotuberontial lesions: Neurowilson<br>**Slit lamp exam**: Absence of Kayser-Fleischer ring | - **Low copper and low ceruloplasmin blood levels**<br>- **High urinary copper**<br>- **CBC**: Normal<br>- **Liver function:** IHC | Ceruloplasmin blood levels Urinary and copper levels | Treatment consists of copper-chelating |

Table XI: Clinical data of patients with diagnosis (suite…)

| Clinical form | Diagnosis | Medical history | Age (year), gender of patients | Age at onset (years) | Clinical features | Radiology results | Biology test results | Means of diagnosis | Prognosis and treatment |
|---|---|---|---|---|---|---|---|---|---|
| | **Menkes disease** | - 2nd degree consanguinity<br>- Liver cirrhosis | 9, ♂ | 5 | Psychomotor delay, Abnormal movement, SMG, seizures | **Brain MRI:** Normal | - **Low copper and low ceruloplasmin blood levels**<br>- **CBC**: Pancytopenia<br>- **Liver function:** IHC | Copper and ceruloplasmin levels | - |
| | **Free Sialic acid storage disease** | No history | 10, ♂ | 7 | Psychomotor delay, Seizures, Facial dysmorphia | **Brain MRI:** Corticosubcortical atrophy | High urinary free sialic acid dosage x2/control | **Spectrophotometry** | - |

Table XI: Clinical data of patients with diagnosis (suite…)

| Clinical form | Diagnosis | Medical history | Age (year), gender of patients | Age at onset (years) | Clinical features | Radiology results | Biology test results | Means of diagnosis | Prognosis and treatment |
|---|---|---|---|---|---|---|---|---|---|
| **Form II (n=7)** | **Gaucher disease (n=2)** | - 1st degree consanguinity<br>- Similar case in family | 11, ♀ | 5 | Psychomotor regression, HMG, SMG | ∅ | - GCase deficiency<br>- **Splenic biopsy**: Gaucher cells "wrinkled tissue pattern" | Enzymatique dosage GCase | Enzyme replacement therapy: CEREZYME Improvement after treatment |
| | | - 1st degree consanguinity<br>- Similar case in family | 7, ♂ | 4 | Psychomotor regression, HMG, SMG | ∅ | - GCase deficiency | Enzymatique dosage GCase | Patient passed at 8 years as a result of the onset of Leukemia Treated with enzyme replacement therapy |

Table XI : Clinical data of patients with diagnosis (suite…)

| Clinical form | Diagnosis | Medical history | Age (year), gender of patients | Age at onset (years) | Clinical features | Radiology results | Biology test results | Means of diagnosis | Prognosis and treatment |
|---|---|---|---|---|---|---|---|---|---|
| | **Niemann-Pick disease (n=2)** | - Cholestatic jaundice - 1st degree consanguinity and Similar case in family | 0.75, ♀ | 0.6 | Psychomotor delay, HMG, SMG, Facial dysmorphia | **Abdominal ultrasound**: Homogeneous hepatosplenomegaly | - ASM deficiency<br>- **CBC**: Anemia<br>- **Liver function**: IHC | Enzymatique dosage ASM | Deceased at the age of 1.5 years. |
| | | 1st degree consanguinity | 0.5, ♀ | 0.5 | Ocular impairment: Nystagmus, HMG | ∅ | - ASM deficiency | Enzymatique dosage ASM | Deceased at the age of 1 year. |

Table XI : Clinical data of patients with diagnosis (suite…)

| Clinical form | Diagnosis | Medical history | Age (year), gender of patients | Age at onset (years) | Clinical features | Radiology results | Biology test results | Means of diagnosis | Prognosis and treatment |
|---|---|---|---|---|---|---|---|---|---|
| | **Ornithine transcarbamylase deficiency** | No history | 7, ♂ | 0.5 | Psychomotor regression, HMG, Delayed growth | ∅ | - **Urine organic acids screening**: elevated Uracil levels | Urine organic acids screening (GC/MS) | Patient is under Arginine supplementation (Arginine Veyron) and Malate citrulline. No improvement |
| | **Tyrosinemia** | - Cholestatic jaundice - 1st degree consanguinity and Death of siblings | 5, ♀ | 0.01 (3 days) | Psychomotor delay, HMG, Ataxia, Delayed growth | ∅ | - **Blood amino acids screening**: elevated Tyrosine levels x3/control | Blood amino-acids dosage (HPLC) | - |

# VI. Prognosis and treatment:

## 1. Treatment:

### 1.1. Symptomatic treatment:

18 patients participating in our study experienced seizures and were treated with DEPAKINE (valproic acid) at a dosage of 30 mg/kg per day. This antiepileptic medication proved effective in improving their seizure symptoms.

Patient diagnosed with Ornithine transcarbamylase deficiency took Arginine supplementation (ARGININE VEYRON) and Malate citrulline (STIMOL) to restore physical strength, prevent body weakness and manage fatigue.

### 1.2. Causal treatment

2 patients diagnosed with Gaucher disease received enzyme replacement therapy, CEREZYME (Imiglucerase) intravenous infusion at a dosage of 60U/kg every 2 weeks for 6 months was administered to one patient, the other's patient treatment protocol wasn't documented.

1 patient was diagnosed with profound Biotinidase deficiency was prescribed a lifelong treatment that consists of oral Biotin supplementation 10mg/j

Copper-chelating treatment was administered to 1 patient diagnosed with Neurowilson, with a posology of D penicillamine (TROLOVOL®) 20mg/kg/day.

1 patient was prescribed GYLENYA (fingolimod) 0.5mg/day orally for his RR Multiple sclerosis, resulting in noticeable improvement.

## 2. Prognosis:

The prognosis of the patients in our study varies widely depending on several factors, including the specific diagnosis, age of onset, and the rate of disease progression. While some patients presented a gradual decline in function and ultimately a fatal outcome, others exhibited periods of stabilization and even improvement (TableXII). We weren't able to reach 2 patients (Patient with Free sialic acid disease and patient with Tyrosinemia) to evaluate the progression of their diseases.

Table XII : Summary of patients' prognosis

| Prognosis | | Number of patients | Details |
|---|---|---|---|
| Favorable | Stabilization | 2 (4.25%) | - 1 patient with RR multiple sclerosis on Gylenya<br>- 1 patient with Neurowilson on copper-chelating treatment |
| | Improvement | 1 (2.12%) | 1 patient with Gaucher disease on CEREZYME |
| Unfavorable | Deterioration | 35 (74.5%) | 29 patients in which 30 patients didn't have a diagnosis and 6 patients were diagnosed with: Tay-Sach disease, NCL, MLD, Menkes disease, Ornithine transcarbamylase deficiency and Biotinidase deficiency |
| | Death | 7 (14.9%) | - 1 patient with decompensated Gaucher disease, evolution towards Leukemia at the age of 8 years.<br>- 1 patient with Krabbe disease at the age of 7 months.<br>- 2 patients with Niemann-Pick disease at the age of. 1 year and 1.5 year.<br>- 2 patients with no diagnosis at the age of 1 year and 2 years.<br>- 1 patient with Metachromatic leukodystrophy at the age of 5 |

# *DISCUSSION*

# Part 1: Review

## I. Hexosylceramides structure, synthesis and degradation:

### 1. Structure

**Glucosylceramides** (**GlcCer**) and **Galactosylceramides** (**GalCer**) also called Monohexosylceramides/Monoglycosylceramides belong to the group of cerebrosides within the sphingolipids' category. They play essential roles in various cellular processes, particularly within the realm of membrane structure and function.

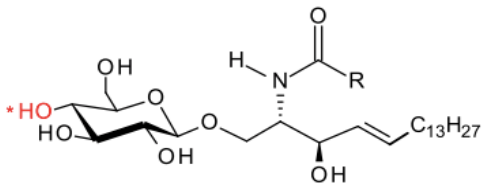
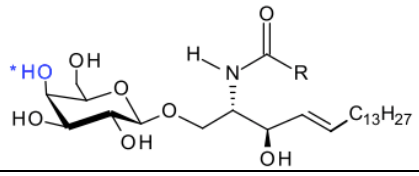
GlcCer, also referred to as Glucocerebroside, consists of a ceramide backbone composed of D-*erythro*-sphingosine and long-chain fatty acid (hydrophobic lipid moiety), attached to a hydrophilic head group sugar: D-glucose through a b glycosidic bond [17]

GalCer (Galactocerebroside) consists of a ceramide backbone composed of D-*erythro*-sphingosine and long-chain fatty acid (hydrophobic lipid moiety), attached to a hydrophilic head group sugar: D-galactose through a b glycosidic bond [17].

Therefore, these two compounds represent very similar structures since d-galactose is an epimer of d-glucose and these two sugars differ only in the configuration at C-4. Fatty acids attached to sphingosine may vary significantly in length (C14-C26), with stearic acid (C18) being the most abundant.

GalCer are enriched in very-long-chain α-hydroxy fatty acids (C18-C26), whereas GlcCer consist of ceramides with primarily nonhydroxylated shorter chain fatty acids (usually C16 or C24) [18].

Table XIII: Structures of GlcCer and GalCer.

| Full name | Abbreviation | Structure |
|---|---|---|
| Glucosylceramide | GlcCer |  |
| Galactosylceramide | GalCer |  |

## 2. Synthesis

Synthesis of GlcCer and GalCer starts with the generation of ceramide in endoplasmic reticulum (ER) (Figure 19). Ceramide made in the ER is transported by ceramide transport protein CERT to the cytosolic side of the trans-Golgi network [19].

Transfer of glucose from UDP-Glc on ceramide is catalyzed by Glucosylceramide synthase (GCS, GlcCer synthase). GCS is localized on the preGolgi, and cis-, medial-, and trans-Golgi membranes as well as perinuclear ER and is encoded by the UGCG gene [17].

The synthesis of GlcCer takes place on the cytosolic side of the trans-Golgi apparatus [20] as glucosylceramide synthase (GCS), along with O-GlcNAc transferase, is the only glycosyltransferase, whose active site is located on the cytoplasmic side of the Golgi [21].

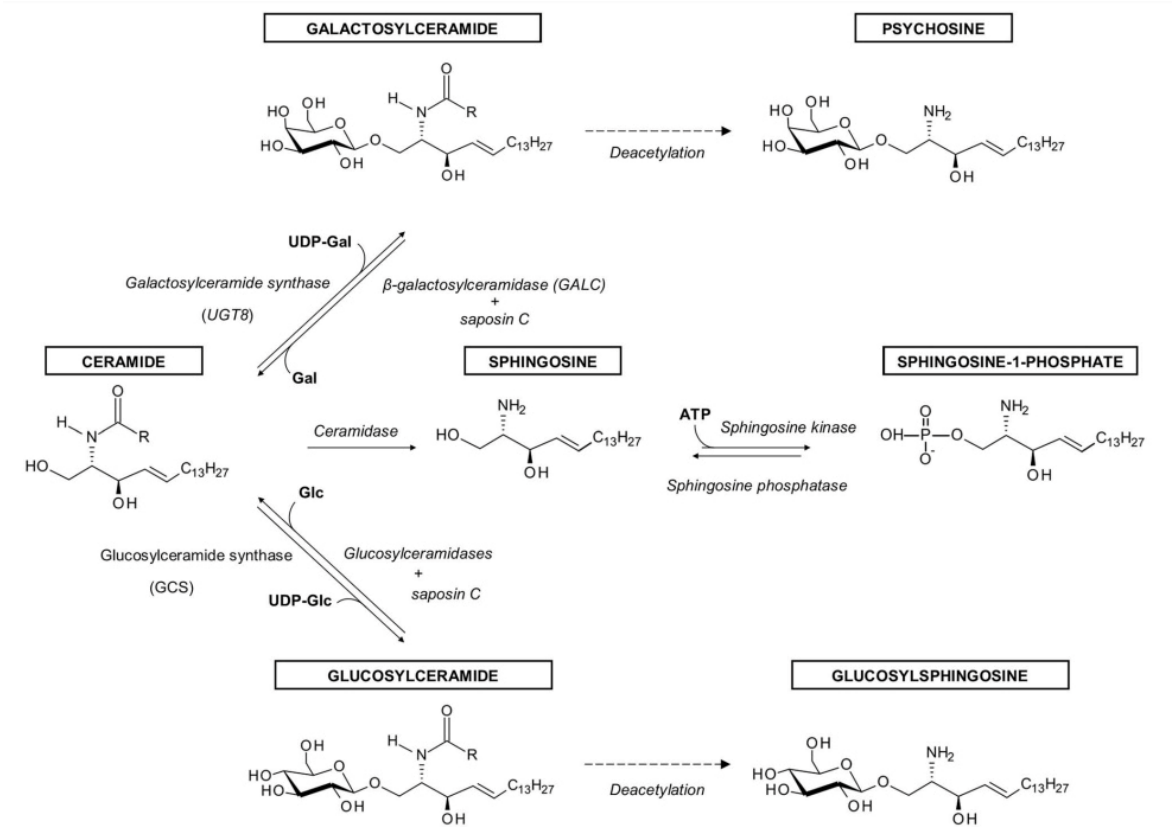



**Figure 19** : The synthesis and degradation of GlcCer and GalCer and their metabolites.

In addition to vesicular transport, the non-vesicular transport of GlcCer from the early Golgi to distal Golgi compartments is mediated by the glycolipid-binding protein FAPP2 (4-phosphate adaptor protein-2) [22].

Most GlcCer is transported back to the cytoplasmic leaflet of ER with the help of the FAPP2. There, GlcCer is translocated to the luminal side and transported again to the trans-Golgi apparatus, where complex GSLs are synthesized. (Figure 20)

Remaining GlcCer is transported by FAPP2 from the cytosolic surface of the TGN to the cytosolic surface of the plasma membrane or endosome. In the plasma membrane, GlcCer is translocated to the cell surface or remains on the cytoplasmic side [23].

GlcCer is a founder molecule for synthesis of hundreds of complex glycosphingolipids (GSLs), which are subdivided according to the sugar sequence and configuration into GSLs of

ganglio-, globo-, isoglobo-, lacto-, and neolacto-series. GlcCer must cross into the lumen of the Golgi in order to be converted to lactosylceramide (LacCer), gangliosides and higher order glycosphingolipids.

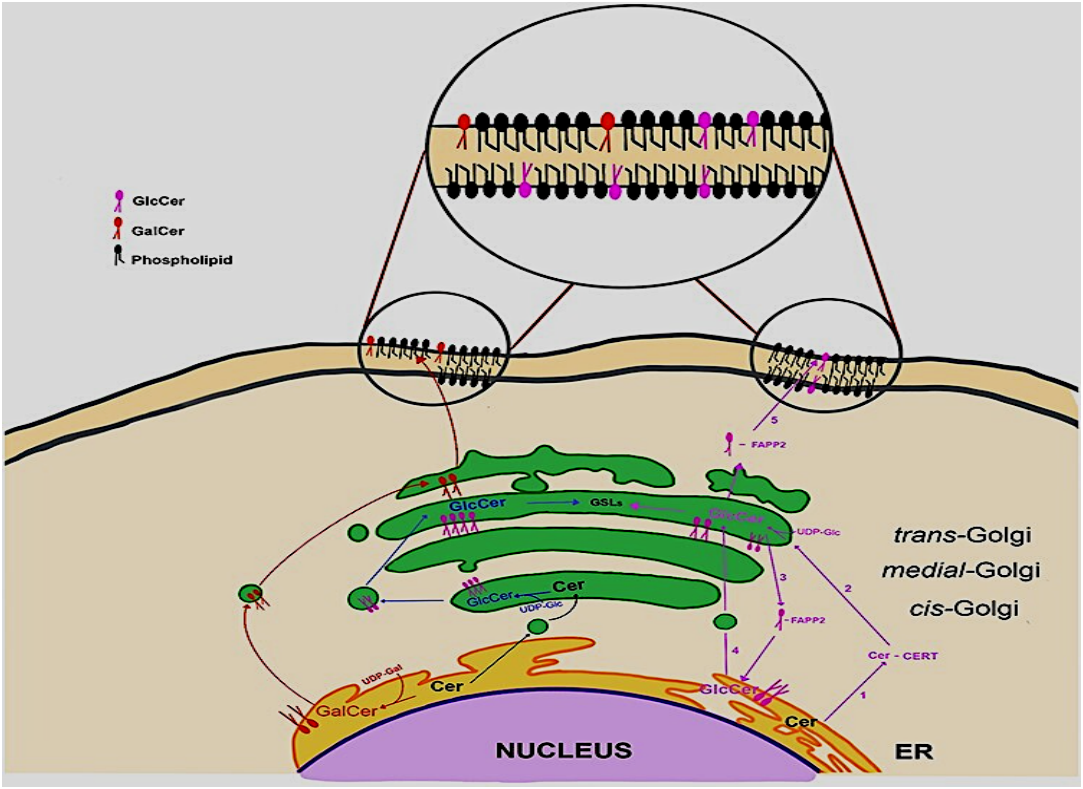



**Figure 20 :** Intra cellular localization and transport of GlcCer and GalCer.

Similar to the synthesis of GlcCer, GalCer is generated by the transfer of galactose from UDP-galactose to the same hydroxyl group at the C-1 position of the ceramide backbone by Galactosylceramide synthase [24],described also as ceramide galactosyltransferase (CGT). CGT is encoded by the UGT8 gene [25].

However, the amino acid sequence of UGT8 shows no significant homology to GCS, which indicates different evolutionary origins of these enzymes [21]

GalCer is synthesized on the luminal side of the ER, as UGT8 is a type I membrane glycoprotein with its active site directed into the lumen of this organelle [26]. From there, GalCer

is transported to the trans-Golgi compartment, where larger galactosphingolipids and sulfatids are synthesized. (Figure 20)

## 3. Degradation

The glucose moiety on GlcCer is removed by lysosomal Glucosylceramidases (GCase) and by the ER-localized *Saposin C* enzyme. GlcCer is then degraded to ceramide which are further hydrolyzed to spingoid base and fatty acid.

The enzyme glucosylceramidases plays a central role in the lysosomal degradation of GlcCer process by cleaving the glucose molecule from the ceramide backbone, leading to the generation of ceramide and glucose. This process is carried out by several glucosylceramidases: GBA1, GBA2 and GBA3 [27].

- GBA1, also known as acid β-glucosidase, β-glucoceramidase, glucocerebrosidase, D-glucosyl-N-acylsphingosine glucohydrolase, GlcCerase, is a lysosomal hydrolase.
- GBA2, bile acid βglucosidase, is a ubiquitous non-lysosomal enzyme located on the cytosolic surface of the ER and/or Golgi apparatus.
- GBA3, known under the names Klotho-related or KLrP, is another cytosolic glucosyl-ceramidase found in the liver, spleen, kidney and in some other tissues, but whose function is presently unclear.

In the case of GalCer degradation, the only enzyme known so far is lysosomal Galactosylceramidase, known as B-galactocerebrosidase (GALC) [28].

In both cases, ceramides that are released from GlcCer and GalCer are hydrolysed by ceramidases to sphingoid bases and fatty acids (Figure 21) [29].

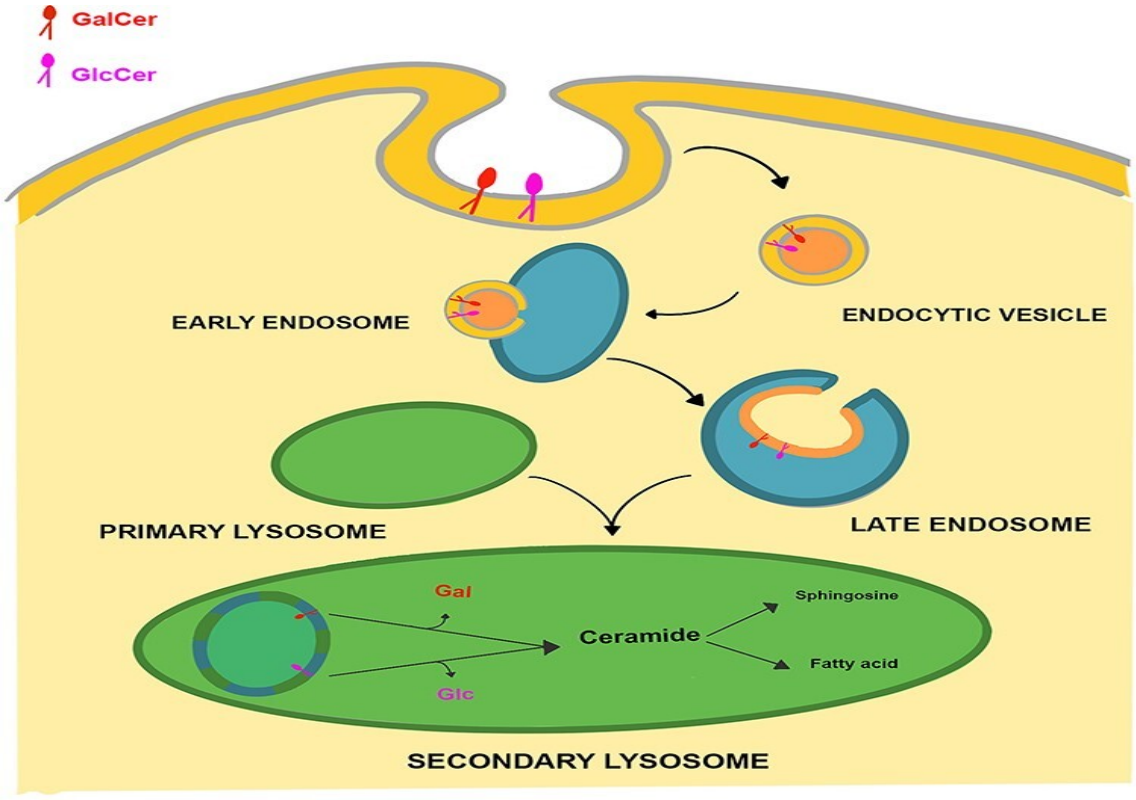



**Figure 21** : Degradation of GlcCer and GalCer.

Besides the enzymes, essential components of the intra-lysosomal degradation of HexCer with less than four sugar residues are sphingolipid activator proteins (SAPs): saposins (Saps) A, B, C and D and GM2-activator protein (GM2-AP) [30].

The four Saposins arise by proteolytic processing of a single precursor protein, prosaposin, which is synthesized in the ER, transported to the Golgi apparatus for glycosylation and finally transported to the lysosomes.

Two mechanisms were proposed to explain the role of saposins in the activation of HexCer degradation:

1- SAPs facilitate the interaction between the HexCer and exohydrolases by binding, extracting and presenting the membrane localized lipids to water-soluble enzymes [31].

2- SAPs bind directly to enzymes, not to HexCer, generating a more active enzymatic complex to hydrolyze HexCer [32].

It was found that deficiency of Saposin C led to accumulation of GlcCer within the cells and resulted in a GlcCer storage disease resembling a neurologic form of Gaucher disease showing the crucial role of this cofactor in the metabolism of this Monohexosylceramides [33].

Also, the saposin A and probably saposin C are necessary components to activate the degradation of GalCer by Galactosylceramidase in vivo [34]. Mice lacking, due to mutation, mature saposin A, accumulate GalCer and develop a late-onset form of chronic globoid cell leukodystrophy [35].

## 4. Occurrence

Despite structural similarities, the cellular and tissue localization of GlcCer and GalCer are different. GlcCer is present in essentially all cell types serving as a precursor for the synthesis of hundreds of different GSLs.

For example, the presence of GlcCer was analyzed in:

- Gastrointestinal tract's the mucosa [36]
- Liver [37]
- Adrenal medulla [38]

Data are also available on the expression of the UGCG gene in normal human tissues. The highest levels of GCS mRNA were found in the kidney, vulva, urinary bladder, stomach, pancreas and colon. Intermediate levels were seen in the prostate, lung, skin, cervix, rectum and thyroid gland, while the lowest levels of GCS mRNA were present in the breast, uterus, ovary and testis [39].

In contrast to GlcCer, GalCer is present only in a limited number of mammalian tissues/cells. GalCer is the most abundant single component of:

- the myelin sheath (20–25% of the total lipid content) produced by oligodendrocytes in the central nervous system (CNS)
- Schwann cells in the peripheral nervous system [40].

- This Monohexosylceramides is also present in larger amounts in the mucosa of the human gastrointestinal tract [36], adrenal medulla [38], liver [36], testis [41] and milk [42].

## 5. Role in the nervous system

Numerous studies indicate that sphingolipids are extensively distributed throughout the central nervous system (CNS), where glycosphingolipids and gangliosides are the major components [43].

Gangliosides are primarily found in neurons, while GalCer is predominantly located in oligodendrocytes [44]. This distribution underscores the crucial role of sphingolipids in the formation of various cell types and neurodevelopment.

### 5.1. HexCer and sphingolipids' role in nervous system maturation

Gangliosides, a major class of sphingolipids, are highly concentrated in the CNS and are vital for nervous system development, axonal growth, and neuronal differentiation [45]. During embryonic development, ganglioside levels fluctuate with maturation stages (Figure 22)

- Early Development: GM3 and GD3 are abundantly synthesized during neural tube formation and play a role in the proliferation and differentiation of neural stem cells [46].
- Later Stages: GM3 derivatives, such as GD1a, GM1, GD1b, and GT1b, contribute to neuronal differentiation, synaptogenesis, and myelination into adulthood [47]. Additionally, sphingomyelin, GalCer, and sulfatide are involved in axonal arborization during development.

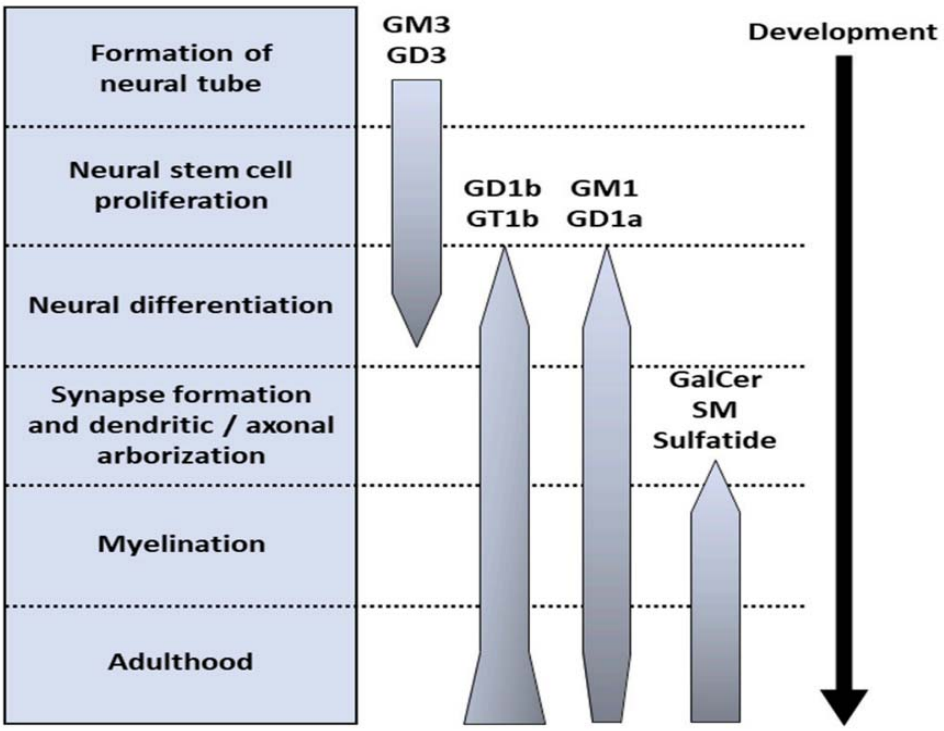



**Figure 22 :** Expression of sphingolipids in the nervous system during development. The gangliosides GD3 and GM3 are produced during the formation of the neural tube, and during neural stem cell proliferation. Galactosylceramide (GalCer), sphingomyelin (SM) and sulfatide are synthesized during synaptogenesis. Their synthesis is maintained until adulthood

### 5.2. Role of HexCer in neuronal differentiation and axonal growth:

Glycosphingolipids, like gangliosides, are key contributors to neuronal proliferation, maturation, and axonal growth [48]. Several glycosphingolipid enzymes, including ceramide and GlcCer synthetic enzymes, ceramide synthase, and UDP-glucose ceramide glycosyltransferase (UGCG), are essential for axonal and dendritic growth.

In summary, sphingolipids, along with the breakdown of GlcCer into ceramide, are critical for nervous system development [49].

# II. Functions and physiopathology of Hexosylceramides:

## 1. Biological functions of Hexosylceramides:

### 1.1. Molecular level:

HexCer plays a role in membrane organization regulating the fluidity of the lipid bilayer and are involved in including or excluding proteins from membrane microdomains (lipid rafts). These microdomains perform a central role in many cellular processes including membrane sorting and trafficking, cell polarization and signal transduction processes.

They also act as bioactive lipid messengers on two levels:

- directly affecting membranous protein functions,
- regulating the expression level of specific genes.

So far, it has been shown that on the molecular level, GlcCer and GalCer are involved in membrane organization. They are localized in the external lipid leaflet of cell membranes and are found predominantly within tightly packed lateral domains of lipids, called membrane rafts [50].

It has been demonstrated that GalCer, using atomistic molecular dynamics simulations [51], significantly increased the thickness of raft membranes, while the average area per lipid and lipid conformational order were unchanged.

They also showed that interdigitation of GalCer slows down the lateral diffusion of raft lipids and affects membrane viscosity between the two membrane leaflets, augmenting the friction between the monolayers.

In addition, interdigitation of GalCer alters the lateral pressure profile, which may lead to a change in membrane protein activation [51].

GalCer has the ability to form multiple hydrogen bonds with surrounding molecules; however, it preferentially interacts with cholesterol molecules shielding them from direct contact with water [52].

Similar to GalCer, GlcCer is also involved in the formation of highly ordered gel domains and increases the order of the membranous fluid phase [53].

The presence of GlcCer promotes morphological alterations in lipid vesicles, which leads to the formation of flexible tubule-like structures extending from the lipid surface.

However, despite tiny differences in the structures of their headgroups—stereochemical orientation of one hydroxyl group in the sugar residues—palmitoyl GalCer and palmitoyl GlcCer differ significantly in their domain forming behavior [54]

GalCer formed ordered domains which dissociated with increasing temperature, and GlcCer at the same concentration formed domains with a more cooperative dissociation behavior, but lower thermostability.

### 1.2. Cellular level:

On the cellular level, HexCers have important functions in such cellular processes as adhesion and recognition, growth, differentiation and development, angiogenesis, inflammation and multidrug resistance in cancer cells [49].

Several lines of evidence suggest that GlcCer affects the proliferation potential of various cell types. Using a mouse model, it was shown that:

- Intraperitoneal injection of emulsified GlcCer and inhibition of glucosylceramidase resulted in enlargement of the liver [17]
- Inhibition of GCS activity in renal epithelial cells decreased their proliferation rate [55].

GlcCer also affected the proliferation of Schwann cells [56] and stimulated mitogenesis of murine epidermis [57].

These proproliferative and antiapoptotic effects are exerted not directly by GCS and GlcCer themselves, but by affecting the intracellular pool of ceramides, as increased synthesis of GlcCer decreases the level of antiproliferative ceramide and decreased synthesis of GlcCer increases the level of proapoptotic ceramide [58].

Treatment of human keratinocytes with exogenous sphingomyelinase, which is known as a potent stress inducer, first caused increased production of ceramide and therefore decreased proliferation of cells and then increased synthesis of GlcCer, which was associated with restoration of cell proliferation [59]

It is now broadly accepted that ceramide is a key molecule involved in specific signaling pathways related to apoptotic and proliferative cellular responses to many stressors, including chemotherapeutics [54]. However, the exact molecular mechanisms of how ceramides affect proliferation and apoptosis are unknown.

In the case of GalCer, it was found that expression of UGT8 and accumulation of GalCer in breast cancer cells increased their resistance to apoptosis induced by doxorubicin (a chemotherapy agent used to treat breast cancer and other cancers such as ovary, bladder, and thyroid) in vitro [60].

## 2. Hexosylceramides under pathological conditions:

Disorders of Hexosylceramides metabolism in lysosomes induced a family diseases identified as lysosomal storage diseases (LSDs). LSDs include Gaucher's disease (GD) and Krabbe's disease (KD) (TableXIV).

Table XIV: Lysosomal storage diseases caused by impairments in HexCer metabolism

| Type of disease | Accumulated compound | Deficient enzyme |
|---|---|---|
| Gaucher disease<br>Glucosylceramide lipidosis | Glucosylceramide<br>(GlcCer) | Glucocerebrosidase<br>(GCase) |
| Krabbe disease<br>Galactosylceramide lipidosis | Galactosylceramide<br>(GalCer) | Galactosylceramidase<br>(GALC) |

### 2.1. Glucosylceramide and Gaucher disease:

Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder (LSD) (Figure 23). and it remains the most common one. It was first described by Philippe Gaucher in 1882 in a patient with massive splenomegaly without leukemia.

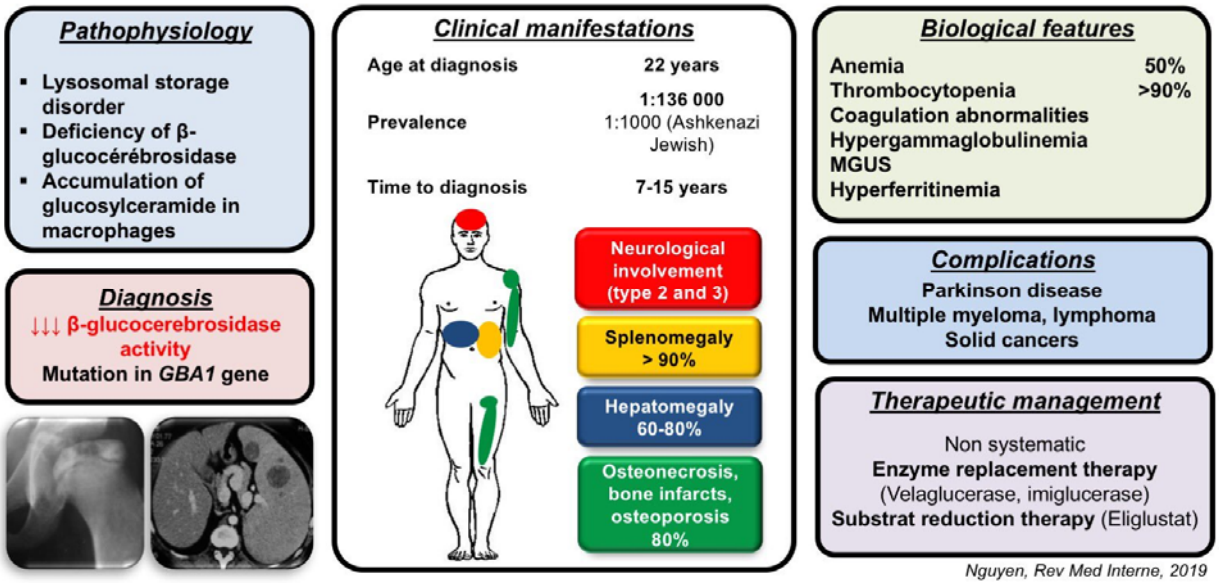



**Figure 23** : A resume on Gaucher disease.

It is mainly caused by mutations in GBA1 gene, located on chromosome 1 (1q21), coding for lysosomal GBA1 [17]. Very rarely, deficiency in the GlcCerase activator, saposin C, could cause GD (Figure24) [61]. The complete loss of function of the GBA1 protein leads to an accumulation of glucosylceramide, which can be the cause of neuropathic forms of the disease.

In the visceral GD variant (type I), mutations lead to GlcCer accumulation in lysosomes of leukocytes, primarily macrophages and antigen presenting cells (APCs) in the spleen, liver, lungs and bone marrow. The GBA1-defective macrophages accumulate GlcCer as the result of ingestion of exogenous lipids from senescent or/and apoptotic erythrocytes, leukocytes and platelets [62].

The exact mechanisms by which GlcCer accumulation leads to GD are not known. However, there are several hypotheses about the role of excessive amounts of GlcCer in the pathogenesis of this disease.

Using cellular models of GD constructed by treatment of human fibroblasts or murine RAW macrophages with inhibitor of GBA1 - conduritol B epoxide (CBE), it was found that an increase in the level of GlcCer induced altered lactosylceramide trafficking from the plasma

membrane to late endosomes and lysosomes, instead of to the Golgi apparatus [63]. These data suggest that accumulation of GlcCer disrupts proper lipid transport and sorting.

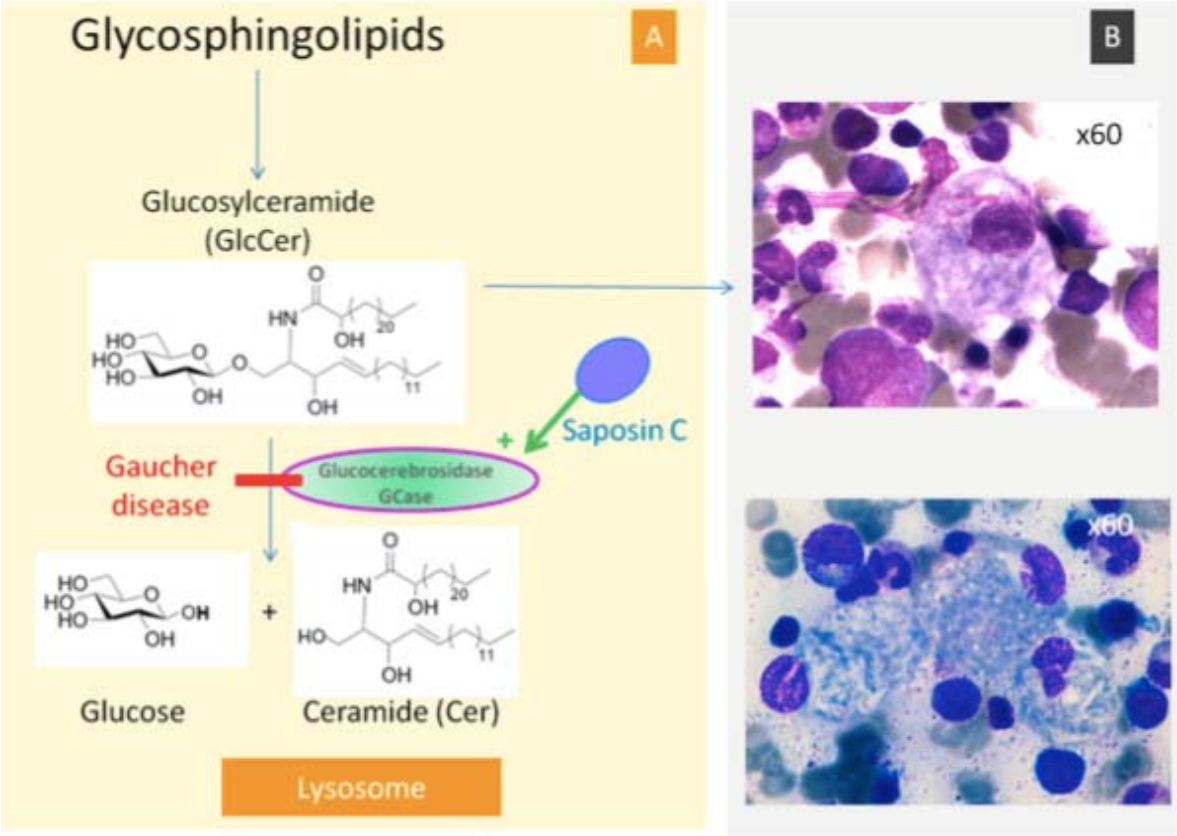



**Figure 24** : A) Hydrolysis of GlcCer by GCase in the lysosome, GCase is activated by saposin C. Deficiency in GCase in Gaucher disease leads to accumulation of GlcCer in the cell lysosome. B) Crumpled tissue paper appearance knows as Gaucher cells, they infiltrate various organs such as bone marrow, spleen and liver.

### 2.2. Galactosylceramide and Krabbe disease:

Krabbe disease (KD), also known as globoid cell leukodystrophy is an autosomal recessive lysosomal storage disorder (LSD) involving the white matter of the peripheral and CNS (Figure 25).

This disease, characterized by the loss of myelin, is caused by mutations in the GALC gene (14q31), resulting in deficiency and/or decreased activity of lysosomal GALC [64], which

leads to accumulation of GalCer and galactosylsphingosine (psychosine) in macrophages and neural cells, especially in oligodendrocytes and Schwann cells [65].

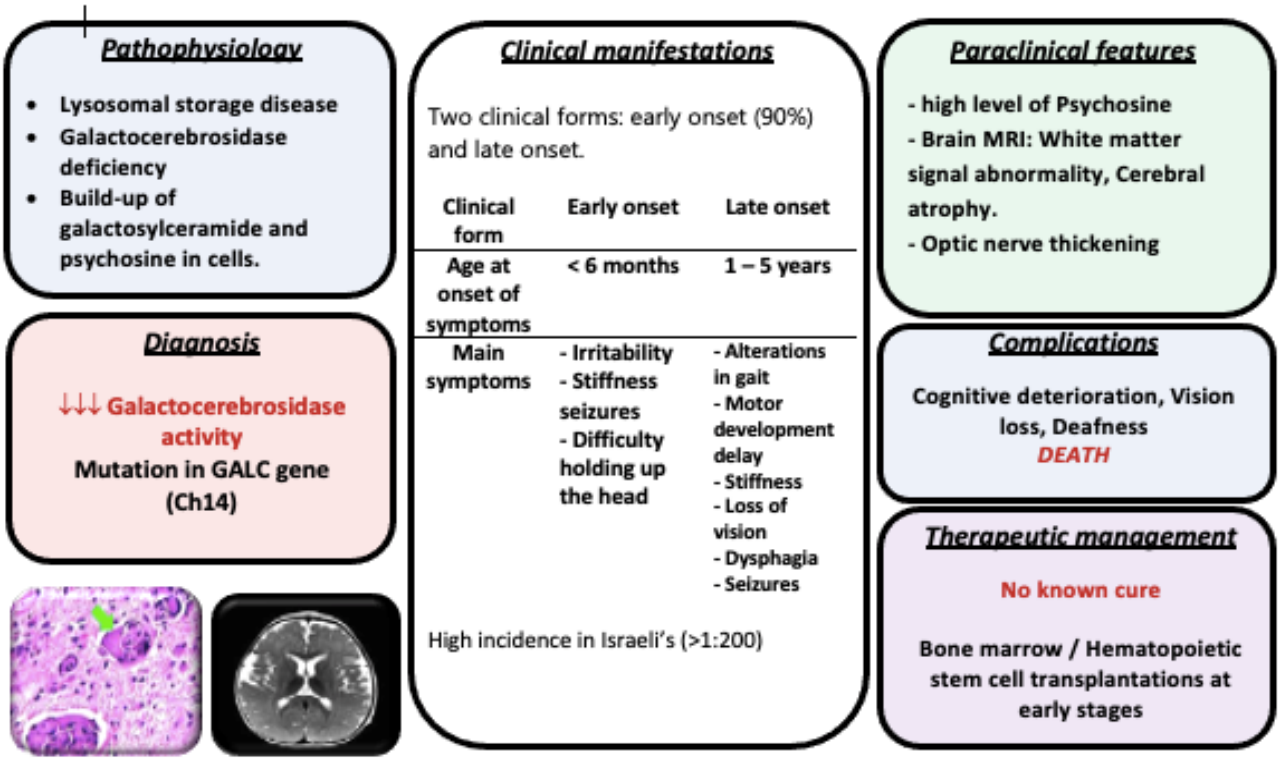



**Figure 25** : A resume on Krabbe disease

According to the "psychosine hypothesis," galactosylsphingosine, not GalCer, is a highly cytotoxic compound directly linked to demyelination of the central and peripheral nervous systems.

When there is a deficiency of GALC, large amounts of psychosine are produced by deacylation of accumulated GalCer by N-deacylase *(Ref74)*, and galactosylation of sphingosine by UGT8 [66], GalCer does not accumulate in the absence of GALC and therefore does not appear to be directly linked to demyelination processes, since in the absence of this enzyme, GalCer is degraded by GM1 β- galactosidase [65].

Psychosine, because of its detergent-like properties, destabilizes cellular membranes, which leads to cell lysis, induces oxidative stress and mitochondrial damage, resulting in cell

apoptosis, and on the cellular level causes inflammation, as well as vascular, neuronal and axonal dysfunction [65].

To sum it up, the pathogenesis of Krabbe's disease is a combination of two phenomena:

- The impaired degradation of GalCer, which leads to globoid cell infiltration,
- The accumulation of the cytotoxic derivative galactosylsphingosine (psychosine), which causes oligodendroglia cell destruction.

### 2.3. Galactosylceramide in juvenile neuronal ceroid lipofuscinosis:

Juvenile neuronal ceroid lipofuscinosis (JNCL) represents one of the genetic diseases known under the collective name of neuronal ceroid lipofuscinoses or Batten disease. It is one of the most common childhood neurodegenerative disorders.

JNCL is caused by mutations in the CLN3 gene and is characterized by abnormal accumulation of lipopigments in lysosomes of neurons [67]. The loss of function by the CLN3 gene is accompanied by massive neuronal death in the cerebrum and cerebellum. This confirms the proposal that the CLN3 protein (battenin) acts as an anti-apoptotic molecule, and the absence of which highly increases the level of the intracellular pool of pro-apoptotic ceramide [68].

CLN3 is a transmembrane protein characterized by the presence of a GalCer binding domain [69]. Normal CLN3, localized in the Golgi apparatus and plasma membranes, takes part in anterograde transport of GalCer from the trans-Golgi to lipid rafts of plasma membrane involving early recycling endosomes [69].

This proposal is supported by observations that mutations in the CLN3 gene found in JNCL patients affect the proper structure of a GalCer binding domain, which prevents mutant CLN3 protein from normal movement between these compartments and therefore prevent delivery of GalCer to plasma membrane, which is associated with accumulation of GalCer in ER and Golgi [70].

The absence of CLN3 protein and GalCer affects the proper composition, structure and function of lipid rafts in the Golgi and plasma membranes, which in turn leads to deregulation of ceramide levels with an end effect of increased apoptosis [69].

### 2.4. Other pathological conditions

Monohexosylceramides have a serious implication in neurodegenerative diseases (see Part 2), cancers and tumor progression in addition to their role in multidrug-resistance of cancer cells.

#### a. Presence of GlcCer and GalCer and expression of UGCG and UGT8 genes in cancer tissues:

❖ *Glucosylceramides and UGCG genes:*

There is a lack of detailed studies comparing the presence of GlcCer in cancer cells and human tumors with corresponding normal cells and tissues.

However, more information are available on the expression of the UGCG gene in human cancers. GCS mRNA expression was significantly up-regulated in metastases of breast cancer in comparison to primary tumors, benign fibroadenoma and normal mammary tissue.

- GCS mRNA levels were significantly higher in tumors of the rectum, small intestine, cervix and breast than in corresponding normal tissues [39].
- GCS is a potential marker of tumor aggressiveness, his expression correlated positively with ER-positive and HER2-positive metastatic breast cancer.
- Ruckhaberle et al. [71], using transcriptome profiling, showed that the expression level of the UGCG gene correlated positively with positive estrogen receptor (ER) status but was inversely associated with lower histological grading, low Ki67 levels and HER2-negativity.
- UGCG is one of several genes whose elevated expression was found in metastatic tumors of clear cell renal cell cancer in comparison to primary tumors [72].

❖ *Galactosylceramides and UGT8 gene:*

There is little information available on GalCer expression in human tumors. In studies on molecular markers in human astrocytomas and oligodendrogliomas, it was found that high amounts of GalCer were present more frequently in oligodendrogliomas than in astrocytomas [73]. However, more is known about expression of the UGT8 gene in cancer cells and tissues.

- Enhanced synthesis of GalCer was found in MDR human colon cancer HT29col cells derived from HT-29 G+ compared with parental HT-29 cells [74].
- Transcriptome profiling of prostate cancer cell lines showed that metastatic cells express much higher levels of UGT8 mRNA than non-metastatic cells [75].
- The UGT8 gene in breast cancer was significantly associated with ER-negativity, and therefore with a more malignant phenotype [76].
- UGT8 is one of six genes whose elevated expression correlated with a significantly increased risk of lung metastases in breast cancer patients. These results were verified by PCR and immunohistochemical staining using antibodies against UGT8 protein [77].
- Expression of UGT8 is significantly higher in breast cancer metastases to the lung than in corresponding primary tumors and in primary tumors of UGT8 node-positive patients than in UGT8 node-negative patients [78].
- The amounts of this enzyme in cancerous tissue correlated with higher malignancy grades. These data suggest that UGT8 could be a significant index of breast tumor malignancy and a potential marker for the prognostic evaluation of lung metastases [79].

**b. The role of Monohexosylceramides in tumor progression:**

Very little is known about the role of GlcCer and GalCer in tumor progression. Inhibition of GCS activity with suppression of UGCG gene expression using an antisense mRNA approach resulted in overall inhibition of ganglioside synthesis and importantly suppressed murine melanoma growth in vivo [80], suggesting that GlcCer may be involved in proliferation of

melanoma cells. However, it is impossible to discriminate between the direct involvement of GlcCer in this process and its role as a precursor molecule for more complex gangliosides.

Studies on the role of GalCer in breast cancer progression suggest that this Monohexosylceramide acts as an antiapoptotic molecule and its presence facilitates tumor cells to survive in the hostile microenvironment of tumors [81]. However, further study revealed that cells with high or low levels of GalCer contain essentially the same amounts of ceramide, suggesting that in breast cancer cells, the key antiapoptotic molecule is GalCer itself [81].

# Part 2: General discussion and conclusions.

Neurodegenerative diseases are progressive, devastating, and terminal, carrying both personal and societal burden. Currently, their diagnosis depends on their clinical presentation and no quantitative biomarkers exist to enable early verdict and commencement of therapy [82].

The biggest challenges are:

- Neurodegeneration starts locally and is confined to a small region of the brain until pathology expands and causes neuroinflammation, leakage of blood brain barrier, and spillover of the molecules into the cerebrospinal fluid (CSF), blood and other biofluids.
- The lack of diagnostic biomarkers stems from the unavailability of brain tissue, the complexity and heterogeneity of the brain and neurodegenerative pathology. Thus, for biomarker discovery we rely on peripheral sources such as CSF, blood, urine, and saliva, or postmortem tissue [82].

In childhood, neurodegenerative diseases are rare [83]. These comprise a heterogeneous group of disorders that result in the progressive loss of motor, mental and perceptual functions.

NDDs in children can be classified according to the topography of the main lesions (gray vs. white matter, cortical vs. subcortical gray matter), to clinical symptoms (seizures, psychomotor regression…), the cause of the disease whether it's caused by metabolic defects (lysosomal, mitochondrial or peroxisomal), the pathophysiology etc. Neurodegenerative diseases can be challenging to diagnose due to their diverse causes and clinical presentations butt we can all agree on the following statements [84]:

- Childhood neurodegenerative diseases (CND) are mostly hereditary.
- Childhood forms contain neurometabolic entities as well (lysosomal and peroxisomal disorders...)
- Childhood forms may combine maldevelopmental and neurodegenerative features (peroxisomal disorders)
- Childhood forms may be multiorgan diseases

- Many CND may show characteristic extracerebral neuropathology: in skin, blood lymphocytes, intestine, muscle which enables diagnostic confirmation of clinical and paraclinical

However, diagnosis can pose a major challenge to the clinician as children can present with nonspecific pediatric problems, such as growth faltering, recurrent vomiting and developmental delay, disorders can be difficult to distinguish from one another clinically, and the same disorder can have different presentations.

# I. Analysis of baseline characteristics:

## 1. Age at admission:

The average age of our patients was 5.22 years, spanning from 27 days to 25 years.

The median age of presentation to the health care facility in other studies done on NDDs in children was 6years old in the Ram Prabhu and al. study [85], 4.7 years in the Tipu Sultan and al. study [86] and 9.2 years in the Annie Ting Gee Chiu and al. study [87]. This diversity may be associated with differences in the selected population and their clinical features.

## 2. Gender:

The gender distribution of our patients found a female predominance, with 53.2% of patients, the male: female ratio was 0.88:1.

In adults, gender is sometimes a significant variable in the prevalence and incidence of neurological disorders and is considered a risk factor in some neurodegenerative diseases. Several hypotheses can be made to explain these sex discrepancies, but it is reasonable to believe that immunological mechanisms may drive the sex and gender bias, driven by hormonal

as well as genetic and epigenetic factors [88]. It should also be noted that access to care and clinician awareness are also factors that influence gender distribution.

The table below shows male and female biased neurodegenerative diseases: (TableXV)

Table XV : Male and Female biased neurodegenerative diseases.

| | NDDs | Sex ratio | Ref |
|---|---|---|---|
| Male-biased NDDS | Parkinson's disease | 2 males to 1 female | [89] |
| | Amyotrophic lateral sclerosis | 1.6 male to 1 female | [90] |
| Female-biased NDDs | Alzheimer's disease | 2 females to 1 male | [91] |
| | Multiple sclerosis | 3 females to 1 male | [92] |

In children, our results were mismatched with studies on pediatric degenerative brain disorders. A study published in August 2020 by the British Pediatric Surveillance unit [93] on NDDs in children showed a slight male predominance with a ratio of 0.8 female to 1 male, on the other hand there was a slight female predominance (51.3%) in a study done in India in 2023 [85]. However, sex was not a significant risk factor in pediatric neurodegenerative disease with the exception of X-linked disorders (CRTR deficiency, X-linked adrenoleukodystrophy or ornithine transcarbamylase deficiency…). The table below (Table XVI) summarizes the female percentage in other studies done on NDDs in children.

Table XVI : Reported female percentage of children who presented neurodegenerative diseases.

| Studies | Country | Year of publication | Number of cases | Female % |
|---|---|---|---|---|
| Our study | Morocco | November 2024 | 47 | 53.2% |
| Ram Prabhu and al. [85] | India | September 2023 | 117 | 51.3% |
| Tipu Sultan and al. [86] | Pakistan | November 2014 | 366 | 41% |
| Annie Chiu and al. [87] | China | September 2022 | 36 | 41.7% |
| Christopher Verity and al. [93] | UK | August 2020 | 2008 | 46% |
| Syed Zubair Shah [94] | Pakistan | November 2023 | 423 | 40.66% |
| Oula A Knuutinen and al. [95] | Finland | May 2021 | 80 | 43.75% |

## 3. Habitat:

In our study, it is crucial to highlight the significance of the patients' habitat, even though this factor may not have been emphasized in other studies. The location where patients reside can have a substantial impact on their health outcomes, particularly in our context. In our study, we found a slight predominance concerning the living area. Although, among those with a history of consanguinity (28 patients), 16 patients (57.1%) were from rural settings.

This correlation underscores the importance of considering environmental and socio-cultural factors associated with rural living, such as limited access to healthcare facilities, lower levels of health education and sensibilization, and cultural practices like consanguinity, which may influence the prevalence of certain neurometabolic inherited diseases.

Given these findings, the patients' habitat becomes a critical variable in understanding the broader health challenges faced by those in rural areas. Therefore, it is necessary to mention and analyze this factor within the scope of our study to provide a comprehensive assessment and to potentially guide future public health interventions tailored to rural populations.

# II. Analysis of Clinical data:

## 1. Medical history:

### 1.1. Personal:

Although 80.9% of our patients had no personal medical history, this does not diminish the significance of this feature in our study. The absence of a personal medical history in the majority of cases may indicate the silent progression or late onset of certain conditions, especially in the context of inherited neurodegenerative disorders.

In fact, perinatal asphyxia, which affected 2 of our patients, is considered to be one of the leading causes of brain neurodegeneration in term newborns The worst consequence of perinatal asphyxia is the aforementioned neurodegenerative brain injury, also known as hypoxic-ischemic encephalopathy [96].

Other clinical features of NDDs are familiar to pediatricians and resemble more common early childhood illnesses such as intrauterine growth retardation [97].

4 of our patients were affected by cholestatic jaundice which can be considered a risk factor for neurodegenerative disorders due to its association with liver diseases that impact overall health and potentially lead to neurological complications. According to a study done by Sri Jayanti and al. [98], Bilirubin and other “yellow players” are involved in neurodegenerative diseases and can have a protective, as well as potentially worsening effects on the brain.

### 1.2. Familial:

Familial history plays a crucial role in understanding and diagnosing pediatric inherited neurodegenerative diseases, particularly in cases of consanguinity. Many of these disorders are passed down through autosomal recessive inheritance. This risk is significantly higher in populations where consanguinity is prevalent.

In our study, 59.6% of our patients were born to consanguineous parents, these data are consistent with previous studies [99], thus highlighting the significant impact of consanguinity and inbreeding in the occurrence and recurrence of inherited neurometabolic and neurodegenerative diseases. Understanding the genetic background within a family can also guide more targeted diagnostic testing and help identify patterns of inheritance that might otherwise be overlooked.

In addition to the studies mentioned in the table below (TableXVII), several other studies have also emphasized the critical role of consanguinity in the prevalence of inherited disorders, particularly in certain populations. 88.89% of the patients' parents were consanguineous in a study done in Pakistan in 2022 [94] vs. 7.5% of the patients in a study done in Northern Finland in 2021 [95].

Table XVII : Familial medical history in our study and other studies on neurodegenerative diseases.

| | Consanguinity | Similar case in family | Death of siblings |
|---|---|---|---|
| Our study | 56.25% | 18.75% | 20.83% |
| Ram Prabhu and al. [85] | 10.3% | 23.9% | - |
| Tipu Sultan and al. [86] | 74% | 32% | - |
| Oula A Knuutinen and al. [95] | 7.5% | 33% | - |

## 2. Age at onset of symptoms:

The average age of symptom onset was 2.68 years, ranging from a maximum of 18 years to a minimum of 3 days after birth. Of the 47 patients included in our study, 46 (97.9%) had an age at onset under 16, with a notable predominance of early infantile onset (1 month to 1 year of age) which was observed in 19 patients (40.4%).

We grouped the patients' age of onset into: Neonatal (≤1month), early infantile (1 month to 1year), late infantile (1 to 4 years), juvenile (5 to 15 years) and adult (≥ 16 years). There is no single standardized method for grouping neurodegenerative diseases based on age of onset, as different classifications can be used depending on the specific context or criteria applied.

In Ram Prabhu and al. study [85], children were categorized into three groups based on the age at onset of disease: below 2 years (48.7%), between 2 and 5 years (27.3%), and between 6 and 12 years (23.9%). Three-fourth (75%) of the children in our study presented before the age of 5 years, and almost half of them had an infantile onset.

Tipu Sultan and al. study [86] evaluated different age groups and revealed that 26% of the children presented with age less than 5 years, 47% with age between 5 to 10 years while 27% were of age above 10 years. Mean age of onset of symptoms was 3.8 years.

Another study by Christopher Verity and al. [93] presented the patients in four different age groups: <1 year: 40%; 1 to 4 years inclusive: 41%; 5 to 9 years inclusive: 13%; 10 to 15 years inclusive 6%; thus 81% presented before 5 years of age.

The mean age of onset in the study done by Oula A Knuutinen and al. [95] in Finland was 5 months with a range of 0-15years.

In the table below (TableXVIII) we grouped some neurodegenerative diseases in children depending on the age of onset [83]:

Table XVIII: Neurodegenerative diseases and the age of onset of the symptoms.

| Age of onset | | Neurodegenerative diseases |
|---|---|---|
| Neonatal (onset at birth) | | GM1 type1 gangliosidosis<br>Type 1 glycogen storage disease<br>Neonatal Adrenoleukodystrophy<br>Mucolipidosis II<br>Alexander's disease |
| Early infantile | 1-3 weeks | Galactosemia<br>Maple syrup urine disease |
| | 1-3 months | Type 2 glycogen storage disease (Pompe's)<br>Infantile type of neuronal ceroid lipofuscinosis |
| | 3-6 months | Gaucher's disease<br>Tay-Sachs disease<br>Krabbe's disease<br>Niemann-Pick disease (type A)<br>Canavan's disease |
| | 3-12 months | Lesch-Nyhan syndrome<br>Palizaeus Merzbacher disease |
| Late infantile | 6 months-2 years | Adrenoleukodystrophy<br>Juvenile GM1 gangliosidosis type 2 |
| | 6 months-4 years | Homocystinuria |
| | 1-3 years | Hurler's syndrome<br>Metachromatic leukodystrophy<br>Neuroaxonal dystrophy<br>Leigh's disease |
| | 1-6 years | Niemann-Pick disease (type C) |
| | 2-5 years | Late infantile type of neuronal ceroid lipofuscinosis |
| | 2-6 years | Hunter's syndrome<br>GM2 type 3 gangliosidosis |
| Late childhood | 4-8 years | Juvenile form of neuronal ceroid lipofuscinosis<br>Sanfilippo syndrome |
| | 5-10 years | Adrenoleukodystrophy<br>Huntington's chorea |
| | 8-15 years | Lafora body disease |
| | 10-12 years | Niemann-Pick disease (type D) |
| | 5-20 years | Juvenile metachromatic leukodystrophy<br>Gaucher's disease type 3<br>Mucolipidosis I<br>Hallervorden-Spatz syndrome |

## 3. Clinical manifestations:

The clinical manifestations of neurodegenerative diseases in children are diverse, complicating the diagnosis process. The chief complaints in our series were psychomotor regression (44.7%) and psychomotor delay (42.5%) followed by seizures (38.3%) and ocular impairment (nystagmus and/or vision alteration) (25.5%).

Tipu Sultan and al. [86] study included 366 children were presented with psychomotor regression (100%), Spasticity was seen in 69% patients, while ataxia was seen in 33% cases versus 8.5% in our study. Children presented with seizures 70%, with visual problems 14% and 17% patients presented with dystonia.

In Oula Knuutinen and al. [95] study, the most common clinical findings were psychomotor delay (79%), intellectual disability (56%), hypotonia (60%), and spasticity (49%). Epilepsy was diagnosed in 36% of the cases, 29% of the children were presented with ataxia, and 23% with extrapyramidal signs vs. 6.4% in our study

The study by Ram Prabhu et al. [85] described the clinical manifestations in the population by categorizing them based on the age of symptom onset:

- In the infantile group, developmental delay (59.6%), tone abnormalities (57.9%), neuromotor regression (52.6%), microcephaly (47.4%), and epilepsy (36.8%) were the predominant presenting features.
- In the late-infantile group presented with regression of milestones (60%), movement disorder (51.8%), seizures (45.2%), cerebellar ataxia (41.9%), and vision impairment (30%).
- In the juvenile group presented with tone abnormalities (89.3%), movement disorder (72.4%), speech disturbances (71.4%), cerebellar signs (42.9%), and extrapyramidal symptoms (35.7%).

Christopher Verity and al. [100] described in their study delayed development in 35% of the children, seizures in 14%, motor dysfunction (ataxia, gait problems) in 13%, developmental regression in 10%, visual abnormality/deterioration in 5%, cognitive decline in 4% and dysmorphic features in 3%.

The heterogeneity observed in these studies is likely due to several factors, including the specific expertise of individual centers, the availability of diagnostic facilities, the research focus of collaborating groups, and the characteristics of the populations studied. Moreover, the lack of a universally accepted definition for NDDs in children often leads to the inclusion of various disorders, such as neurometabolic conditions and infections, under this broad category, contributing to the diversity of the study population and their clinical features.

None of these studies reported hepatosplenomegaly, which affected 21.3% as part of the clinical manifestations in their patients. However, it is important to note that patients with neurodegenerative diseases can still be affected by this condition. For instance, lysosomal storage diseases can cause liver dysfunction ranging from asymptomatic hepatomegaly to cirrhosis and portal hypertension. Some of the diseases that may manifest with a hepatomegaly are [101]:

- Major liver dysfunction: Gaucher's disease (Type III) and Niemann-Pick disease.
- Minor liver dysfunction: GM gangliosidosis, Farber, Mucolipidosis, Galactosialodosis, Fucosidosis, α-Mannosidosis and Mucopolysaccharidoses.

The table below gathers examples of non-neurological features in some neurodegenerative diseases [99]:

Table XIX : Non-neurological features in some degenerative brain disorders.

| Non-neurological feature | Neurodegenerative disease |
|---|---|
| **Ophthalmology** | - Cherry red spot: GM1 and 11, Niemann-Pick disease A and B<br>- Kayser-Fleischer: Wilson disease<br>- Cataract: Peroxisomal disorders, Fabry disease… |
| **Deafness** | - Zellweger spectrum<br>- Mitochondrial diseases |
| **Abnormalities of skin or hair** | - Brittle hair: Menkes disease, Biotinidase deficiency<br>- Angiokeratoma: Fabry disease, Sialidosis |
| **Dysmorphic features** | - Peroxisomal disorders<br>- Lysosomal storage diseases |
| **Hepatomegaly and/or Splenomegaly** | - Niemann-Pick disease<br>- Gaucher disease |
| **Cardiac** | - Conduction defects: Mitochondrial diseases |
| **Macrocephaly** | - Alexander disease, Canavan disease, GM2 Gangliosidosis |
| **Hematological abnormalities** | - Pancytopenia in Gaucher type1 |

# III. Analysis of paraclinical data:

The diagnostic strategy of pediatric neurodegenerative diseases rests upon clinical clues and MRI patterns, complemented by appropriately selected electrophysiological and laboratory testing.

In our study, following investigations were done including complete blood count (CBC), liver function (ASAT/ALAT and LDH) and hemostasis test (TP). Radiology tests consisted of brain magnetic resonance imaging (MRI) and brain CT scan, Electrophysiological tests including electroencephalography (EEG), visual evoked potential (VEP) and slit lamp examination for Kayser-Fleischer ring. Additionally, after highlighting the urinary excretion of Hexosylceramides in our patients using Thin-Layer Chromatography, enzymatic assay was done in 25 patients (53.2%) to confirm or eliminate a suspected diagnosis.

## 1. Brain MRI and brain scan:

Brain MRI was the most used diagnostic tool in the evaluation of NDDs in children in many studies and is considered the most important test in a patient suspected of having a degenerative brain disease. Correlation between the MRI brain results with clinical findings may vary, still it is believed to be one of the best tool for clinicians [102].

In our study, brain MRI was performed in 31 cases (65.9%) in which 11 patients (23.4%) had normal Brain MRI results and 20 patients (42.5%) had different abnormalities. The most common abnormalities included brain atrophy (corticosubcortical atrophy) and/or leukodystrophy (white matter signal abnormality) and dysmyelination (TableXX).

Table XX : Our study's brain MRI results and their corresponding disorders.

| Brain MRIs results | Disorders |
|---|---|
| No abnormalities (n=11) | - Krabbe disease (n=1)<br>- Menkes disease (n=1)<br>- Tyrosinemia (type not identified) (n=1)<br>- Unspecified disorder (n=8) |
| Corticosubcortical/Cerebral atrophy only (n=8) | - Neuronal ceroid lipofuscinosis (n=1)<br>- Tay-Sach disease (n=1)<br>- Free sialic acid disease (n=1)<br>- Unspecified disorders (n=5) |
| White matter signal abnormality (Leukodystrophy) only (n=8) | - Biotidinase deficiency (n=1)<br>- Metachromatic leukodystrophy (n=2)<br>- Unspecified disorders (n=4) |
| White matter signal abnormality and corticosubcortical atrophy (n=2) | - Unspecified disorders (n=2) |
| Bi-pallidal and bulboprotuberontial Lesions (n=1) | - Neurowilson (n=1) |
| White matter signal abnormality, corticosubcortical atrophy and Dysmyelinating disease (n=1) | - Unspecified disorder (n=1) |
| Dysmyelinating plaques (n=1) | - RR Multiple sclerosis (n=1) |

Contrary to our results, Tipu and al. findings showed a predominance in white matter abnormalities with a predominance of Metachromatic leukodystrophy (21%) followed by Adrenoleukodystrophy (19.7%) among other leukodystrophies. In another study reviewing children or young adults with degenerative diseases [103], leukodystrophies were also the predominant MRI abnormality with a percentage of 24.5 %.

Neuroimaging, particularly brain magnetic resonance imaging (MRI), is critical in all children with suspected NDD. Characteristic MRI features are noted in several white and gray matter NDD, it would also exclude slow growing brain tumors, which may result in developmental regression simulating degenerative brain disorders [104].

Even if all pediatric neurodegenerative disorders do not present with structural abnormalities at MRI, neuroimaging remains a valuable support [105]. These diseases can manifest differently depending on the severity of symptoms and age at onset and other factors but there are definitely some pathognomic MRI appearances in some NDDs such as predominant demyelination in the parieto-occipital regions with caudorostral progression in X-linked adrenoleukodystrophy [106], predominant white signal abnormality in the frontal region with increased density in the subventricular and subpial regions in Alexander disease and some classic leukodystrophies with typical imaging findings in MRI, which have led to use of the pattern recognition approach as a primary diagnostic method [107]. Brain MRI interpretation and pattern recognition may help to target specific biochemical or single-gene tests [108].

Brain scan is less sensitive than MRI in defining the extent of lesions in demyelinating and dysmyelinating disorders, hence why clinicians tend to use brain MRI [109]. This makes MRI the neuroimaging modality of choice in neurodegenerative diseases.

## 2. EEG and VEP:

In our study, 7 (14.6%) underwent EEG testing. Among these, 4 EEGs revealed normal activity, while 3 showed generalized paroxysmal discharges. In Tipu Sultan and al. study [86] 19% of the patients had findings suggestive of generalized epileptiform activity. The other studies mainly focused on using brain MRI or brain scan.

In neurodegenerative, EEG is indicated because of the increased incidence of epilepsy or epileptic encephalopathy (Rett syndrome, lysosomal storage disease, Neuronal ceroid lipofuscinosis…) [110]. Though generally non-specific, some EEG findings may be helpful with a differential list. With diffuse cortical and subcortical gray matter disease there are bilaterally synchronous paroxysmal discharges, in white matter disease EEG may show continuous non-paroxysmal slow wave activity. In diseases involving both gray and white matter, there will be bilaterally synchronous paroxysmal discharges and a marked increase in slow wave activity [109]. Patients with late infantile NCL can have a classic spike followed by slow wave with photic stimulation, other patients with Alpers disease can have a pathognomonic finding of unilateral occipital rhythmic high-amplitude delta waves with superimposed polyspikes (RHADS) on EEG.

The VEP is usually abnormal in lesions of the anterior visual pathway, optic nerves and optic chiasm. In axonal lesions (optic hypoplasia and compressive lesions of the optic nerve), the amplitude of P100 is decreased and the VEP wave form is distorted. In demyelinating lesions (optic neuritis and leukodystrophy), the latency of P100 is increased and the amplitude and waveform are normal [109].

## 3. Enzymatic activity:

Enzymatic dosage plays a critical role in the diagnosis of pediatric neurodegenerative diseases. It serves as a diagnosis tool and helps guiding treatment decisions while monitoring the therapeutic response.

In our study, 22 patients (46.8%) benefited from measurement of enzymatic activity using Tandem mass spectrometry (TMS) from Dried blood spot (DBS). 9 patients had low enzymatic activity and were diagnosed with Krabbe disease, Gaucher disease, Niemann-Pick disease, Tay-Sach disease, Neuronal ceroid lipofuscinosis and Metachromatic leukodystrophy (Table 11).

Dried blood spot (DBS) methods are currently available for identification of different disorders including lysosomal storage disorders (LSDs). These disorders are generally characterized by a deficiency of activity of a lysosomal enzyme. Diagnosis of LSD patients is often delayed, which is of particular concern as therapeutic outcomes; such as enzyme replacement therapy (ERT); are generally more favorable in early disease stages [111].

DBS screening using High-throughput tandem mass spectrometry has proven to be a sensitive technology for large-scale identification of several LSDs, including Gaucher disease, Fabry disease, mucopolysaccharidosis I [112], Krabbe disease [113] and Niemann-Pick A or B diseases. With the MS/MS technology, several lysosomal enzymes can be determined at the same time (multiplexing) using newly developed substrates and internal standards [114] [115].

Even though DBS testing has proven to be a quick and practical method for identification of a range of LSDs, it still comes with its challenges. One of the main diagnostic challenges is that the 'infantile' presentations of LSDs are generally accompanied by absent or very low residual enzyme activity and these cases can be accurately identified in the first-tier DBS test. Also, the test results may be less conclusive in patients with late-onset LSDs, for example, a "variant" Fabry phenotype, or in a heterozygous female with Fabry disease results would fall within the low-normal range [116].

Therefore, DBS assay results must be interpreted in conjunction with clinical findings and in collaboration with clinicians. Patients with low enzyme activity or those suspected of having an LSD but with non-diagnostic DBS results should be referred for additional confirmatory tests [116].

## 4. Thin-Layer Chromatography:

Thin-Layer Chromatography (TLC) is the earliest and widely established chromatographic method used for lipid assessment that continues to be employed today as reported by several researchers [117] .

Hammoud M. and al. developed a procedure based on the characterization of lipids using thin-layer chromatography (TLC) as a first step to orient the diagnosis process in a rapid, efficient and affordable manner [11]. In line with our study, the presence of glucosylceramides (GlcCer) led to conducting a β-glucocerebrosidase (GBA) assay to confirm Gaucher disease. the realization of galactocerebrosidase (GALC) assay to confirm a Krabbe disease was performed when galactosylceramides (GalCer) were visible in TLC analysis. With the combination of clinical and paraclinical features, their study [11] allowed to develop a rapid diagnosis method using TLC analysis of urinary lipid extracts. In our study, TLC was used as a screening tool to guide us in the diagnosing process.

In our context, TLC can stand as a simple, versatile, quite inexpensive and fast procedure for lipid analysis, notably for hexosylceramides and other sphingolipids and phospholipids (lactosylceramide, sphingomyelin, sulfatides, Gb3, Gb4...). TLC proved to be an indispensable tool in our diagnostic approach. By guiding our selection of the most appropriate enzymatic assay, TLC facilitated the accurate and timely diagnosis of the condition.

To our knowledge, our study is the first of its kind assessing the involvement of HexCer in neurodegenerative diseases using TLC analysis of urine samples.

Our current data indicates that Hexosylceramides increase during the course of different neurodegenerative diseases; however, their suitability as biomarkers requires determination of specificity via comparison with other neurological diseases but our research findings demonstrated the reliability of HexCer in reflecting disease burden and monitoring treatment response.

Furthermore, the urinary excretion of Hexosylceramides isn't only an indicator of disease severity in neurodegenerative diseases, but can also be a predictive biomarker in patients who don't present any neurological symptoms yet or a marker of poor prognosis in other non-neurological diseases. While studying the natural history of type 1 GD, it was noted that a few rare patients developed parkinsonian manifestations. Alternatively, parkinsonism could arise from the loss of GCase activity, where glucosylceramide accumulation could change lipid homeostasis, resulting in altered α-syn processing [118]. These findings confirm our speculation about the role of HexCer in predicting neurological impairment and degeneration.

Hexosylceramides were also associated with shorter overall survival in patients with pancreatic cancer [119] and it's increase during late stages of ovarian cancer enhances the involvement of HexCer in poor cancer prognosis [120].

To further emphasize the involvement of Hexosylceramides and show their role in disease progression and prognosis, we compared the TLCs of some the patients in our study to other patients in our platform presenting the same disease but with different severity:

- **Hexosylceramides in Tay-Sach disease:**

Tay-Sach is an inherited neurodegenerative disease that manifests in3 forms: infantile, juvenile, and adult-onset, with infantile Tay-Sachs being the most severe [121]. In the table below (Table XXI), we compared 2 patients both diagnosed with Tay-Sach disease, patient P1 diagnosed with the infantile form and his TLC showed the presence of HexCer while patient P2 had the juvenile form of this didn't excrete HexCer (both patients' urine samples were collected under the same circumstances and their TLCs were handled by the same operator).

Table XXI : Comparative table between P1 diagnosed with infantile Tay-Sach disease and P2 diagnosed with juvenile form of the disease and their clinical features, Brain MRI results and TLC results.

| Tay-Sach disease | | |
|---|---|---|
| **Patient** | **P1** | **P2** |
| **Form** | Infantile Tay-Sach disease | Juvenile Tay-Sach disease |
| **Age at onset** | 6 months | 7 years |
| **Gender** | Male | Male |
| **Symptoms** | - Psychomotor regression<br>- Seizures<br>- Hypotonia<br>- Blindness | - Psychomotor regression<br>- Seizures |
| **Medical history** | 1st degree consanguinity | 1st degree consanguinity |
| **Brain MRI** | Corticosubcortical atrophy | Moderate reduction of neuronal density |
| **TLC** | **Presence of HexCer** | **No HexCer** |

Our findings provide compelling evidence for the role of HexCer in the progression of neurodegenerative diseases. The observed correlation between HexCer levels and disease severity reinforces this association.

Moreover, a comparative analysis of P1 and P2, considering both MRI findings and clinical features, suggests a more severe disease course and poorer prognosis for P1. These results highlight the potential of HexCer as a valuable biomarker for disease severity and unfavorable outcome.

- **Hexosylceramides in Gaucher disease:**

Gaucher disease is a rare autosomal recessive genetic disease. The rarity of Gaucher disease and its wide variability in clinical presentations usually leads to diagnosis delays [122]. The prognosis of Gaucher disease varies significantly depending on several factors, including the type of the disease, the age at which it is diagnosed, the promptness of treatment initiation, and the individual's overall health [123].

With appropriate treatment (ERT), individuals with Type 1 Gaucher disease can achieve a near-normal life expectancy. Type 2 Gaucher disease is a severe, rapidly progressive form that

primarily affects infants. Unfortunately, the prognosis for individuals with Type 2 is grim, with a significantly shortened lifespan. Type 3 Gaucher disease, a less severe form than Type 2, typically manifests in childhood or adolescence [123]. The prognosis for individuals with Type 3 can range from mild to severe.

In the table below (TableXXII), we compared the clinical features in 2 siblings (P and P') born to consanguineous parents and diagnosed with Gaucher disease. Even though the 2 patients are siblings, they presented different phenotypes and prognosis of GD. Patient P presented with a severe acute neuropathic form (Type2 GD) and died four years after symptom onset due to disease progression and the onset of leukemia. In contrast, the sibling, patient P', presented with hepatosplenomegaly (Type1 GD) and has responded favorably to enzyme replacement therapy (ERT).

We note that Hexosylceramides were excreted in the urine of the sibling (P) with the severe neuropathic form of Gaucher disease which highlights the involvement of Hexosylceramides in the unfavorable prognosis and outcome of these diseases.

Further studies are needed to extend the results of our research and consider urinary Hexosylceramides as a predictive marker of the severe phenotype of Gaucher disease or other neurodegenerative diseases.

Table XXII : A comparative table between siblings diagnosed with Gaucher disease presenting 2 different phenotypes. (ERT= Enzyme replacement therapy)

| Gaucher disease | | |
|---|---|---|
| **Patient** | **P** | **P'** |
| **Form** | Type2 Gaucher disease | Type1 Gaucher disease |
| **Age at onset** | 4 years | 7 years |
| **Gender** | Male | Female |
| **Symptoms** | - Psychomotor regression<br>- HMG<br>- SMG | - HMG<br>- SMG |
| **Medical history** | 1st degree consanguinity | 1st degree consanguinity |
| **Paraclinical results** | - | Bicytopenia: Anemia and Thrombocytopenia<br>Myelogram: Gaucher cells |
| **Prognosis** | Patient passed at 8 years as result of decompensation and the onset of Leukemia<br>Patient was treated with ERT before his decompensation | Patient treated with ERT with noticeable improvement after treatment |
| **TLC** | **Presence of HexCer** | **No HexCer** |

M Biegstraaten and al. [124] report on monozygotic twin sisters, born to consanguineous Moroccan parents, who exhibited significant phenotypic variability in Gaucher disease despite sharing the same genotype. This highlights the limitations of genotype-based predictions for disease onset and progression. These findings encourage us to use Hexosylceramides as a valuable biomarker for identifying severe phenotype of Gaucher disease, particularly among siblings and in individuals suspected of having this disease.

- **Hexosylceramides in Multiple Sclerosis:**

In our study, 1 male patient was diagnosed with Remitting-Relapsing Multiple sclerosis at the age of 12years with an age at onset of 10. His disease was revealed by decrease of visual acuity, binocular diplopia, nystagmus and left facial paralysis. His VEP confirmed demyelinating optic neuropathy and his brain MRI showed different dysmyelinating lesions. The patient

presented to the metabolic platform for urinary screening of a suspected neurometabolic disease during a decompensation episode on the 21/02/2022. Thin-layer chromatography performed during the flare-up revealed the presence of Hexosylceramides. Following a diagnosis of R-R multiple sclerosis, the patient was prescribed oral Gylenya 0.5 mg/day, resulting in a noticeable improvement in symptoms. A subsequent TLC analysis of the patient's urine on 17/10/2024 showed no evidence of Hexosylceramides.

The figure below (Figure 24) compares both TLCs before and after the treatment. The HexCer spot was no longer detectable after treatment and resolution of the flare-up.

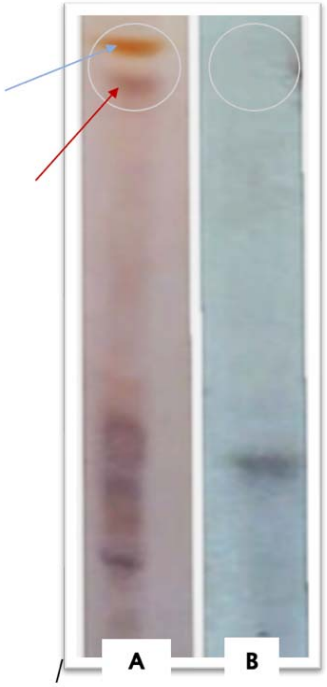



**Figure 26** : Thin-Layer Chromatography (TLC) plates of urinary lipid extract of a patient diagnosed with RR Multiple sclerosis. (A)= TLC during disease flare-up, (B)= TLC after treatment and improvement of symptoms. Red arrow: Hexosylceramides, Blue arrow: Phospholipids.

In line with our findings, Antonio Checa and al. [125] used liquid chromatography tandem mass spectrometry to analyze sphingolipids in Cerebrospinal fluid (CSF). Their results indicated that levels of HexCer increases in the CSF of Multiple sclerosis patients with disease worsening

and that CSF levels of HexCer may be suitable to monitor worsening disability status in patients with RRMS. We conclude that Hexosylceramide is a promising candidate biomarker of disease progression and a therapeutic response biomarker in MS. We should also note that our method is advantageous due to the non-invasive nature of urine collection for Hexosylceramide detection, unlike the invasive procedure of CSF collection.

# IV. Diagnosis:

We were able to diagnose 16 patients (42.1%) in our study with a predominance of Gaucher disease, Niemann-Pick disease and Metachromatic leukodystrophy (2.12% each). In Tipu Sultan and al. [86] study 94.3% of the patients were diagnosed with a predominance of Metachromatic leukodystrophy (21%) followed by Adrenoleukodystrophy (19.7%). Neurowilson disease affected 3.3% of the patients versus 2.12% in our study. Multiple sclerosis was diagnosed in 1.4% compared to 2.12% in our study and Gaucher disease was seen in 0.5% of the patients of the study versus 4.25% in ours. In another study done by S A Mirowitz and al. [103] 20.6% of the patients were diagnosed with different leukodystrophies including Metachromatic leukodystrophy (1.6%) and Krabbe disease (4.7%). In addition to these leukodystrophies, 1 patient was diagnosed with Ornithine transcarbamylase deficiency and 1 other patient with Neurowilson disease. We summarized the spectrum of diagnosis in our study and these 2 studies the table below (TableXXIII):

Table XXIII: The spectrum of diagnosis in our study compared to Tipu Sultan and Mirowitz studies.

| Name of disease | Our study (n=47) | Tipu Sultan and al. (n=366) | S A Mirowitz and al. (n=63) |
|---|---|---|---|
| Gaucher disease | 4.25% | 0.5% | - |
| Krabbe disease | 2.12% | - | 4.7% |
| Niemann-Pick disease | 4.25% | - | - |
| Metachromatic leukodystrophy | 4.25% | 21% | 1.6% |
| Neurowilson | 2.12% | 3.3% | 1.6% |
| Multiple sclerosis | 2.12% | 1.4% | - |
| Menkes disease | 2.12% | - | - |
| Neuronal ceroid lipofuscinosis | 2.12% | - | 4.7% |
| Ornithine transcarbamylase deficiency | 2.12% | - | 1.6% |
| Adrenoleukodystrophy | - | 19.7% | 4.7% |
| Unclassified | 65.9% | 5.7% | 27% |

# V. Prognosis and treatment:

The treatment and prognosis of pediatric neurodegenerative diseases vary widely depending on the specific disorder, its underlying mechanisms, and the timing of intervention.

## 1. Treatment approaches

Treatment in pediatric degenerative brain disorders is directed towards the underlying disorder, other associated features, and complications.

The treatable complications include; epilepsy, sleep disorder, behavioral symptoms, feeding difficulties, gastroesophageal reflux, spasticity, drooling, skeletal deformities, and recurrent chest infections. These children require a multidisciplinary team approach with the involvement of several specialties including pediatrics, neurology, genetics, orthopedics, physiotherapy, and occupational therapy.

Many new antiepileptic drugs have been developed to treat intractable epilepsy [126]. A 3mg dose of melatonin taken at bedtime has been documented to regulate the sleep-wake cycle, particularly in those with ocular impairment [127]. Lioresal or diazepam can help alleviate spasticity, improve limb movement, and reduce pain.

The table below summarizes examples of specific treatments designed to counteract the offending metabolite, replace the dysfunctional enzyme, or provide vitamin therapy (TableXXIV) [104]:

Table XXIV : Specific treatment of some important neurodegenerative disorders.

| Neurodegenerative disorders | Specific treatment modality |
|---|---|
| Gaucher disease | ERT: Imiglucerase, Velaglucerase alfa |
| Krabbe disease | Bone marrow transplantation |
| Metachromatic leukodystrophy | Bone marrow transplantation/gene therapy |
| Mucopolysaccharidosis | - Bone marrow transplantation<br>- Recombinant human α-L-iduronidase |
| Menkes disease | Copper sulfate |
| Mitochondrial encephalopathies | Nicotinamide, riboflavin, dichloroacetate, L-carnitine, CoQ10 |
| Neurowilson disease | - D-penicillamine, trietine, zinc acetate,<br>- Liver transplantation |
| Adrenoleukodystrophy | - Glyceryl trioleate and trierucate,<br>- Steroids for adrenal insufficiency,<br>- Diet low in VLCFA,<br>- Bone marrow transplantation |

Unfortunately, not all degenerative brain disorders are treatable. This lack of effective therapies often leads to a progressive decline in cognitive function, physical abilities, and quality of life for affected individuals. As a result, the prognosis for many patients is poor, with limited options for slowing or halting disease progression. While some progress has been made, significant challenges remain in understanding the underlying mechanisms of these diseases and translating research findings into clinical applications.

## 2. Prognosis:

The prognosis of pediatric neurodegenerative diseases can be assessed using various criteria that encompass clinical manifestations, severity and progression, age of onset, quality of life and lack of effective treatment.

Children with these conditions suffer from progressive cognitive and physical decline, eventually leading to death. Despite their rarity, these disorders collectively contribute significantly to the burden of disease in the pediatric population [128].

The age at onset of symptoms in pediatric neurodegenerative diseases plays a crucial role in determining the clinical course and prognosis. Early-onset disorders tend to have more severe outcomes compared to those that manifest later in childhood. NDDs in children, like Tay-Sachs disease, in generally have a poor prognosis for infantile forms, with most children not surviving beyond age 4 due to rapid progression of symptoms including severe psychomotor regression and seizures [129]. In our study, 80.9% of our patients were presented with infantile age at onset ( $\leq$ 4years). Even though the remaining 19.1% of patients had an older age at onset, the overall prognosis for these patients remains poor.

Psychomotor regression (44.7%), psychomotor delay (42.5%) and seizures (38.3 %) are the chief complaints in our study, affecting 28 patients. These findings suggest a poor prognosis for nearly 60% of our patients [130][131]. It is important to note that these results do not imply a positive prognosis for the other patients. While the identified most commun symptoms in our study are associated with a poorer outcome, it is important to acknowledge that other factors may also influence disease progression. With some neurodegenerative diseases manifesting with primary severe developmental disorders, others diseases initially develop normally and then progressively deteriorate which is the case in Metachromatic leukodystrophy [132].

Another factor influencing the prognosis of NDDs is the limited availability of effective treatments. While some treatments, such as enzyme replacement therapy (ERT) [133], substrate

reduction therapy (SRT) [134], and gene therapy [135], have been developed for certain NDDs, many patients still lack access to these therapies or benefit from them. In our study, only 5 patients (10.6%) patients benefited from causal treatment in which 2 still presented gradual deterioration of their symptoms. Treatment was symptomatic for 36.1% and the rest of the patients didn't benefit from any interventions.

The high prevalence of poor prognostic factors in our cohort underscores the urgency of early diagnosis and intervention. HexCer, as a potential biomarker, could facilitate timely detection, allowing for prompt initiation of treatment. By identifying patients at risk early, we can potentially improve clinical outcomes and enhance the quality of life for individuals with neurodegenerative diseases.

Furthermore, early diagnosis can enable targeted therapeutic interventions, such as enzyme replacement therapy or substrate reduction therapy, which may slow disease progression and alleviate symptoms. Ultimately, the use of HexCer as a biomarker could revolutionize the management of neurodegenerative diseases, leading to improved patient outcomes and a better quality of life.

# VI. Involvement of Hexosylceramides in Neurodegenerative disorders:

The table below (TableXXV), summarizes the involvement of Hexosylceramides in neurodegenerative diseases in different studies using lipidomics.

**TableXXV: Hexosylceramides evaluation by lipidomics in different neurodegenerative diseases.**

| Disease | Compounds | Profile | Profile details | Methods of detection/Instruments | Tissue | References |
|---|---|---|---|---|---|---|
| **Gaucher Disease** | HexCer<br>*Urine + Plasma:*<br>(d18:1/16:0)<br>(d18:1/22:0)<br>(d18:1/24:1)<br><br>*Urine only:*<br>(d18:1/20:0)<br><br>*Plasma only:*<br>(d18:1/24:0) | ↑ | More than 2-fold difference between controls and GD patients In urine & 3-fold higher in plasma.<br><br>HexCer species would serve as excellent candidates in developing an early diagnostic test for GD using both plasma and urine. | LC/ESI/MS | Plasma<br>+<br>Urine | **[136]** |
| **Niemann-Pick Disease** | HexCer<br>(16:0, 18:0, 20:0, 22:0, 24:0) | ↑ | 2 to 2.5-fold elevations in all HexCer species | HPLC/MS/MS | Plasma | **[137]** |

TableXXV: Hexosylceramides evaluation by lipidomics in different neurodegenerative diseases (suite..).

| Disease | Compounds | Profile | Profile details | Methods of detection/Instruments | Tissue | References |
|---|---|---|---|---|---|---|
| **Alzheimer's disease** | HexCer (m19:0_21:2) | ↑ | AD/control = 1.99 | LC-FTMS | Urine samples | **[138]** |
| | HexCer | ↑ | HexCer significantly higher concentrations in AD brains + capable of measuring disease severity | LC/MS/MS | Brain tissue (neocortex biopsy) | **[139]** |
| | HexCer (18:1, 24:1) | ↑ | HexCer are elevated in Patients with AD compared to control patients | ESI/MS/MS | Plasma | **[140]** |
| **Parkinson's disease** | HexCer (16:0, 20:0, 24:0) | ↑ | Increased in PD patients with cognitive impairment | HPLC coupled to/ESI/MS/MS | Plasma | **[141]** |
| | GlcCer | ↑ | Glucosylceramide as a Pathway biomarker for GBA-PD patients | LC/MS/MS | CSF | **[142]** |
| | HexCer | ↑ | Increased in the GBA mutation carriers | HPLC | Serum | **[143]** |
| | HexCer (34:1, 42:1) | ↑ | Increased in L444P-GBA by 70% compared to control and idiopathic PD | Mass Spectrometry (Shotgun lipidomics) | Fibroblasts (skin biopsy) | **[144]** |

TableXXV: Hexosylceramides evaluation by lipidomics in different neurodegenerative diseases (suite…).

| Disease | Compounds | Profile | Profile details | Methods of detection/Instruments | Tissue | References |
|---|---|---|---|---|---|---|
| **Multiple sclerosis** | HexCer (16:0) | ↑ | Increased concentrations of HexCer induce mitochondrial dysfunction and axonal damage | Mass Spectrometry | CSF | **[145]** |
| | HexCer (16:0) | ↑ | Increased in Progressive MS (marker of worsening) HexCer16:0 is a promising candidate marker of disease progression in MS, especially in RRMS (relapsing/remitting MS). | UHPLC/MS/MS | CSF | **[125]** |
| | HexCer (20:0, 18:1) | ↑ | HexCer plasma levels correlated with increased brain atrophy especially in patients with progressed disability | LC/MS/MS | Plasma | **[146]** |
| | HexCer (16:0, 18:0, 18:1, 24:0, 24:1) | ↑ | HexCer increased in IN-MS lesions (inactive MS lesions) | HPLC/MS/MS | Brain tissue (white matter biopsy) | **[147]** |

TableXXV: Hexosylceramides evaluation by lipidomics in different neurodegenerative diseases (suite…).

| Disease | Compounds | Profile | Profile details | Methods of detection/Instruments | Tissue | References |
|---|---|---|---|---|---|---|
| **Amyotrophic lateral sclerosis** | HexCer (Glucosylceramide) | ↑ | Increase of levels of GlcCer observed in the CSF of ALS patients + Inhibition of GlcCer degradation by CBE (Conduritol B epoxide: a GCase inhibitor) improves in vitro axonal plasticity and in vivo nerve regeneration. | HPLC | CSF | **[148]** |
| **Age related maculopathy** | HexCer (16:1, 18:1) | ↑ | Increasing HexCer were significantly associated with increasing odds of suffering from GA (Geographic atrophy) or late AMD (i.e. GA + CNV: Choroidal neovascularization) | ESI/MS/MS (Electrospray ionization) | Serum | **[149]** |

TableXXV: Hexosylceramides evaluation by lipidomics in different neurodegenerative diseases (suite..).

| Disease | Compounds | Profile | Profile details | Methods of detection/Instruments | Tissue | References |
|---|---|---|---|---|---|---|
| **Lewy Body Dementia (Autopsy confirmed LBD)** | HexCer (GlcCer + GalCer) | ↑ | Increased levels of HexCer in LBD patients carrying GBA mutations compared to control patients | Lipidomic analysis | Postmortem brain Tissue (Lipidomic analysis) | **[150]** |
| | HexCer (18:1, 24:1) | ↑ | HexCer are elevated in patients with LBD compared to control patients (higher in high likelihood DLB groups than intermediate likelihood groups = CN) | ESI/MS/MS | Plasma | **[140]** |
| **Long-Chain 3-Hydroxyacyl -CoA Deficiency (LCHADD)** | HexCer | ↑ | 2-fold increase of HexCer Compared to healthy control patients | Mass spectrometry | Fibroblasts | **[151]** |
| **Neurodegeneration In type 2 Diabetes** | HexCer (34:1, d16:0_22:6) | ↑ | HexCer were higher in Type 2 diabetes with MCI (mild cognitive impairment) compared to NC (normal cognitive) patients | Targeted lipidomic analysis | Serum | **[152]** |
| **Adreno-leukodystrophy** | HexCer 44:1 (d18:1/C26:0) | ↑ | HexCer showed a significant abundance in X-ALD patients' fibroblasts | LC/ESI/MS | Fibroblasts | **[153]** |

# VII. Strengths and limitations of the study

Several limitations should be acknowledged. Firstly, this is a single-center retrospective study of prospectively collected data with a small sample size and a relatively short follow-up period. Secondly, the retrospective nature of our study poses some challenges such as data loss and incomplete or inconsistent information. We also note that in some cases the patients were unreachable hence the lack of information (our result might be prone to selection bias). And lastly, the study relies on TLC outcomes, which is a very efficient technique in our context, but isn't enough to confirm nor put the diagnosis for each patient, so we had to rely on other tests such as enzymatic assays or molecular analysis to make a diagnosis. But unfortunately, most our patients didn't have the means to undergo more specialized tests.

However, the major strength of this study is the detailed description of the association between the HexCer metabolism disorders and the neurodegenerative disease pathogenesis. Moreover, considering the limited number of reports about the use of TLC to highlight the presence of HexCer in urine (most of the reports we found focused on other biofluids such as blood samples or CSF samples and brain tissue...), this study allowed us to consider the use of TLC analysis of crude urine samples and urinary lipid extracts as a rapid diagnosis method based on the combination of the clinical symptoms and the detection of known biomarkers.

# VIII. Recommendations:

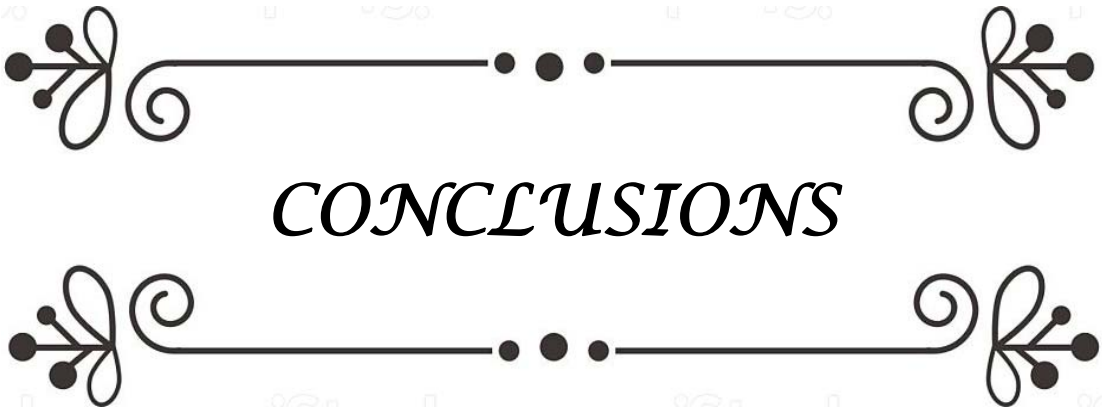
Based on our findings, we propose the following recommendations to improve the diagnosis and management of neurodegenerative diseases:

→ Endorse HexCer as a potential predictive biomarker of neurodegeneration in particular and as a biomarker of unfavorable prognosis in general.

→ Implement routine screening for HexCer in at-risk populations, particularly those with a family history of neurodegenerative disorders or unexplained neurological symptoms.

→ Explore the potential of targeting HexCer metabolism as a therapeutic strategy for neurodegenerative diseases.

→ Counseling the families and educating the public about these potentially preventable disorders is very important. A national campaign to increase the public awareness about these devastating disorders and discourage consanguineous marriages is urgently needed in our region.



# *CONCLUSIONS*

In conclusion, our study underscores the significant challenges posed by the diverse and often overlapping clinical presentations of neurodegenerative diseases, particularly in pediatric populations.

Early and accurate diagnosis is crucial for timely intervention and improved patient outcomes. Our findings highlight the potential of Hexosylceramides (HexCer) as a valuable biomarker for identifying individuals at risk of developing severe neurodegenerative disease. The non-invasive nature of urine testing, coupled with the simplicity and cost-effectiveness of thin-layer chromatography (TLC), makes HexCer a promising tool for routine clinical practice.

By implementing HexCer-based screening strategies, we can improve early diagnosis, optimize treatment plans, and ultimately enhance the quality of life for affected individuals and their families.

Further research is needed to validate these findings in larger, more diverse populations and to explore the potential of HexCer as a therapeutic target. Additionally, investigating the underlying molecular mechanisms that link HexCer levels to disease severity may provide valuable insights into the pathophysiology of neurodegenerative diseases. By advancing our understanding of these complex disorders, we can develop more effective diagnostic tools and therapeutic interventions.

# ABSTRACT

# Abstract

**Introduction**:

Neurodegenerative diseases in children are common in Morocco as a result of the high rate of consanguinity; they are classified into different disorders and characterized by a variety of clinical manifestations, complex molecular biology, and a long list of potential investigations. Our study is the first of its kind assessing the involvement of HexCer in neurodegenerative diseases using TLC analysis of urine samples and to our knowledge, the first report in the North African region.

**Objective**:

This study aimed to highlight the association between abnormal urinary excretion of Hexosylceramides (HexCer), and the neurodegeneration in patients suspected with inherited metabolic diseases and their potential implication in the evolution and prognosis of those diseases.

**Patients and methods**:

This is a retrospective study over 5 years describing the available data concerning 47 patients referred to the Metabolic platform suspected or diagnosed with a neurodegenerative disease, whose Thin-Layer Chromatography (TLC) analysis revealed the presence of HexCer in urine.

**Results**:

During this period 47 patients were sent to the Metabolic platform for further testing to aid in their diagnosis. The mean age was 5.22 years (27 days – 25 years), 53.2% of patients were female and 51.1% were located in a rural area.

2 clinical forms were described in our study, the first form with strictly neurological symptoms (79.2%) and the second one with neurological and visceral symptoms (20.8%). Consanguinity was most prevalent medical history in our cohort with a percentage of 59.6%.

The average age at onset was 2.63 years (3days-18years) with a predominance of the infantile age group (80.8%).

The chief onset symptoms in in our series were psychomotor regression (44.7%) followed with psychomotor delay (42.5%) and seizures (38.3%).

Brain MRI were performed for 31 patients over 47, among them 11 (23.4%) had normal brain MRI and 20 patients presenting pathological features such as cerebral atrophy and/or white matter signal abnormalities and dysmyelinating lesions.

Using enzymatic essays and other tests, we managed to obtain the diagnosis for 16 patients (42.1%) with a predominance of Metachromatic leukodystrophy, Gaucher disease and Niemann-Pick disease.

The prognosis for 42 patients (89.4%) was unfavorable including 7 deaths.

**Conclusion**:

In conjunction with clinical and paraclinical features, we conclude that Hexosylceramides play a major role in neurodegenerative diseases and is a viable biomarker in predicting the progression and severity of these diseases. Additionally, many studies associated increased levels of HexCer in different biofluids in neurodegenerative diseases and considered it a promising marker of neurological impairment and poor prognosis.

# Résumé

**Introduction** :

Les maladies neurodégénératives chez les enfants sont fréquentes au Maroc en raison du taux élevé de consanguinité, elles sont classées en différents troubles et caractérisées par une variété de manifestations cliniques, une biologie moléculaire complexe et une longue liste d'investigations potentielles. Notre étude est la première du genre à évaluer l'implication de l'HexCer dans les maladies neurodégénératives en utilisant la Chromatographie à couche mince (CCM) d'échantillons d'urine et, à notre connaissance, le premier rapport dans la région de l'Afrique du Nord.

**Objectif :**

Cette étude avait pour but de mettre en évidence l'association entre une excrétion urinaire anormale d'hexosylcéramides et la neurodégénérescence chez des patients suspectés de maladies métaboliques héréditaires et leur implication potentielle dans l'évolution et le pronostic de ces maladies.

**Patients et méthodes :**

Il s'agit d'une étude rétrospective sur 5 ans décrivant les données disponibles concernant 47 patients adressés à la plateforme métabolique, suspectés ou diagnostiqués avec une maladie neurodégénérative, dont l'analyse par chromatographie en couche mince (CCM) a révélé la présence d'HexCer dans les urines.

**Résultats :**

Au cours de cette période, 47 patients ont été envoyés à la plateforme métabolique pour y subir des tests supplémentaires afin de faciliter leur diagnostic. L'âge moyen était de 5,22 ans (27 jours - 25 ans), 53,2 % des patients étaient des femmes et 51,1 % vivaient dans une zone rurale.

2 formes cliniques ont été décrites dans notre étude, la première avec des symptômes strictement neurologiques (79,2%) et la seconde avec des symptômes neurologiques et viscéraux (20,8%). La consanguinité était l'antécédent médical le plus fréquent dans notre cohorte avec un pourcentage de 59,6 %.

L'âge moyen d'apparition de la maladie était de 2,63 ans (3 jours-18 ans) avec une prédominance du groupe d'âge infantile (80,8 %).

Les principaux symptômes d'apparition dans notre série étaient la régression psychomotrice (44,7 %), suivie d'un retard psychomoteur (42,5 %) et de crises d'épilepsie (38,3 %).

Une IRM cérébrale a été réalisée chez 31 patients, parmi lesquels 11 (23,4%) avaient une IRM cérébrale normale et 20 patients présentaient des caractéristiques pathologiques telles qu'une atrophie cérébrale et/ou des anomalies du signal de la substance blanche et des lésions de démyélinisation.

En utilisant le dosage de l'activité enzymatiques et d'autres tests diagnostiques, nous avons réussi à obtenir le diagnostic pour 16 patients (42,1%) avec une prédominance de Leukodystrophy métachromatique, de maladie de Gaucher et de maladie de Niemann-Pick.

Le pronostic de 42 patients (89,4 %) était défavorable, dont 7 décès.

## Conclusion

En conjonction avec les caractéristiques cliniques et paracliniques, nous concluons que les hexosylcéramides jouent un rôle majeur dans les maladies neurodégénératives et constituent un biomarqueur viable pour prédire la progression et la gravité de ces maladies. En outre, de nombreuses études ont associé des niveaux accrus d'HexCer dans différents bio fluides dans les maladies neurodégénératives et l'ont considéré comme un marqueur prometteur de déficience neurologique et de mauvais pronostic.

# ملخص

**مقدمة**

تعد الأمراض العصبية التنكسية لدى الأطفال شائعة في المغرب نتيجة لارتفاع معدل زواج الأقارب، وهي تصنف إلى اضطرابات مختلفة وتتميز بمجموعة متنوعة من المظاهر السريرية والبيولوجيا الجزيئية المعقدة وقائمة طويلة من الفحوصات المحتملة .تعتبر دراستنا هي الأولى من نوعها التي تقيّم مشاركة هيكسوسيلسيراميد في الأمراض العصبية التنكسية باستخدام تحليل الطبقات اللونية الرقيقة لعينات البول، وعلى حد علمنا، أول تقرير في منطقة شمال أفريقيا

**الأهداف**

هدفت هذه الدراسة إلى تسليط الضوء على الارتباط بين الإفراز غير الطبيعي لهيكسوسيلسيراميد في البول، والتنكس العصبي لدى المرضى المشتبه في إصابتهم بأمراض التمثيل الغذائي الوراثية وتأثيرها المحتمل في تطور تلك الأمراض وتشخيصها.

**المرضى والطرق :**

هذه دراسة بأثر رجعي على مدى 5سنوات تصف البيانات المتاحة بشأن 47مريضًا أُحيلوا إلى منصة التمثيل الغذائي للاشتباه في إصابتهم أو تشخيص إصابتهم بمرض تنكسي عصبي، وكشف تحليل الطبقات اللونية الرقيقة عن وجود هيكسوسيلسيراميد في البول

**النتائج**

خلال هذه الفترة، تمت إحالة 47مريضًا إلى منصة التمثيل الغذائي لإجراء المزيد من الاختبارات للمساعدة في التشخيص .كان متوسط العمر 5.22عامًا 27)يومًا 25 -عامًا(، وكان 53.2%من المرضى من النساء و 51.1%منهم يعيشون في منطقة ريفية.

تم وصف شكلين سريريين في دراستنا، الأول بأعراض عصبية بحتة (79.2%) والثاني بأعراض عصبية وحشوية (20.8%). كانت صلة القرابة هي التاريخ الطبي الأكثر شيوعًا في مجموعتنا بنسبة .59.6%

كان متوسط عمر بداية المرض 2.63سنة 3) أيام 18 -سنة(، مع غلبة الفئة العمرية الطفولية .(80.8%)

كانت الأعراض الرئيسية لبداية المرض في سلسلتنا هي التراجع الحركي النفسي (44.7%)، يليه التخلف الحركي النفسي (42.5%)ونوبات الصرع .(38.3%)

تم إجراء التصوير بالرنين المغناطيسي للدماغ لدى 31 مريضًا تزيد أعمارهم عن 47عامًا، منهم 11مريضًا (23.4%) كان التصوير بالرنين المغناطيسي للدماغ طبيعيًا و20 مريضًا لديهم سمات مرضية مثل ضمور الدماغ و/أو تشوهات في إشارة المادة البيضاء وآفات خلل النخاع.

باستخدام مقايسات الإنزيمات، تمكنا من الحصول على تشخيص 16مريضًا (42.1%)مع غلبة داء حثل المادة البيضاء المتبدل اللون وداء غوشيه وداء نيمان -بيك.

كان تشخيص 42 مريضاً (89.4%) غير مواتٍ، بما في ذلك 7حالات وفاة.

**استنتاج**

وبالاقتران مع السمات السريرية وشبه السريرية، نستنتج أن الهكسوسيلسيراميد يلعب دورًا رئيسيًا في الأمراض العصبية التنكسية وهو مؤشر حيوي قابل للتطبيق في التنبؤ بتطور هذه الأمراض وشدتها .بالإضافة إلى ذلك، ربطت العديد من الدراسات بين زيادة مستويات الهيكسوسيلسيراميد في السوائل الحيوية المختلفة في الأمراض العصبية التنكسية واعتبرته علامة واعدة على ضعف الجهاز العصبي وسوء التشخيص.

# *APPENDICES*

# APPENDIX: OPERATING SHEET – INVOLVEMENT OF HEXOSYLCERAMIDES IN NEURODEGENERATIVE DISEASES

## A- Identity:

- Name:
- File number:
- Age/Date of birth:
- Gender:
  - o Male
  - o Female
- Habitat:
  - o Rural
  - o Urban

## B- Medical history:

### 1) Personal:

- o Cholestatic jaundice
- o Liver cirrhosis
- o Perinatal asphyxia
- o Intra uterine growth delay
- o No history

### 2) Familial:

- o ··Consanguinity
- o ··Similar case in family
- o ··Death of siblings
- o ··No history

## C- Clinical data:

- Age at onset of symptoms
- Revelation mode:
  - o ··Psychomotor regression
  - o ··Psychomotor delay
  - o ··Hepatomegaly
  - o ··Splenomegaly
  - o ··Epilepsy / Seizures

- ··Ataxia
- ··Hypotonia
- ··Ocular impairment
- ··Delayed growth
- ··Facial dysmorphia
- ··Extrapyramidal symptoms
- ··Abnormal movements

## D- Paraclinical data:

### 1) General tests:

- **Biology:**

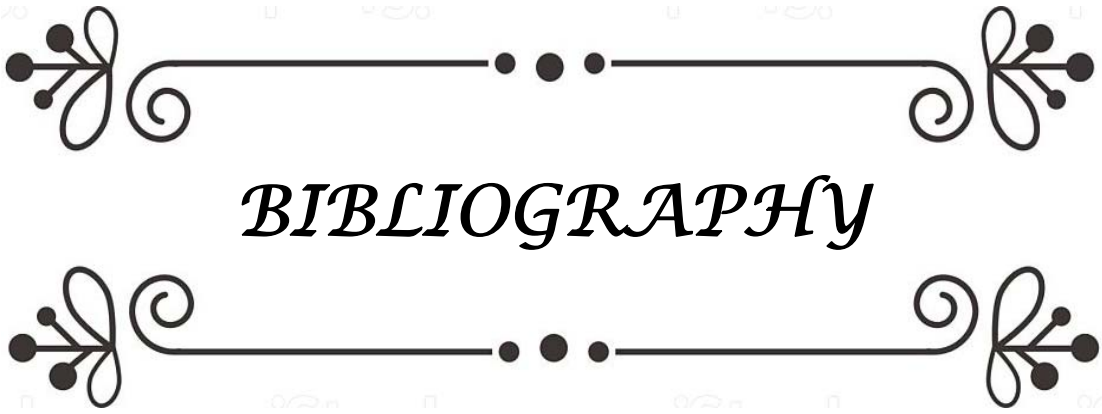
| Test | Results |
|---|---|
| CBC (complete blood count) | |
| Liver function | |
| Haemostasis blood test | |

- **Radiology:**

| Test | Results |
|---|---|
| EEG | |
| Brain MRI | |
| Bone X-ray | |
| Abdominal ultrasound | |

### 2) Specialized tests:

| Test | Results |
|---|---|
| Enzymatic dosage | |
| Thin-Layer Chromatography | |



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# قسم الطبيب

## أقْسِم بِالله العَظِيم

**أن أراقبَ الله في مِهْنَتِي.**

**وأن أصُونَ حياة الإنسان في كآفّةِ أطوَارهَا في كل الظروف والأحَوال باذلة وسْعِي في إنقاذها مِن الهَلاكِ والمرَضِ والألَم والقَلَق.**

**وأن أحفَظَ لِلنَاسِ كرَامَتهُم، وأسْتر عَوْرَتهُم، وأكتمَ سِرَّهُمْ.**

**وأن أكونَ عَلى الدوَام من وسائِل رحمة الله، باذلة رِعَايَتي الطبية للقريب والبعيد، للصالح والطالح، والصديق والعدو.**

**وأن أثابر على طلب العلم، وأسَخِّرَه لِنَفْعِ الإنْسَان لا لأذَاه.**

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**وأن تكون حياتي مِصْدَاق إيمَاني في سِرّي وَعَلانيَتي، نَقِيَّة مِمّا يُشينهَا تجَاهَ الله وَرَسُولِهِ وَالمؤمِنين.**

## والله على ما أقول شهيد

كليـــــــة الطب
والصيدلة - مراكش
FACULTÉ DE MÉDECINE
ET DE PHARMACIE - MARRAKECH

MARRAKECH
جامعة القاضي عياض
UNIVERSITÉ CADI AYYAD

سنة 2024 أطروحة رقم 335

# تورط هيكسوسيل سيراميد في الأمراض العصبية التنكسية: دراسة بأثر رجعي.

## الأطروحة

قدمت ونوقشت علانية يوم **2024/11/11**

من طرف

**السيدة هجر مشاشتي أبختي**

المزدادة في 14 شتنبر 1996 بمراكش

## لنيل شهادة الدكتوراه في الطب

**الكلمات الأساسية:**

جلوكوزيل سيراميد - جالاكتوسيل سيراميد - هيكسوسيل سيراميد - الأمراض العصبية التنكسية - كروماتوغرافيا الطبقة الرقيقة.

## اللجنة

| | | |
|---|---|---|
| السيدة | ل. السعدوني<br>أستاذة في الطب الباطني | الرئيسة |
| السيدة | ن. فضيل<br>أستاذة في كيمياء التنسيق البيولوجي العضوي | المشرفة |
| السيدة | ص. زحلان<br>أستاذة في الطب الباطني | الحكام |
| السيدة | ن. لوهاب<br>أستاذة في طب الأعصاب | |
| السيد | ن. راضـى<br>أستاذ في طب الأطفال | |