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MYCOBACTERIUM ABSCESSUS – DIAGNOSTIC AND THERAPEUTIC FRONTIERS IN INFECTION MANAGEMENT

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Abstract

Mycobacterium (M.) abscessus, a highly pathogenic non-tuberculous mycobacterium, is responsible for several clinical manifestations. A very frequent occurrence is proven in patient with various lung diseases. Furthermore, it can result in complications such as skin and soft tissue diseases, central nervous system infections, bacteremia, eye infections, and others. *M. abscessus* is a clinical contraindication in cystic fibrosis patients awaiting a lung transplant, as it can exacerbate disease progression. Its pathogenicity and the emergence of resistance are influenced by factors including the composition of the cell envelope, rough and smooth *M. abscessus* morphotypes, efflux pumps, antibiotic-modifying/inactivating enzymes, and genetic polymorphisms in target genes. Management of the infection requires multicomponent therapy due to the high level of resistance. The following antibiotics are recommended according to the guidelines from the year 2017: amikacin, tigecycline, and imipenem with a macrolide. In order to properly manage patients with *M. abscessus* infection, correct identification of the subspecies as well as determination of resistance is essential. To achieve this goal, molecular-genetic techniques, such as whole-genome sequencing, are becoming increasingly favored in modern clinical practice. In this review, we provide up-to-date information on the issue of infections caused by non-tuberculous *M. abscessus*. We focus on its characteristics, possible infectious diseases, cystic fibrosis, and resistance, as well as the benefits of whole-genome sequencing.

Keywords: Mycobacterium abscessus, resistance, whole-genome sequencing

INTRODUCTION

While non-tuberculous mycobacteria (NTM), enviromental opportunistic pathogenic bacteria, are not responsible for causing tuberculosis, they present a distinct set of challenges in clinical practice. Therefore, an accurate identification is critically important in setting the correct treatment regimen (1).

Mycobacterium (M.) tuberculosis (MTB) is characterised by inhalation way of transmission by patients with a symptomatic disease, while NTM spread from the environment to the patient. After the inhalation, both are stored in the form of persisters, which are antibiotic-resistant bacteria cells found inside macrophages (2).

NTM are bacterial species stained acid-fast (3) and are generally less virulent compared to MTB. They consist of more than 190 species and subspecies (4), including rapidly-growing species like *M. abscessus* (5). NTM can cause diseases in both immunocompromised and immunocompetent hosts. The most common NTM clinical manifestation is lung disease and it mainly occurs in patients with pre-existing lung conditions (6). Importantly, many of the

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patients do not have obvious risk factors. The treatment of NTM lung disease is challenging, prolonged, and costly (3).

The incidence of NTM infectious diseases and related deaths is steadily on the rise. Studies from North America, Europe, and Asia have shown a rising incidence of NTM disease over the past two decades. For clarification, the estimated prevalence of NTM disease in the United States increased from 2.4 cases per 100,000 in the early 1980s to 15.2 cases per 100,000 by 2013. Between 1997 and 2007, there was a more than twofold increase in prevalence among the elderly population (>65 years), rising from 20 cases per 100,000 to 47 cases per 100,000. A study conducted in the UK revealed similar trends, as NTM infection rates more than tripled, increasing from 0.9 cases per 100,000 in 1995 to 2.9 cases per 100,000 in 2006 (7). A summary of this information is provided in the table (Tab. 1). Similar trends were observed in Germany and Denmark (7–9).

Table 1 An overview of the increase in NTM cases in United States (7)

Year duration	Increase in cases per 100 people
from the early 1980s to 2013	from 2.4 to 15.2
1997-2007 among the elderly population (>65 years)	from 20 to 47
1995-2006	from 0.9 to 2.9

These findings also confirmed that the prevalence of infections increases with the age as a result of age-related immune system changes. The number of NTM pulmonary infections rises globally primarily due to the availability of molecular-based detection. On the other side, not all of these detected isolates indicate actual lung disease. Despite its importance, long-term data are not available for many countries (e.g., there is only a limited number of studies from Eastern Europe and South America). The identification and interpretation of results has become more difficult because of Covid-19 pandemic, lower reporting, and the war in Ukraine (10) .

In addition to lung diseases, NTM are also responsible for infections in other sites of the body, e.g skin and soft tissue, central nervous system, bacteremia, and eyes (11).

MYCOBACTERIUM ABSCESSUS

M. abscessus was first isolated in 1953 by Moore and Frerichs from a knee infection (12). Several studies indicate that *M. abscessus* belongs to the most pathogenic bacteria among NTM (13). It is characterized as a rod-shaped acid-fast bacillus (14) and is considered as one of the most antibiotic-resistant organisms within the NTM group (15).

Mycobacterium abscessus complex (MABC) belongs to the phylum *Actinobacteria* and is a part of the *M. chelonae-abscessus* group which consists of three species: *M. chelonae, M. abscessus*, and *M. immunogenum* (11,12). These microorganisms are characterized by a high content of guanine and cytosine in their DNA. Their distinct outer membrane possesses various characteristics enhancing their resistance to antibiotics. Outer membrane consists of mycolic acid and an arabinogalactan layer (Fig. 1). Other components, such as glycopeptidolipids (GPLs), trehalose-6,6-dimycolate (TDM), trehalose monomycolate (TMM), trehalose polyphleates (TPPs), and phosphatidyl-myo-inositol dimannoside (PIM) are involved in their pathogenity (16).

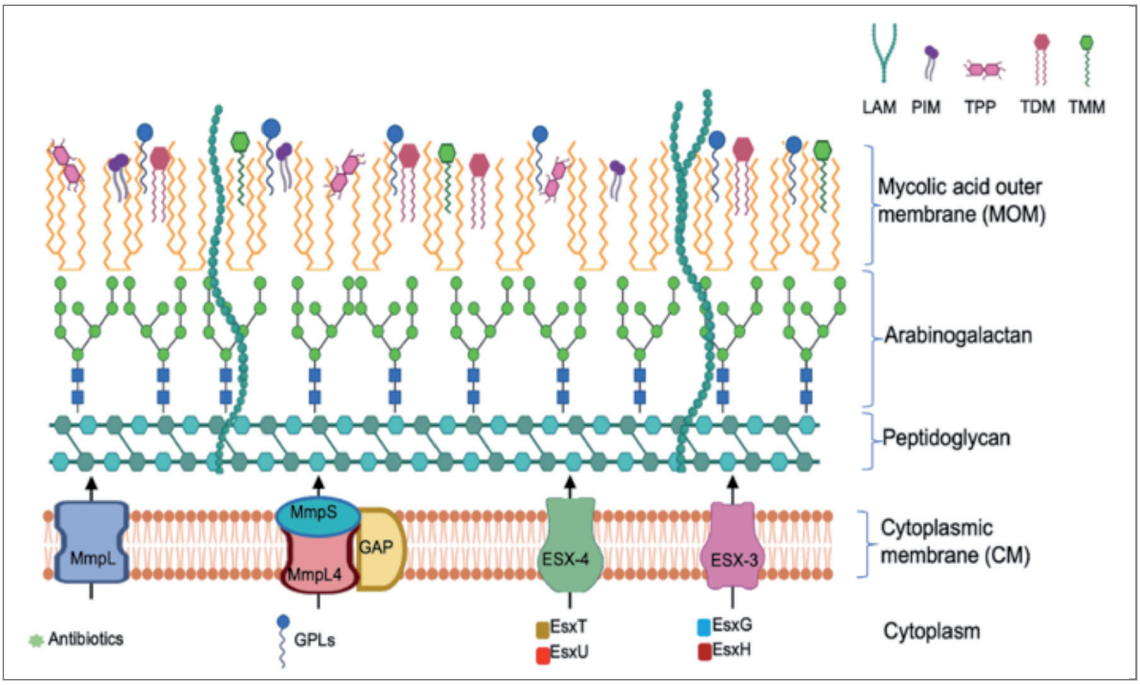

Fig. 1 Modification of components in the cell envelope of *M. abscessus* (16)

The composition of the cell wall plays a crucial role in the antibiotic resistance. Drug efflux through membrane pumps, biofilm formation, and glycopeptidolipids (GPLs) in mycobacterial outer membrane (MOM) are other factors contributing to the resistance. A reduced amount of GLPs leads to an increased hydrophobicity of the MOM. Several antibiotic substances are hydrophilic; therefore, a low sensitivity to these therapeutic agents was documented (16).

ROUGH AND SMOOTH COLONY VARIANTS OF MYCOBACTERIUM ABSCESSUS

M. abscessus is present in two unique morphological types (*M. avium* or *M. smegmatis*), and these types impact their characteristics. The first one is the smooth type, non-cording, but motile and can form biofilms. The second one is the rough type, immobile, and not able to form biofilms (Fig. 2) (17). Smooth colonies represent a wild-type due to their conversion to the rough type. This transformation is mediated by a reduction of surface glycopeptidolipids in the cell envelope of rough form. As mentioned above, a decrease in GLPs leads to an increase in hydrophobicity of the membrane. MABC strains involved in chronic infectious respiratory diseases were shown to be predominantly rough type. In contrast, the smooth morphotype is typically associated with skin abscesses and transient airway colonization (18).

In summary, the morphological variations of *M. abscessus* are important to be characterized especially in cystic fibrosis (CF) patients. In the early stages of this dissease, the MABC smooth forms of the bacterium are prevalent in lung infections, producing GLPs, and forming biofilms. In a more advanced stage, rough types become dominant in more invasive infection, and associated production of trehalose dimycolate leads to the cord formation (20).

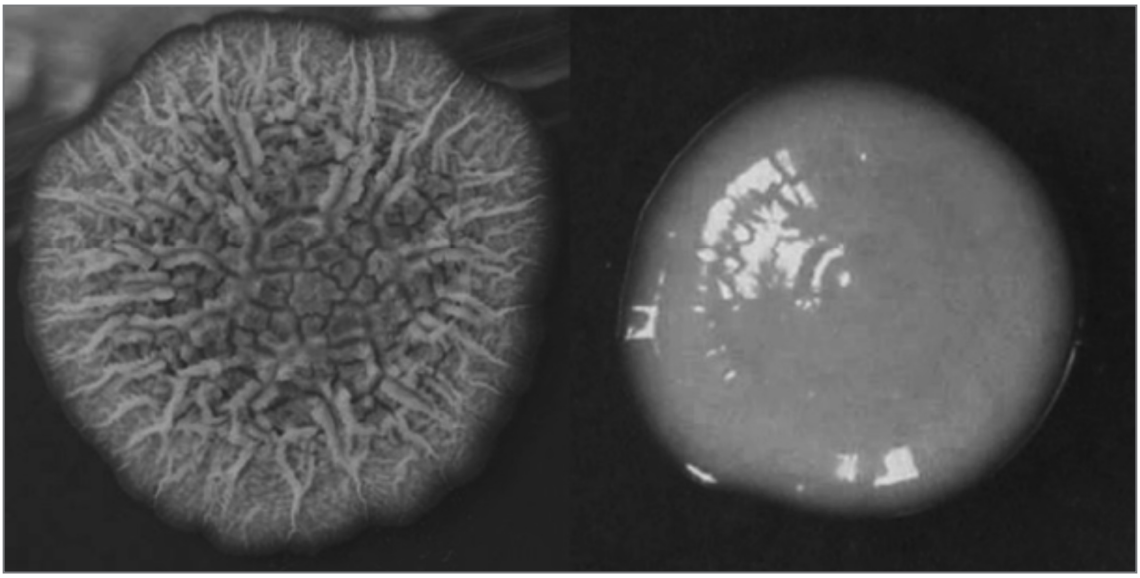

Fig. 2 Rough (left) and smooth (right) colonies of *M. abscessus* on solid agar media (19)

CYSTIC FIBROSIS

NTM, such as *M. abscessus*, commonly affect patients with ongoing lung disease. In patients with CF, infection by *M. abscessus* can exacerbate the underlying disease and lead to the prolongation of its treatment (21).

CF is a multisystemic, chronic, and genetic disease. It is inherited in an autosomal recessive manner (Fig. 3), primarily caused by genetic mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, located on the long arm of chromosome 7 (22,23).

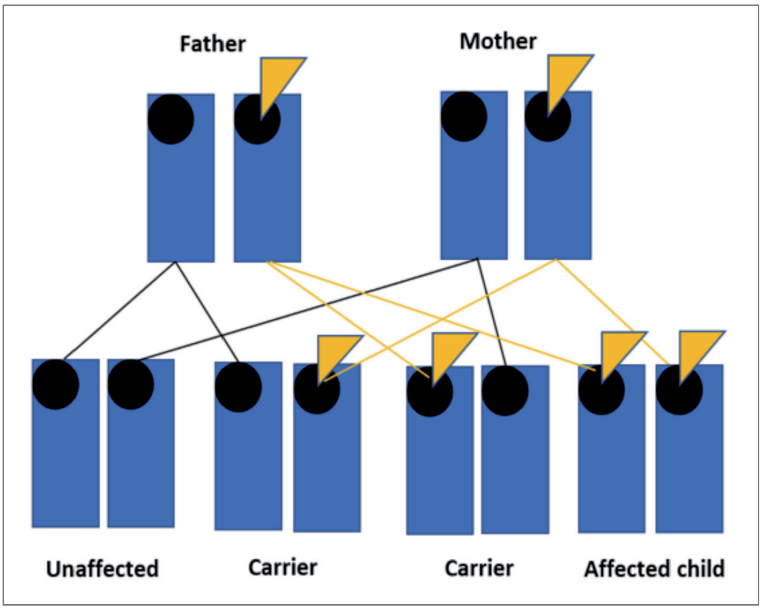

Fig. 3 Recessive inheritance in CF (adjusted based on the work of Gulani a Weiler [25])

This leads to an abnormal CFTR (chloride) channel function, unregulated chloride (Cl^-) excretion, and concomitant sodium (Na^+) release. It is associated with increased concentrations of salt (NaCl) in the sweat of patients, which characterizes the abnormal transport of electrolytes from the sweat gland (22).

Malfunction of CFTR proteins leads to a decrease in chloride secretion into the cellular spaces and, concurrently, an increase in sodium reabsorption. This increased sodium absorption causes the retention of water. As a result of these processes, there is a certain pathological organ manifestation, related to a specific gene mutation of the CFTR channel. It leads to thicker mucus, and to clogging of organ systems. Furthermore, loosing the hypotonicity of periciliar fluid leads to a higher risk of bacterial colonisation. CF primarily affects the lungs, pancreas, liver, biliary system, intestines, and sweat glands (25).

Over the past few decades, there have been improvements in the treatment of people with CF. Previously, this disease was often fatal in infants and young children (26). Nowadays, the majority of individuals are diagnosed for CF either through newborn screening at birth or before the age of two years (27). The progression of the disease in young children was primarily observed in the period of 6 months. A difference in treatment outcomes was noted at initiation of treatment at 4 to 13 months. Improvement in both the respiratory and digestive systems in children under six years of age were observed, if the treatment is initiated within two months from birth (28).

The lung environment with thick mucus creates suitable conditions for the survival of *M. abscessus* (29). Factors such as reduced immunity, reinfection, and repeated lung damage further support the growth of these bacteria in such environment (30).

NTM infections in patients with CF can contribute to many complications and worsen already existing progression of the disease. This also includes an increased number of hospitalizations, severe and progressive decline in lung function, and a risk of reinfections, which may require more aggressive and complicated treatment regimens, and leads to a reduced quality of life (31). Consequently, contamination by *M. abscessus* is considered as a contraindication for CF patients awaiting a lung transplant.

Management of infection is difficult and requires a correct choice of the antibiotic regimen with limited undesirable side effects. These can include e.g. severe nausea, deafness, impaired liver function, etc. (30,32).

CF is disease occured worldwide, related to region and population. It occurs mostly in developed countries, e.g. United States, Canada, the United Kingdom, Ireland, and Australia. Reduced prevalence is documented in Asia, Africa, and certain parts of Southern Europe, most probably as a result of different genetic backgrounds of the disease (33).

TREATMENT AND RESISTANCE

The management of *M. abscessus* infections requires a tailored and personalized strategy due to the frequent and significant resistance. This strategy comprises a prolonged course of therapy and a combination of multiple antibiotics.

Bacteria commonly responsible for lung diseases are primarily associated with the Mycobacterium avium complex (34). Additionally, M. abscessus is the second most common NTM associated in lung diseases and is further classified into three subspecies, i.e. abscessus, massiliense, and bolletii. Bacteria that cause lung disease are most often members of Mycobacterium avium complex. M. abscessus subsp. abscessus is characterized as the most resistant mycobacterial species due to acquired and innate drug resistance (32). These subspecies are associated with different clinical outcomes and antibiotic susceptibility. Subspecies abscessus and bolletii are characterized by an inducible gene (erm(41)) responsible for intrinsic resistance to macrolides. In contrast, subspecies massiliense does not contain the active erm(41), which means that it is naturally sensitive to this group of antibiotics. In comparison to azithromycin (AZM), clarithromycin (CLR) has been shown to

induce the erm(41) gene more effectively, with higher mRNA expression, and MIC decreases during a longer incubation time. Therefore, AZM is more preferred for the therapy of M. abscessus infections. In addition, this species is susceptible to acquired resistance to mutational macrolides, which occurs at low rates due to point mutations at 2058 or 2059 of the 23S rRNA rrl gene positions. It is worth to mention that M. abscessus is naturally resistant to antituberculotics such as rifampicin and ethambutol (15).

In summary, among the most important treatment priorities is the preservation of susceptibility of M. abscessus strains to macrolides, because these antibiotics represent a crucial part of the multidrug therapy. This can be ensured by combining macrolides with other antibiotics (5,23,32). The British Thoracic Society guidelines from the year 2017 recommended a revision of the drug combination as follows: intravenous amikacin, tigecycline, and imipenem with a peroral macrolide, e.g., clarithromycin, for the initial treatment phase (35,36). Based on previous information, in the treatment of MABC lung disease (MABC-LD), a combination of intravenous drugs together with effective oral antibiotics, especially new-generation macrolides, is recommended. However, patient compliance can be reduced and potentially influenced by adverse effects and insufficient evidence of their effectiveness (24).

For amikacin, successful treatment outcomes have been demonstrated. In the case of MABC-LD caused by drug-resistant strains, the use of amikacin and tobramycin in the form of aerosols can be also considered. This helps to get the drug directly to the lungs where the infection is most problematic (36–38). However, a resistance to aminoglycosides has also been demonstrated in *M. abscessus*. This is primarily mediated by mutation in rrs gene with an increased resistance to amikacin and kanamycin. Furthermore, it is also charateristic by a distinct resistance determinants, AAC(2 '), Eis2 and Eis1 through gene deletion (39), shown in the table (Tab. 2).

Table 2 Enhanced susceptibility of NTM to aminoglycosides through gene deletion (39)

Deletion of gene	Increased susceptibility to antibiotic
AAC(2 ')	kanamycin B, tobramycin, dibekacin, and gentamicin C
Eis2	capreomycin, hygromycin B, amikacin, and kanamycin
Eis1	no affect on drug susceptibility

On the other side, low MICs of apramycin, arbekacin, isepamicin, and kanamycin A is not associated with an inactivation by either AAC(2 ') or Eis enzymes (39).

However, in the case of intravenous tigecycline, the efficacy for MABC-LD needs to be further investigated and confirmated (24).

Fluoroquinolones, such as moxifloxacin, and to a lesser extent levofloxacin and ciprofloxacin, have shown potential for the treatment of infections caused by *M. abscessus in- vivo* (40). In general, these antibiotics are not recommended in pediatric population and information on their use is limited due to concerns about the development of arthropathy (41). On the other hand, the use of these drugs in pediatric patients with CF represents a potential exception in their indication. Therefore, fluoroquinolones can be also considered to use in the treatment, therefore, it is important to update treatment guidelines based on targeted clinical trials in children (42).

WHOLE-GENOME SEQUENCING

Understanding the connection between pathological phenomena and DNA variations is one of the fundamental goals of human genetics. One approach involves the cataloging of genetic variations, known as single nucleotide polymorphisms (SNPs), throughout the

genome, aiming to identify distinct variants associated with specific phenotypes (43). In recent decades, there have been significant advancements in methods based on functional genome analysis. They have changed from traditional real-time polymerase chain reaction to more complex methods, e.g. next-generation sequencing, whole-genome sequencing (WGS), or mass spectrometry. They are designed to analyze various aspects, including genomics, epigenomics, proteomics, and interactomics (44).

WGS is one of preferred technologies that provides a detailed inshights into various aspects related to bacterial genome (45). The method helps to analyze the entire genome of bacteria and helps to determine genetic variation and diversity within this species. Thanks to WGS it is possible to identify single nucleotide polymorphisms (SNPs), deletions, insertions, copy number variations, or structural variants in the genome (46–48). Technological advancements in recent years have significantly simplified the process, lowered the costs, and reduced the time required for sequencing (48).

WGS offers various other valuable applications. One of these is phylogenetic analysis, which offers insight into the evolutionary relationships between genes and species through phylogenetic trees. This analytical tool aids in the identification of unknown species or strains by comparing their genetic data with reference sequences (49). Additionally, WGS has been utilized in several studies to characterize transmission patterns using sets and combinations of SNPs. They represent a measure of epidemiological data and/or phylogeny or genetic distance and this information serves to confirm the transmission of bacterial strains between patients. Furthermore, WGS brings the possibility to distinguish variation between isolates due to its higher resolving power. Another option is a statistical framework that helps determine the direction of transmission. This framework integrates data with additional information and facilitates the estimation of probabilities associated with hypothetical transmission chains, rather than exclusively pinpointing specific transmission events (50).

This technology also enables the identification of antibiotic resistance, playing a vital role in effective patient care (21), as we can rapidly identify resistance mechanisms based on genetic mutations. Early detection of these processes also has implications for clinical trial design and also becomes essential in ongoing clinical trials. In general it helps to distinguish an exogenous reinfection from a relapse of primary infection. This distinction plays a crucial role in evaluating the effectiveness of investigational drugs or treatment regimens (51).

WGS can be used in specific cases, such as tracking and identifying outbreaks of infections in healthcare facilities and prevent them from further transmission (52). In relation with MABC diagnostics, it can be utilized in strain identification, antibiotic susceptibility testing, differentiation between other mycobacteria, or reccurence monitoring (53).

The choice of methods depends on the set objectives. Research often uses a combination of these methods to achieve comprehensive insights from whole-genome sequencing data.

CONCLUSION

M. abscessus represents a challenge in healthcare and microbiology. It is characterized by high pathogenicity, complex subspecies, and genetic variability. Its pathology is associated with many clinical manifestations. Consequently, tailored treatment regimens and a multidisciplinary approach are needed due to its natural antibiotic resistance and other factors. Here, WGS plays an important role in understanding genetics, identifying subspecies, and determining antibiotic resistance and thus offers new ways of understanding and more effective management of *M. abscessus* infections.

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AN OBSERVATIONAL STUDY OF GLYCEMIC STATUS IN NEW ONSET ACUTE STROKE AND THEIR CLINICAL OUTCOME IN A TERTIARY CARE TEACHING HOSPITAL

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Abstract

Objective: The study was aimed to test the blood glucose level in acute stroke patients to find out any correlation with types and prognosis of different glycaemic groups.

Method: It was a prospective observational study of patients admitted to hospital in Nizamabad due to an acute ischemic or hemorrhagic stroke (<24Hrs after the onset). Clinical parameters including history and clinical examination findings were recorded. CT scan of brain, blood glucose level, HbA1c, and other laboratory tests were taken in all the patients. 2D echocardiography (2D-ECHO) and X- Ray chest picture were also performed. According to glycaemia, the patients were classified into four groups such as Nondiabetic/euglycemic, known Diabetics on glycemic control therapy with or without glycemic control, Newly detected diabetics, and Stress hyperglycemia patients. On the day 1 and 15 National Institutes of Health Stroke Scale (NIHSS) was calculated to evaluate the severity of stroke and its outcome. Good prognosis was considered when NIHSS 5, moderate prognosis when NIHSS was 6-15, and poor prognosis when NIHSS was 16-20. Patients with NIHSS 20 died within 3 days of admission.

Results: Finally, 158 patients with acute stroke were included. Hemorrhagic stroke was seen in 43 (27.2%) and ischaemic stroke in 115 (72.8%) patients. Maximum cases were found in 51-60 years age group (37.9%) followed by 41-50 (20.3%) and 61-70 years age group (20.3%). Majority of study subjects were males, 65.8 % (n=104). Maximum cases were Euglycemics (44.3%), followed by stress hyperglycemic (27.8%), known diabetes (15.9%), and newly diagnosed diabetes (12%) presenting with acute stroke. Higher NIHSS score was found in stress hyperglycemic patients (19.4%) followed by known diabetics (17.3%), newly detected diabetics (16.3%), and euglycemics (9.5%). Maximum proportion (81.8%) of ischaemic stroke cases were found with stress hyperglycemia compared to other glycemic conditions but in hemorrhagic stroke maximum proportion (47.3%) were newly diagnosed diabetics. Maximum proportion of large size of lesion (47.4%) was found in newly diagnosed diabetes followed by stress hyperglycemia (45.55). Medium size of the lesions was found more in newly diagnosed diabetes (52.6%) followed by known diabetes cases (52%). Good outcome was found in 51 cases (32.2%), moderate outcome in 34 (21.5%), and poor outcome was in 30 (18.9%). 43 patients with NIHSS score >20 died within 3 days of admission (27.2%). Maximum proportion of death cases in ischemic group those belongs to newly diagnosed diabetes cases (8/9=88.8%). Higher deaths were found in blood sugar <110 mg/dl and blood sugar >199 mg/dl with an incidence rates of 41.2% and 53.4%.

Conclusion: Hyperglycaemia in non-diabetic patients after acute stroke is a stress response reflecting more severe neurological damage. Elevated HbA1c presenting stroke glycaemia status has a significant trend in increasing the risk of cause mortality. The management of hyperglycaemia in patients with diabetes and non-diabetes is an important aspect of the emergency management of stroke.

Keywords: Ischemic stroke, Hyperglycemia, NIHSS score, Hemorrhage.

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INTRODUCTION

A stroke or cerebrovascular accident is defined as the abrupt onset of a neurologic deficit that is attributable to a focal vascular cause (1). Stroke is one of the major causes of disability and mortality all over the world (2,3). Given its major socioeconomic burden, there is always a need to improve our understanding of its high risk population, complications, and prognosis.

Diabetes is a major risk factor for stroke occurrence. A high incidence of patients who developed stroke may have hyperglycemia, even without a previous history of diabetes (4). As reported by Frederic, 11.3% of cases of stroke gave history of diabetes when compared with 2% in the general population (5). The incidence of diabetes was found to be twice as high in patients admitted to hospital with any type of stroke than in patients with other neurologic diseases. The increased risk of stroke in diabetes and the increased prevalence rate of stroke in diabetes as compared to the general population was confirmed by a study done by Wolf et al(6). Diabetics as well as patients with stress hyperglycaemia have severe stroke and these patients are associated with poor prognosis (7). The mortality rate from stroke in diabetics was twice that of the general population (8). Glucose tolerance also deteriorates with age (9). Multivariant studies also show that blood glucose is a significant predictor of death (10). Diabetic macrovascular diseases including coronary heart diseases, stroke, and peripheral vascular diseases were common causes of morbidity and mortality among people with diabetes mellitus (11). As reported by several studies, it was found that hyperglycaemia in non-diabetic patients after acute stroke is a stress response reflecting more severe neurological damage. However, it is also suggested that hyperglycaemia influences the outcome of stroke severity.

Increased blood glucose concentration at or around the time of a cerebral ischaemic event may worsen outcome; even mild hyperglycaemia (6.6 mmol/L) may result in an increased brain damage and delayed recovery. Studies in rats showed that insulin improved the functional recovery from brain ischemia, probably through its effects on glucose and lactate levels (12).

Many investigators predict that this is not a benign condition and that stress-induced hyperglycemia is associated with a high mortality after stroke (13). Despite these observations, the relationship between admission glucose level and stroke outcome is still a field for ongoing research. This study aimed to assess the glycemic status after acute stroke and its role on stroke outcome.

MATERIALS AND METHODS

The present study was conducted in the critical care unit of the Department of General medicine, Government medical college and hospital, Nizamabad.

Study setting: The present study was conducted in the Department of General medicine, Government medical college and hospital, Nizamabad.

Study population: Patients admitted in Government medical college and hospital, Nizamabad in the Department of General medicine with stroke after taking their consent.

Study design: A hospital based prospective, observational study.

Sample size with justification: Calculated sample size was 158

$N=4PQ/L^2$

P = 3%; Q=47%; L= Allowable error (15% of p)

N = 4×53×47/7.95×7.95= 157.6

N = 158.

Duration of the study: From November 2017 to November 2018. Inclusion criteria: Patients above the age of 40yrs. Patients admitted within twenty-four hours of onset of stroke. Patients

with new onset cerebrovascular accident (stroke). Blood sugar recorded on admission with in twenty-four hours of the onset of stroke. Stroke that can be medically managed i.e. anti-coagulation therapy, antiplatelet therapy, neuroprotective drugs, and supportive treatment.

Exclusion criteria: Patients admitted after twenty-four hours of stroke. Those patients who received intravenous glucose before or during study period with stroke. Stroke with complications i.e. stroke requiring thrombolysis, surgical management [decompression] size of lesion >10mm, embolectomy, massive infarct/haemorrhage with altered behaviour, tumours, obstructive hydrocephalus, syndromes, other systemic complications requiring intensive care.

Methodology

Sample selection: 158 patients were selected by using randomized table method. Written informed and valid consents were taken from the patients after providing adequate information and answering their question and queries in detail.

Method: All patients were studied for clinical history, blood pressure, blood sugar, urea, creatinine, electrolytes, haemoglobin, total cell count, differential count, urine sugar, albumin, electrocardiogram, chest X-ray, CT scan brain (if require), and 2D-ECHO. The severity of stroke for each patient was calculated based on NIH stroke scale, NIHSS which takes the following clinical findings into account and each criteria awarded specific points (14,15).

The points were added, with a maximum of thirty points.

Once clinical diagnosis of acute stroke was made venous blood sample was taken, with in twenty-four hours of onset of symptoms, and sent to laboratory for glucose estimation.

In patients with blood sugar more than 6.1 mmol/L (110 mg/dl), Haemoglobin A1c (HbA1c) was performed, and diabetic status noted.

(HbA1c is structurally similar to haemoglobin A except for the addition of glucose Group to the terminal amino acid of the beta chain of the haemoglobin Molecule glycosylation).

Therefore, HbA1c is a function of the exposure of the red blood cells to glucose. Since the glucose linkage to haemoglobin is relatively stable, Hemoglobin A1c accumulates through out of the life span of erythrocyte and its concentration reflects the integrated blood glucose concentration over a period approximating to the half-life of erythrocytes, i.e. six to eight weeks. Measurement of HbA1c helps to monitor the overall degree of diabetic control achieved. The normal range of Hemoglobin A1c is 3.8% to 6.4%.

The patients can be classified into four groups (16):

1. Blood sugar < 6.1 mmol/l [110mg/dl]: Nondiabetic/euglycemic.
2. With history of diabetes: Known diabetics on glycemic control therapy with or without glycemic control.
3. Blood sugar > 6.1mmol/l[110mg/dl], no history of diabetes, and HbA1c > 6.4: Newly detected diabetics.
4. Blood sugar more than 6.1 mmol/l [110mg/dl], no history of diabetes, and HbA1c< 6.4: Stress hyperglycemias.

Then computerized tomography (CT) of the brain was performed in all patients:

- CT scan immediately done to rule out haemorrhagic stroke.
- Repeat CT scan after 48hours to confirm ischemic stroke.
- Detecting the type of stroke and location the site of lesion.
- Detecting the size of lesion (small <5mm; Medium 5-10 mm; Large >10 mm or involving more than one vascular territory).
- Identifying the presence of cerebral oedema or midline shift.

Patient was given corticosteroid according to protocol with vasogenic edema hemodynamically stable patients. Injection dexamethasone 8mg was given thrice daily and tappered accordingly after improvement on repeat CT scan.

The patients were assessed on the day of admission NIHSS score and the day 15 NIHSS Score outcome observed in the form of death, poor, moderate, and good prognosis.

Patients who were unable to return to any form of work, persistent disability, need for residential placement, dependent in activities of daily living, and stable deficit with no recovery were classified as those with poor outcome.

Patient whose symptoms improved, who were independent in attending day to day activities, improvement in motor function and aphasia, and no persistent disability were grouped as patients with good outcome.

Patients who fared in between these two groups were grouped as those with moderate outcome.

Statistical Analysis: The collected data entered into the Excel spread sheet and transferred to the SPSS version 23 for statistical analyses (IBM, Chicago, Illinois). Chi-square test was used as test of significance for categorical data. Unpaired t test was used for continuous data. P value less than 0.05 was considered as statistically significant.

RESULTS

Maximum cases were found in 51-60 years age group (37.9%) followed by 41-50 (20.3%) and 61–70 years age group (20.3%). Majority of study subjects were males, i.e. 65.8 % (n=104).

Table 1 Distribution of study subjects according to risk factors incidence in relation to gender

	Male	%	Female	%	Total	Total %
RISK FACTORS						
Hypertension	66	63.6	38	36.36	104	65.8
Smoking	63	100	0	0	63	39.8
Diabetes	27	60.7	17	39.3	44	27.8
Alcohol	39	96.1	2	3.85	41	25.9
Hyper Cholesterolemia	16	71.4	6	28.56	22	13.9
Atrial Fibrillation	0	0	2	100	2	1.26
Coronary Artery Disease	6	66.6	3	33.33	9	5.69
CLINICAL PRESENTATION						
Right hemiplegia	60	69.1	27	30.9	87	55
Left hemiplegia	39	64.1	22	35.9	61	38.6
Faciobrachial Monoplegia	3	37.5	5	62.5	8	5
Cerebellar symptoms	2	100	0	0	2	1.26
Loss of consciousness	49	60.8	31	39.2	80	50.6
Hemianopia	3	60	2	30	5	3.16
Aphasia	35	58.3	25	41.6	60	37.9
Bladder and Bowel involvement	25	59.6	17	40.4	42	26.5

Risk factors: Major risk factor was hypertension, i.e. 65.8% (104 cases), followed by smoking 39.8 % (63 cases), Diabetes in 27.8 % (44 cases) and Alcohol 25.9% (41 cases). Among major risk factor in males, smoking was found in 100%, alcohol was found in 96.1%, hypercholesterolemia was found in 71.4%, and hypertension was 63.6%. Whereas in females Diabetes was found in 39.3% and hypertension was found in 36.4% (Table 1).

Among neurological symptoms, maximum cases were presented with right hemiplegia in 55% (n=87), followed by loss of consciousness in 50.6% (n=80), left hemiplegia in 38.6% (n=61), aphasia in 37.9 %(n=60), and bladder & bowel involvement in 26.5%(n=42). Male patients were commonly presented with cerebellar symptoms, right hemiplegia, left hemiplegia, and loss of consciousness. Whereas faciobrachial monoplegia was predominantly seen in female patients (Table 1).

Glycemic status: 44.3% cases were euglycemic, 27.8% cases were stress hyperglycemic, 15.9% cases were known diabetic, and 12% cases were newly diagnosed diabetics.

Maximum NIHSS score was found in stress hyperglycemic patients (19.4%), followed by known diabetics (17.3%), newly detected diabetics (16.3%), and euglycemics (9.5%). This association was statistically significant (P<0.05) (Table 2).

Table 2 Stroke severity according to NIHSS score on admission day

Glycemic status	N	NIHSS score Mean ± SD	One-way ANOVA F test*
Euglycemia	70	9.5 ± 6.760	
Stress hyperglycemia	44	19.4±5.248	
Known diabetes	25	17.3±6.561	F=401.1,
Newly diagnosed diabetes	19	16.3±7.040	P<0.001

*The mean NIHSS score was tested in 4 glycemic groups using One-way F test analysis of variance (ANOVA) test. F-test in ANOVA is used to assess whether the expected values of a quantitative variable within four pre-defined groups with various glycemic condition differ from each other.

Table 3 Association between Glycemic status and type of stroke

Glycemic Status	Ischaemic Stroke		Hemorrhagic Stroke		TOTAL	
	No.	%	No.	%	No.	%
Euglycemia	54	77.1%	16	22.9%	70	44.3%
Stress hyperglycemia	36	81.8%	8	18.2%	44	27.8%
Known Diabetes	15	60.0%	10	40.0%	25	15.8%
Newly diagnosed Diabetes	10	52.7%	9	47.3%	19	12.1%
TOTAL	115	72.7%	43	27.3%	158	100%

$\chi^2 = 8.44$, df=3, $p<0.05$.

Maximum proportion (81.8%) of ischaemic stroke cases were found with stress hyperglycemia compared to other glycemic conditions but in hemorrhagic stroke maximum proportion (47.3%) were newly diagnosed diabetics. This association was statistically significant (P<0.05) (Table 3).

Table 4 Association between Glycemic status and size of the lesion

Glycemic status	Small		Medium		Large		Total
	Freq	%	Freq	%	Freq	%	
Euglycemia	46	65.7	13	18.6	11	15.7	70
Stress hyperglycemia	2	4.5	22	50	20	45.5	44
Known diabetes	3	12	13	52	9	36	25
Newly diagnosed diabetes	0	0	10	52.6	9	47.4	19
Total	51	32.2	58	36.8	49	31	158

Chi-square = 65.42, df=6, p<0.001.

Maximum proportion of large size of lesion (47.4%) was found in newly diagnosed diabetes followed by stress hyperglycemia (45.55). Medium size of the lesions were found more in newly diagnosed diabetes (52.6%) followed by known diabetes cases (52%). This association was statistically significant (P<0.05) (Table 4).
Among the 158 study subjects, hemorrhagic stroke was seen in 43 (27.2%) patients and ischaemic stroke was seen in 115 (72.8%) patients (Table 5).

Table 5 Distribution of NIHSS score on Day 15 among prognosis groups

Prognosis groups	N	NIHSS score (Mean ± SD)	ANOVA
Good	51	2.51±1.94	F=897.4, P=0.042*
Moderate	34	10.41±2.24	
Poor	30	18.23±1.45	
Death	43	0.000	
Total	158	6.513	

***significant**

Association between glycemic status and outcome: As NIHSS score increases, the severity of the disease was also increased. Maximum percentage (50%) of deaths were seen in newly diagnosed diabetes cases compared to other groups. Maximum cases of good outcome belonged to euglycemic group (65.9%).This association was statistically significant (P<0.05); (Figure 1).

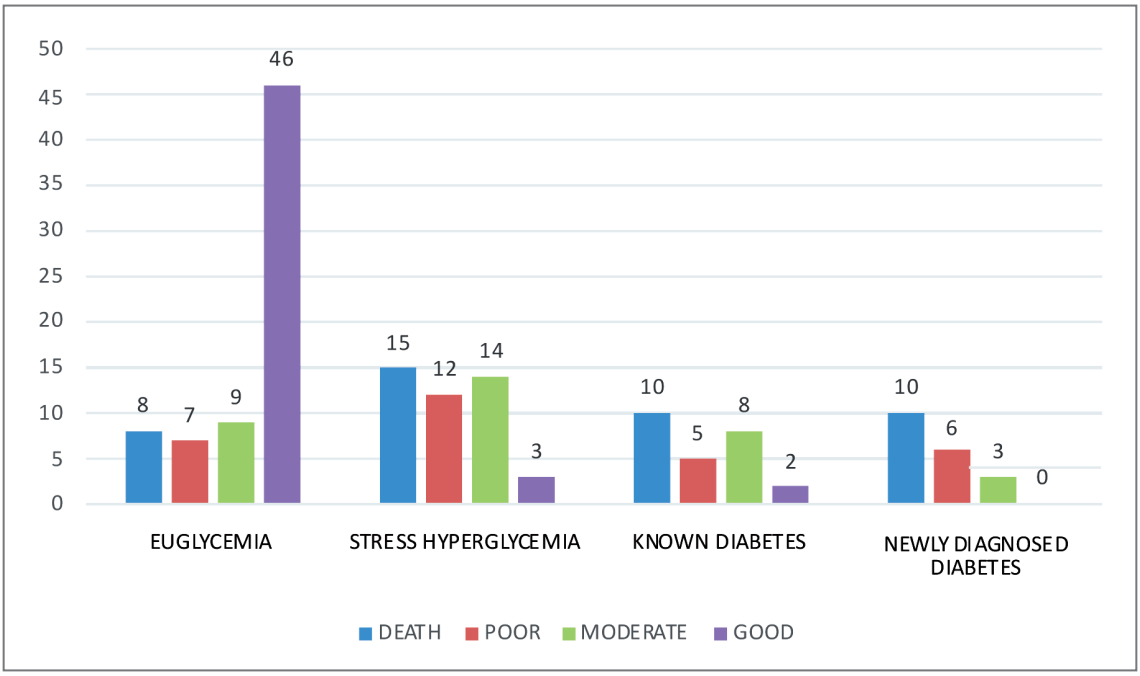

Fig. 1 Association between glycemic status and outcome. Chi-square=69.3, df= 9, P<0.001.

Table 6 Association between glycemic status and outcome in stroke groups

	Glycemic Status	Outcome				Total
		Good	Moderate	Poor	Death	
Hemorrhage group	Euglycemia	4	5	0	6	15
	Stress hyperglycemia	0	2	1	5	8
	Known diabetes	0	5	3	2	10
	Newly detected diabetes	0	3	5	2	10
	Total	4	15	9	15	43
Ischaemic group	Euglycemia	40	5	3	5	53
	Stress hyperglycemia	2	13	11	11	37
	Known diabetes	2	3	2	9	16
	Newly detected diabetes	0	0	1	8	9
	Total	44	21	17	33	115

All the good outcome cases in hemorrhagic stroke belonged to euglycemia group and in ischemia group also maximum cases of good outcome belonged to euglycemia. Maximum proportion of death cases belong to stress hyperglycemia in hemorrhagic stroke group (5/8=62.5%) (Table 6).

Maximum proportion of death cases in ischemic group belongs to newly diagnosed diabetes cases (8/9=88.8%).

Table 7 Correlation of Sugar Levels and Outcome in stroke Groups

Group			Outcome				
			Good	Moderate	Poor	Death	Total
HEMORRHAGE	SUGAR	A	5	5	0	7	17
		B	0	2	2	0	4
		C	0	7	0	0	7
		D	0	0	7	8	15
	Total		5	14	9	15	43
ISCHAEMIC	SUGAR	A	40	5	3	5	53
		B	2	12	11	0	25
		C	2	3	0	5	10
		D	0	0	3	24	27
	Total		44	20	17	34	115

A <110 mg/dl; **B =** 110-125 mg/dl; **C =** 126-199 mg/dl ; **D >**199 mg/dl

Association between outcome and sugar levels: More proportion of deaths were found in blood sugar (<110 mg/dl) and blood sugar (>199 mg/dl) such as 41.2% and 53.4%. Mortality was high in hyperglycemic cases in hemorrhagic stroke cases. In ishchaemic stroke maximum mortality was found with hyperglycemic patients (88.9%) (Table 7).

DISCUSSION

Clinical outcome after stroke is highly variable and depends on many factors (17). Accurate assessment of the expected outcome is important for clinical decision- making, to guide patient management (18).

Majority of the patients 60(37.9%) in our study were within the age group of 51 to 60 yrs, which is in agreement with the studies of Topie E et al (19) and Kyadav K et al (20). Study by Singh K Get al[21] shows that stroke majorly in age group of 51-60 i.e. 23(46%). Other studies found the prevalence of 41 – 50 years age group (10). Our study shows male preponderance, which is also seen in Prasad BNR et al (22). In our study of 158 patients showing male preponderance (65.8%) which is similar to Prasad BNR et al., also showed same male preponderance.

Risk factors incidence:

Among the 158 patients, 104(65.8%) cases were hypertensive with Male preponderance of in 66(63.6%) than females in 8(36.36%). More than half of diabetic patients were males in 27(60.7%) when compared to females in 17(39.3%). The risk of diabetes mellitus was 27.8%. More than two third of hypercholesterolemia patients were males in 16(71.4%) than in females 6(28.56%).

O' Donnell MJ et al[2] showed concordance with our study. This study showed patients with risk factors of hypertension (99%), diabetes mellitus (5%), alcohol intake (3.8%), cardiac causes (6.7%). Therefore, showing hypertension, smoking, diabetes, alcohol intake were significant risk factors for hemorrhagic stroke.

Glycemic status in study population:

Among the 158 patients in our study group 70 patients had normal blood glucose values. Raised blood glucose levels on admission were noticed in 19(12%) newly diagnosed diabetes, 25(15.9%) known diabetes, and 44(27.8%) stress hyperglycemia patients. Other study Singh KG et al showed preponderance of known diabetes 14(28%) in hyperglycemic group.

In ischemic stroke group stress hyperglycemia amounted to one third of the patients and one fifth in hemorrhagic group. The present study shows 15.9% (25/158) prevalence of known diabetics presenting with acute stroke; whereas the prevalence of stroke in known diabetics was 8.5%. But in a study, Kyadav K et al (20) known diabetics were high compared to the present study (24%).

The study also shows a high prevalence of newly diagnosed diabetics (12%) in patients presenting with acute stroke. In another study, the incident of stroke was 16% and 12% in known diabetics and newly diagnosed diabetics (10), which is similar to the present study. These are in agreement with the observations in other series (23). Previous study found a range of prevalence of undiagnosed diabetes in acute stroke population from 6% to 42% (24).

Severity of Stroke:

Severity of stroke was assessed with NIH Stroke scaling system (NIHSS) score. On the admission day hyperglycemic patients had a higher score when compared to euglycemic patients (17.27 vs. 9.5) which was statistically significant with $p = 0.001$. Among the admission day hyperglycemic patient's stress hyperglycemics had higher NIHSS score (19.4%). As score increases severity also increases.

Similar concordance was seen in Al- Weshshy A et al (25) showing NIHSS score high in hyperglycemia patients (14.9±5.9 vs 7.8±3.5, p=0.000). 38.1% of patients with stress hyperglycemia had high NIHSS score versus 10% diabetics and 5% control. 30 days mortality higher in stress hyperglycemia compared to diabetics.

Size of stroke lesion:

The size of the lesion was analyzed with the help of CT scan brain. Most of the euglycemic 46(65.7%) patients had small sized infarcts and hemorrhage whereas majority of the admission day hyperglycemic patients had large sized lesion with edema and midline shift. Among the hyperglycemic patients large sized lesion observed in newly diagnosed diabetes in 9(47.7%) and stress hyperglycaemia group in 20(45.5%). Similar result found in Singh KG et al (21) showing large sized lesions in stress hyperglycemia 5(100%) and also in newly diagnosed diabetes 11(100%).

Type of stroke:

Among the euglycemic group three fourth of the patients had ischemic stroke and one fourth had hemorrhagic stroke. Among patients with admission day hyperglycemia one third of the patients had hemorrhagic stroke. In newly detected diabetic patients, half of them had hemorrhagic stroke. Our study shows an increasing incidence of hemorrhagic stroke among diabetes patients.

Outcome of stroke:

In present study euglycemic patients had a better outcome when compared to the admission day hyperglycemic patients. Euglycemic patients had a better recovery after acute stroke. Sixty five percent of euglycemic patients had a good functional recovery. On the contrary, only three percent of admission day hyperglycemic patients had good functional recovery at the end of thirty day follow up. Early inpatient mortality was high in the admission day hyperglycemic patients. Fifty percent of the admission day hyperglycemic patients died within the first thirty days. In the euglycemic patients the early case fatality rate was only fifteen percent. Hence, there was a threefold increased risk of early mortality in the admission day hyperglycemic patients when compared to euglycemics. Poor outcome was noticed in 33.3% of the admission day hyperglycemic patients and in four percent of euglycemic patients.

The present study shows that the admission day elevated blood glucose level was associated with a high early mortality rate and an increased risk of poor functional recovery.

Several studies have confirmed that stress hyperglycemia is associated with a poor outcome (26,27,28). Jorgensen H et al (28), in their large prospective Danish study found that plasma glucose level >11 mmol/L (>198 mg/dL) was associated with a hospital mortality of 17% for non-diabetic patients, 24% for those with known diabetes, and 32% for patients with hyperglycemia with no history of previous diabetes.

Glycemic status and clinical outcome of hyperglycemia:

In the ischemic stroke group early mortality rate was 9.43 % in euglycemic patients and 56.25% in hyperglycemic patients. Poor outcome was noticed in 5.66 % in euglycemics and 12.5% in hyperglycemics. Hence, hyperglycemia was associated with an increased early mortality rate and poor functional outcome in ischemic stroke group.

In the hemorrhagic patients the early mortality in hyperglycemic patients was 20% and 40 % in euglycemic patients.

In the non-diabetes ischemic stroke patients stress hyperglycemia had worst outcome when compared to euglycemic group. The early mortality rate was 29.7 % in stress hyperglycemics and 9.43% in euglycemics. Hence, this study shows a three-and-a-half-fold increased risk of mortality in non-diabetes stress hyperglycemic patients when compared to non-diabetic euglycemic patients.

The study by Singh KG et al revealed a higher mortality with stress hyperglycaemics and diabetic groups, which was also consistent with other series as reported by some authors (29,30). However, some other studies did not show any significant increase in diabetic stroke deaths in diabetics compared to non- diabetics (31).

Outcome of stroke according to NIHSS score on day 15:

In our study group of 158, patients with NIHSS score <5 had good prognosis n=51 (2.51±1.94), patients with score between 5- 15 had moderate prognosis n=34(10.41± 2.24), patients with score between 15 to 20 had had poor prognosis n=30(18.23±1.45), patients score more than 20 died within 3 days of admission n=43(0.000). Therefore, as score increases severity of outcome increased.

Similar result found in Gajurel BP et al (32), showing better outcome with mean NIHSS score of 4.6±2.2, and poor outcome with mean NIHSS score of 14.16±7.96.

According to Lindsberg PJ and Roine RO (33), hyperglycemia found in two thirds (66%) of all ischemic stroke patients. In our study, hyperglycemia was noticed in 56% of patients in general and in 53% of patients with ischemic stroke. In Lindsberg PJ and Roine RO study, known diabetes and newly diagnosed diabetes contributed to one third of cases (33%). In our study the same group contributed to 28%.

A study by Christensen H and Boysen G (34) demonstrated that an elevated glucose level after acute stroke was associated with higher stroke severity than those with normal level. The mean NIHSS was 9.5 in euglycemics and 17.3 in hyperglycemic patients.

A study by Umpierrez GE et al (35) confirmed that patients with newly detected hyperglycemia had a significant higher early mortality and a lower functional outcome than patients with a history of diabetes or normoglycemia.

Capes SE et al in their metaanalysis concluded that hyperglycemic patients had threefold increased early mortality than euglycemic patients. After ischemic stroke admission hyperglycemia was associated with threefold increased 30 day mortality than euglcemics. After hemorrhagic stroke, admission hyperglycemia was not associated with higher mortality in either diabetic or non-diabetic patients. In our study, in ischemic patients who had elevated admission day glucose level experienced a three and a half fold increased early mortality than euglycemics.

The study clearly shows an increased early mortality rate and poor functional recovery in patients with diabetes and stress hyperglycemia when compared to euglycemics. Hence, there is an urgent need to confirm the improvement in these patients by normalizing blood sugar. Several trails are now under way to improve the outcome of Stroke by normalizing the blood glucose with human recombinant insulin.

Vinychuk SM et al (36) showed that administration of insulin to patients with hyperglycemia improves the functional recovery and vital activity of mild to moderate ischemic stroke patients. However, other clinical benefits of the insulin therapy remain to be determined.

Recommendations of our study includes:

Studies on larger number of patients and investigating the effect of thrombolytic therapy and lowering hyperglycemia on stroke outcome were recommended.

To improve the outcome, intensive glucose lowering therapies should be tried for a minimum of 72 hrs after acute ischemic stroke.

CONCLUSION

Maximum NIHSS score found in stress hyperglycemic patients compared to all other patients. Commonly large size of lesion was significantly found in stress hyperglycemic patients. Commonly higher death cases were found in patients with known diabetes. Severity of stroke correlates with the glycemic status of the patients in diabetics and non-diabetics. Hyperglycaemia in non-diabetic patients after acute stroke is a stress response reflecting more severe neurological damage. Management of hyperglycaemia in patients with diabetes and non-diabetes is an important aspect of the emergency management of stroke.

Ethical Approval

This protocol was approved by the institutional ethics committee, Government Medical College, Khaleelwadi, Nizamabad, Telangana State.

Conflict of Interests

All authors declared no potential conflicts of interest related to the research, authorship, and publication of this article.

Authors' Contributions

Both authors contributed to design, contributed to acquisition, analyses, and drafted the manuscript. Both authors contributed to conception and design, contributed to analysis and interpretation. Both authors were revised the manuscript critically and approved the content of this manuscript.

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IRON CITRATE (SYNTHESIT) SUPPLEMENTATION DURING PANCREAS CANCER SHOWED SURPRISING RESULTS – CASE STUDY

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Abstract

Iron is a crucial mineral for our organism and its deficiency can cause serious health problems such as anaemia, fatigue, and impaired physical fitness. It has been shown that anaemia or iron deficiency is very common in patients with cancer. These patients benefit from iron supplementation either in intravenous or oral form. Our patient is a 67-year-old Russian woman with pancreatic cancer diagnosed in 2019. She fought off lymphocytic leukaemia in 2015. She refused treatment for her pancreatic cancer. The specific type of pancreatic cancer was not specified as the patient chose not to undergo targeted testing. Between March 2020 and February 2023, she took the dietary supplement Synthesit for three cycles (1 cycle lasted about a month).

After taking the dietary supplement, a total percentage of neutrophils became in the reference range. Subjectively, the patient started to feel better after taking Synthesit and her quality of life and well-being has improved as well. It might be supposed that the dietary supplement could have some effect on her well-being and various blood parameters such as white cells count.

Even though the dietary supplement is not supposed to be used for treatment of diseases, it can change some blood parameters and improve the immune system.

This short case study presents the patient with pancreatic cancer who started to take the dietary supplement Synthesit which contains iron in the form of citrate salt in a dosage of 800 µg per capsule, 1 capsule per day. The dietary supplement was administered over three treatment cycles (1 cycle took about a month) from March 2020 to February 2023. It describes a difference in blood test results before taking Synthesit and after the administration of Synthesit.

Keywords: pancreatic cancer, iron supplementation, iron citrate

INTRODUCTION

Iron (Fe) is a vital mineral for various physiological processes in our organism including oxygen metabolism and uptake, electron transport in mitochondria, and energy metabolism. Additionally, iron plays a critical role in maintaining proper muscle function and haematopoiesis, making it indispensable for overall physical functioning and well-being (1,2).

However, the balance of iron homeostasis is crucial, as the presence of free bivalent iron can lead to the generation of free oxygen radicals, causing tissue damage. Therefore, precise control of iron levels is necessary to minimize the concentration of free iron (1).

In the context of cancer, iron regulation and homeostasis may be compromised (3). This can result in an inadequate iron supply for erythroblasts leading to weakness, fatigue, impaired physical fitness, well-being, and anaemia (2). Notably, deficient iron levels are prevalent in patients with pancreatic cancer (63%), underscoring the significance of addressing iron imbalances in this population. This study has indicated that iron supplementation can offer benefits to individuals with cancer (4).

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Pancreatic cancer has one of the lowest combined five-year survival rates among various cancers, with only 5 to 10 percent of patients still alive five years after the diagnosis (5).

If pancreatic cancer has spread, patients may observe new symptoms. It commonly metastasizes to the liver and it can also extend to the lymph nodes, abdomen, lungs, and occasionally the bones. The symptoms of advanced pancreatic cancer include weight loss, abdominal discomfort, a general feeling of being unwell, jaundice, ascites, lack of appetite (6).

Pancreatic cancer's poor prognosis profoundly impacts patients' quality of life, leading to substantial deterioration, including mental changes, cognitive decline, and coping with the disease. Comorbid depression is common and overall health-related quality of life is notably low (7).

In this case study, it is presented that the patient diagnosed with pancreatic cancer started taking the dietary supplement Synthesit containing iron citrate (800 µg per capsule), 1 capsule per day, for a total of 3 months over 3 years. A comparison of blood test results before and after the administration of Synthesit is examined to explore any potential impacts of the dietary supplement.

Case description

This case study presents a unique clinical scenario involving a 67-year-old woman from Russia diagnosed with pancreatic cancer in 2019. The patient declined to undergo any testing for a specific type of pancreatic cancer. The patient's medical history includes a dental intervention for tooth extraction in 2015. After the tooth extraction, a complication of infection emerged. Subsequent laboratory analyses revealed the presence of chronic B-lymphocytic leukaemia (B-CLL), specifically categorised as stage B according to the Binet staging system, with CD20+ positivity. To address the B-CLL diagnosis, the patient underwent a six-month chemotherapy regimen consisting of 6 treatment cycles. She completed her chemotherapy regimen after half a year, in May 2016. Eventually, a remission from leukaemia was achieved in May 2016. In an effort to support her immune system after chemotherapy, she incorporated the use of thymus extract intravenously in June 2016 (after a month of the 6th cycle of chemotherapy). The treatment involved administering thymus extract intramuscularly at a dose of 20 mg/day for 10 days, followed by a break of 10 days. This procedure was repeated thrice and continued until July 2016. Following a monthly interruption, the regimen was repeated once more as a 10-day administration period. After an extended interval, the patient resumed the administration of thymus extract in the spring and autumn of 2017.

However, this therapeutic approach of chemotherapy was not without challenges, as the patient experienced complications, including pneumonia episodes after the first and the sixth treatment cycles. Furthermore, nephropathy manifested during chemotherapy, leading to a decline in renal function to 35% of its efficiency. The patient also endured various severe adverse effects of chemotherapy such as joint pain, a cataract, gastropathy, elevated hepatic enzyme levels, and oral candidiasis.

In December 2019, a haematologic examination revealed elevated levels of gamma-immunoglobulins (IgG), surpassing 17% of the overall immunoglobulin levels (Table 1). This finding raised a suspicion of an underlying a pancreatic pathology, which was later confirmed to be pancreatic cancer, possibly related to her prior history of leukaemia.

The specific type of pancreatic cancer was not specified as the patient chose not to undergo targeted testing. Despite the diagnosis, the patient made an informed decision to decline conventional anticancer treatments and instead chose to alter her lifestyle significantly. She also didn't undergo pancreatic cancer surgery.

Opting for a shift from urban to rural living, the patient embraced a lifestyle characterised by regular physical exercise and adherence to a healthful dietary regimen.

Additionally, the patient initiated the consumption of a dietary supplement Synthesit, enriched with iron citrate at a dose of 800 µg/capsule, 1 capsule per day. The dietary

supplement was administered over three treatment cycles (1 cycle took about a month) from March 2020 to February 2023, during which her blood parameters exhibited notable improvements (Figure 1).

RESULTS

After the administration of three treatment cycles of the dietary supplement Synthesit, a comparative evaluation of blood analyses conducted in July 2021 and March 2023 demonstrated that the total percentage of neutrophils had improved and now fell within the reference range. Notably, the values observed three years prior to Synthesit intake were lower than the reference range for neutrophils.

Conversely, the analysis conducted in July 2021 indicated that the thrombocyte count was within the reference range, whereas in March 2023, thrombocytopenia and lymphocytosis persisted (Table 2).

In conjunction with the objective findings, the patient also reported experiencing a notable improvement in her overall well-being and quality of life following the administration of Synthesit. Subjectively, she expressed enhanced satisfaction with her health status, an essential aspect during cancer treatment.

These results suggest a potential beneficial impact of Synthesit on specific haematological parameters, particularly neutrophils and thrombocytes. However, the continued presence of thrombocytopenia and lymphocytosis indicates that additional evaluation and monitoring are required to comprehensively assess the supplement's long-term effects on haematological profiles.

It is imperative to acknowledge that while subjective improvements in the patient's well-being are noteworthy, further investigations encompassing a larger cohort and controlled clinical studies are warranted to establish a robust correlation between Synthesit supplementation and haematological parameters in the context of cancer treatment.

Table 1 Analyses of blood plasma proteins done in December 2019 and March 2023. Parameters that were out of the reference range are highlighted. Values marked as dash mean no data available.

December 2019				March 2023	
Fraction	**%**	**Reference range %**	**g/l**	**g/l**	**Reference range**
Albumin	62.9	60.3 – 72.8	44.0	41.4	37.5 – 50.1
Alpha1-globulins	2.2	1.0 – 2.6	1.5	3.0	1.9 – 4.6
Alpha2-globulins	8.6	7.2 – 11.8	6.0	6.1	4.8 – 10.5
Beta1-globulins	6.0	5.6 – 9.1	4.2	-	-
Beta2-globulins	3.3	2.2 – 5.7	2.3	-	-
Beta-globulins	-	-	-	6.8	4.8 – 11.0
Gamma-globulins	**17.0>**	6.2 – 15.4	11.9	11.7	6.2 – 15.1
Albumin/Globulin ratio	1.70	1.2 – 2.0	-	1.5	1.2 – 2.0
Monoclonal IgG kappa	0.9	-	0.6	-	-
Total protein	69.9	62 – 81	-	67	62 – 81

Table 2 Blood analyses before taking Synthesit in February 2020, during taking in July 2021, and after taking it in March 2023. Parameters that were out of the reference range are highlighted. Values marked as dash mean no data available.

Parameter	Before taking Synthesit **02/2020**	During taking Synthesit **7/2021**	After taking Synthesit **03/2023**	**Reference range**	**Unit of measurement**
Haematocrit	40.9	-	42.7	35.0 – 47.0	%
Haemoglobin	14.0	14.8	14.5	11.7 – 16.0	g/dl
Erythrocytes	4.58	4.90	4.72	3.80 – 5.30	$*10^6/\mu l$
Thrombocytes	**116**	260	**123**	150 – 400	$*10^3/\mu l$
Leukocytes	7.07	**4.20**	6.80	4.50 – 11	$*10^3/\mu l$
Neutrophils (total), %	**40.30**	66	48.40	48.0 – 78.0	%
Lymphocytes, %	**50.5**	-	**44.1**	19.0 – 37.0	%
Monocytes, %	7.5	-	5.6	3.0 – 11.0	%
Eosinophils, %	1.4	3	1.6	1.0 – 5.0	%
Basophils, %	0.3	-	0.3	<1.0	%
Neutrophils abs.	2.85	-	3.29	1.56 – 6.13	$*10^3/\mu l$
Lymphocytes abs.	3.57	-	3.00	1.18 – 3.74	$*10^3/\mu l$
Monocytes abs.	0.53	**1**	0.38	0.20 – 0.95	$*10^3/\mu l$
Eosinophils abs.	0.10	-	0.11	0.00 – 0.70	$*10^3/\mu l$
Basophils abs.	0.02	-	0.02	0.00 – 0.20	$*10^3/\mu l$
Erythrocyte Sedimentation Rate (ESR)	12	4	7	<30	mm/h

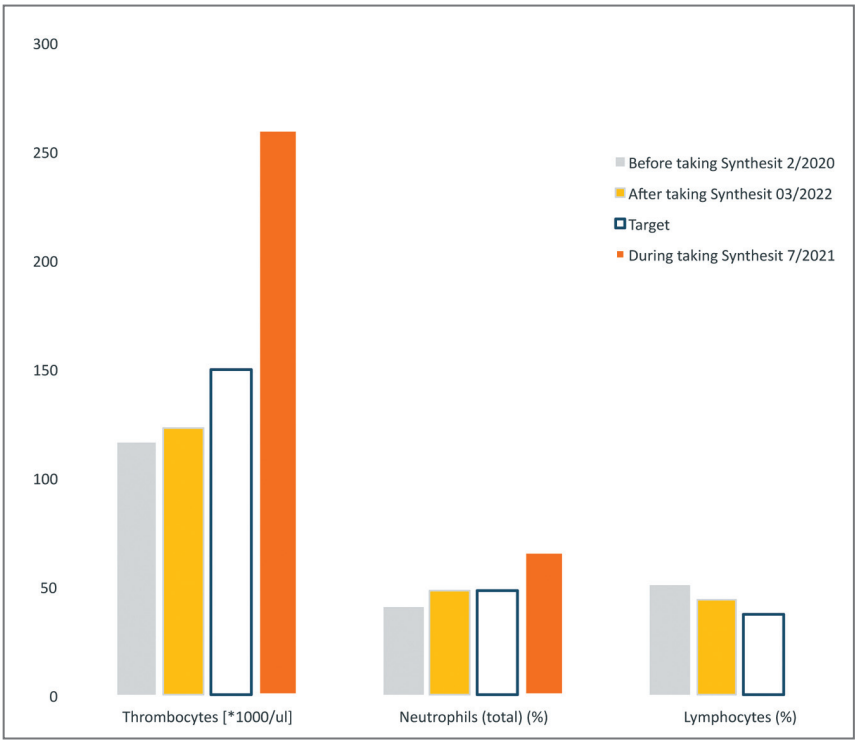

Fig. 1 Comparison of various haematological parameters

DISCUSSION

Pancreatic cancer is a highly aggressive and deadly malignancy with limited treatment options. However, there have been promising advancements in understanding the molecular mechanisms of pancreatic cancer and identifying potential therapeutic approaches (8). Here are some possibilities for pancreatic cancer treatment:

Surgery

Surgery offers a potential cure for pancreatic cancer, but only 20% of patients reach a questionable "cure". Conventional surgery involves pancreatoduodenectomy, distal pancreatectomy, or total pancreatectomy for extensive tumour growth. 5-year survival rates are less than 5% (8,9).

Pancreatic cancer chemotherapeutics

Pancreatic cancer recurrence rates are high, making chemotherapy an inevitable choice for patients after surgery. However, the overall prognosis of patients undergoing adjuvant chemo-treatments remains dismal due to low vasculature and the buildup of immunosuppressive microenvironment around the pancreas (9). Gemcitabine, the first FDA-approved agent for treating pancreatic cancer patients, is used in chemotherapy and has modest overall survival rates. Its clinical beneficial response appears more positive than other drugs. Although, it faces challenges due to the establishment and development of cancer microenvironment in progressive cancerous lesions, which reduces or blocks persistent drug penetration (10).

5-Fluorouracil (5-FU) is another important anti-cancer drug, promoting the incorporation of fluoridine triphosphate into RNAs, of fluorodeoxyuridine triphosphate into DNAs and

suppressing thymidalate synthase, resulting in severe genomic damages for eliminating cancer cells. It is often used in combination with other anticancer drugs gemcitabine, cisplatin, doxorubicin, or others (11).

Folfirinox is a relatively effective but aggressive combination treatment for pancreatic cancer patients, composed of four anticancer drugs: 5-FU, irinotecan, oxaliplatin, and leucovorin (12).

Immunotherapy

Pancreatic cancer lesions form a solid fibrotic and cancerous microenvironment, preventing drug penetration, and suppressing immune reactions. This microenvironment is caused by immunosuppressive cytokines which stimulate further expansion of immunosuppressive lymphocytes to antagonize anticancer responses (8). Immunotherapy is promising but more complex than we anticipate. Ipilimumab is an antibody-based treatment (13). The drug is shown to improve the overall survival of pancreatic cancer patients. Nivolumab and pembrolizumab are used clinically for melanoma treatment and still under clinical trials for treating pancreatic cancer. Therapeutics targeting immunosuppressive cells to reach the pancreatic tumour microenvironment may enhance the efficacy of immune-based therapies (8).

Other approaches for pancreatic cancer therapy

Pancreatic tumour-associated microenvironment cells promote epithelial-mesenchymal transition and remodelling surrounding tissues. Hyaluronan, a major element in cancer extracellular matrix, binds to cell surface receptors to maintain tumour cell survival and activate downstream signalling pathways related to tumour proliferation, migration, and invasion. Clinical trials have investigated a pegylated hyaluronidase (PEGPH20) that can break down hyaluronan. However, overall survival rates have not improved and hyaluronan derived drugs are more toxic (14).

Mutant *K-ras* gene is a potential drug target, but inhibitors against this oncoprotein have not been successful due to its complexity. Small molecules that can covalently bind to the mutant *K-ras* offer hope for treating oncogenic *K-ras* cancers. Several candidates are currently in clinical trials for solid tumours like pancreatic, colon and lung cancers (15). Sotorasib is the first one approved by FDA for treating *K-ras* lung cancer patients (8). Introducing wild type *p-53* protein (*wt-p53*) into cancer cells has increased the cytotoxicity of gemcitabine for eliminating pancreatic tumours (16).

Cyclopamine, an inhibitor of formation of cancer stroma, has been explored for treating pancreatic cancer (8). The Janus kinase and activator of transcription (JAK/STAT) signalling pathway is believed to induce inflammation in host tissues and tumour lesions. Inhibitors of these pathways such as napabucasin were shown to be very promising in treating pancreatic cancer. Early clinical trials targeting JAK/STAT signalling pathway showed no obvious toxicity, indicating the ongoing development of drugs inhibiting this pathway (17).

Dietary supplementation during pancreatic cancer

Weight loss before cancer diagnosis often indicates malnutrition, as side effects and metabolic changes contribute to this issue. One in three cancer patients are malnourished, with pancreatic cancer having the highest prevalence (18). For example, low selenium levels have been shown in pancreatic cancer patients (19). Moreover, selenium supplementation is quite rare in those patients (18).

The risk of pancreatic cancer was inversely associated with total vitamin E intake. A high level of vitamin E might be a protective factor for populations at risk for pancreatic cancer (20).

Other common options are methionine and folate as nutritional supplementation. Methionine and folate are precursors for the methyldonor of S-adenosylmethionine, which showed anti-tumoural effects in liver cancer (21). Rather, when it comes to supplementing

with folate and methionine for pancreatic cancer, current research only finds a few correlations between these nutrients' dietary intake and a decreased risk of the disease's onset; however, no investigation is currently being done to examine these nutrients' impact on the treatment of these cancer patients or when combined with gemcitabine (22).

Curcumin has been shown to have anti-neoplastic properties by inhibiting pathways involved in proliferation and inflammation, which can support the development of colorectal cancer. Studies have shown that curcumin can decrease cancer incidence, colonic inflammation, and adenoma/adenocarcinoma appearance in colorectal cancer mice. Preclinical studies have also shown that curcumin can reduce tumour volume and chemoresistance when combined with common chemotherapy drugs. Curcumin's potential in treating pancreatic ductal adenocarcinoma (PDAC) is still under investigation, but it has shown promising effects in both cases (23).

Many pancreatic cancer patients present with cachexia at diagnosis. L-carnitine supplementation showed an improved quality of life and an increase in lean body mass (24). Presently, cutting-edge dietary approaches to alleviate cachexia involve the use of natural substances like silibinin, supplementing with 3-polyunsaturated fatty acids, and implementing a ketogenic diet. Increased ketone bodies have anticachectic and anticancer properties. It has been demonstrated that silibinin inhibits pancreatic cancer cell proliferation, induces metabolic alterations, and lessens myofiber degradation. It has been demonstrated that consumption of 3-polyunsaturated fatty acids greatly reduces resting energy expenditure and controls metabolic dysfunction (25).

It has been shown that oral high-fat supplementation improved nutritional risk index by increasing oral caloric and meal intake in postoperative pancreatic cancer patients. Serum metabolites associated with pancreatic cancer were altered in benefit of the surgical patients receiving high fat oral supplementation compared with the control group. Oral high fat supplementation may have positive effects on the health status of postoperative pancreaticobiliary cancer patients (26).

The cancer-associated fibroblasts (CAFs) in pancreatic ductal adenocarcinoma (PDAC) are well known to play a dominant role in distant metastasis. Patients with PDAC tend to develop distant metastases with a greater number of CAFs. The number of CAFs is increased by radiotherapy if patients do not have adequate levels of vitamin D in the plasma. Therefore, vitamin D supplementation would be effective in inhibiting metastasis by inactivating CAFs (27).

Ferric citrate and its usage

Ferric citrate (FC) as a medicine has been approved as an oral iron replacement product for patients with iron-deficiency anaemia (28).

It is intriguing to note that, even in the case where there is no apparent iron deficiency, the dietary supplement appears to potentially assist the patient in maintaining normal haemoglobin levels (Table 2). However, the extent of its efficacy in this situation remains uncertain.

Additionally, the patient experienced decreased kidney efficiency in 2019. In cases of chronic kidney disease (CKD), ferric citrate has been shown to be an effective source of enteral iron (29). A randomized, placebo-controlled trial in CKD patients demonstrated that ferric citrate significantly improved iron status (30). Considering the patient’s nephropathy as an adverse effect of chemotherapy, the dietary supplement containing iron citrate might assist in maintaining normal iron levels.

Furthermore, the study revealed that ferric citrate significantly reduced the concentration of fibroblast growth factor 23 (FGF23) (30). FGF23 is involved in phosphate regulation and can mitigate hyperphosphatemia in CKD patients. However, elevated FGF23 levels are associated with CKD progression (31). Given that both dietary phosphate absorption (32) and iron deficiency (33) potently stimulate FGF23 production, ferric citrate treatment targets both factors to lower circulating FGF23 levels (29).

In a more recent study, ferric citrate administration in mice resulted in reduced serum phosphate concentrations and increased serum iron levels. This effect, along with the potential improvement in iron status, led to decreased circulating FGF23 levels. Notably, ferric citrate also demonstrated anti-inflammatory properties, improved kidney function, reduced albuminuria, and decreased kidney inflammation and fibrosis. These results suggest potential renoprotective effects of ferric citrate (29).

Based on this study, it is reasonable to consider that the dietary supplement containing ferric citrate may have some protective effects on kidneys, particularly when administered in larger doses. However, further scientific investigations and clinical trials are necessary to validate these observations and establish the safety and efficacy of ferric citrate supplementation for renal protection in human subjects.

From 2016 to 2017, the patient took extract of thymus which can enhance the immune system of cancer patients. The patient started to take Synthesit after four years, in 2020. It can be supposed that thymus extract didn't have any effect on immune system at time when the patient took Synthesit.

CONCLUSION

This case study serves as an intriguing example of chronic B-lymphocytic leukaemia and pancreatic cancer in a patient who opted for a non-conventional treatment approach. The outcomes observed during the course of this study warrant further investigation into the potential synergetic effects of lifestyle modifications and dietary supplements in the management of such complex medical conditions. Nevertheless, this case underscores the importance of personalised and integrative approaches to healthcare and individualized therapeutic decisions.

It is important to highlight that the dietary supplement Synthesit is not classified as a medicine and is not intended to diagnose, treat, cure, or prevent any diseases, including chronic-B-lymphocytic leukaemia and pancreatic cancer. As a dietary supplement, its purpose is to complement a balanced diet and support general well-being.

Patient consent

The participant has voluntarily provided informed consent to participate in the case study.

Conflicts of interest

The author is an employee of Research Centre Synthestech, Sochi, Russia.

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