Issue 1
2023

ISSN: 3049-1525
Volume 4

SOPHIA COLLEGE FOR WOMEN
ऊर्ध्वं मूलः
Empowered Autonomous

SCRIBE

Annual Science Journal

(Supported by DBT Star Status)

Sophia College for Women (Empowered Autonomous)

SCRIBE

Volume 4, Issue 1, 2023

Annual Science Journal, Sophia College for Women
(Empowered Autonomous), Mumbai
(Supported by DBT Star Status)

Peer-Reviewed **Multi-Disciplinary** **Open-Access**

Publisher:
Sophia College for Women (Empowered Autonomous)
Bhulabhai Desai Road,
Mumbai – 400026,
India

GENERAL PUBLICATION DETAILS

Starting year of publication: 2020

Frequency of publication: Annual

Format: Print Journal

Author guidelines: Requirements will be mentioned in the call for papers of each issue

Language: English

Aims and Scope: SCRIBE is a peer-reviewed open access annual journal published by Sophia College for Women (Empowered Autonomous), Mumbai. Supported by the DBT Star Status, SCRIBE aims to promote and disseminate high-quality research and scholarly work in the multidisciplinary and interdisciplinary realms of science, technology, and education.

The journal's primary goal is to provide a platform for researchers, educators, and students to share innovative ideas, methodologies, and pedagogical approaches across various scientific disciplines. SCRIBE seeks to bridge the gap between fundamental research and applied sciences, fostering a deeper understanding of how scientific advancements, discussed through the review articles, can effectively be integrated into educational practices.

SCRIBE welcomes original research articles, review papers, and perspective pieces that contribute to the advancement of knowledge in areas or contemporary issues including, but not limited to:

- Life Sciences and Biotechnology
- Physical Sciences and Materials Research
- Environmental Sciences and Sustainability
- Science Education and STEM Pedagogy
- Interdisciplinary Applications of Science and Technology

The journal emphasizes the importance of investigation-based learning and aims to highlight research that demonstrates innovative approaches to science education at all levels. SCRIBE also encourages submissions that explore the intersection of traditional scientific disciplines, recognizing the growing importance of multi as well as interdisciplinary research in addressing complex global challenges.

A double-blind peer review process ensures the quality, originality, and significance of published content. SCRIBE is committed to promoting science and making research findings accessible to under- and post-graduate students, seeking to inspire the next generation of researchers and educators.

SCRIBE (Volume 4, Issue 1, 2023)

CONTENTS

Editorial

Tressa Jacob, Rajbinder Kaur Dehiya, Hema Subramaniam

We are delighted to present to you the fourth issue of SCRIBE, our Annual In-house Peer-Reviewed Multidisciplinary Science Journal, supported by DBT Star Status.

We are deeply grateful to our Principal, Dr. Anagha Tendulkar Patil, and our Dean and Administrator, Dr. (Sr.) Ananda Amritmahal for their continued and unwavering support. Thanks are due to our expert Reviewers for sparing their valuable time for critically evaluating and contributing to elevating the standards of the journal.

Major reforms have been sweeping through the education sector in India with the state of Maharashtra implementing the National Education Policy 2020 for autonomous institutions with effect from the academic year 2023-24. As is the case with any transition, this too has been an intensive exercise for the teaching community. In this backdrop the staff and students have strived to make SCRIBE possible.

In the current issue of SCRIBE, we are pleased to include eight review articles, one research article along with the regular three articles on Nobel Prize winning works in the fields of Chemistry, Physics and Physiology or Medicine.

The ‘Invited Article’ by Dr. Harinath Chakrapani, Professor, IISER, Pune gives an exclusive perspective on commonly shunned malodorous gases. Dr. Chakrapani’s laboratory is involved in synthesizing and evaluating therapeutics for controlling reactive oxygen species derived from Sulfur, Nitrogen and Oxygen that are produced during normal metabolism. However, elevated levels can cause irreparable damage to cells, he explains.

We bring to you a research article that gives a comparative account of preliminary studies on the efficacy of the antimicrobial action of chemically and biologically synthesized silver nanoparticles on dental biofilm forming bacteria.

The year 2023 being the international year of Millets, we have a review article that examines the myriad possible applications of millet grains, across healthcare and industry, including the role of millets in boosting gut microflora, in fermentative products, in millet-based food products and in biofuel production.

The next one extensively reviews the beneficial effects of curcumin in *Caenorhabditis elegans* maintained on a high glucose diet**.** “Swimming with anxiety” provides a comprehensive overview of current knowledge on anxiety perception, detailing how it is studied in model vertebrate organisms such as zebrafish. It emphasizes the role of Schreckstoff as a significant inducer of anxiety in zebrafish, and sheds light on the mechanisms behind this phenomenon.

With an increase in human lifespan, degenerative diseases of the nervous system are a significant medical and public health concern all over the world. The prevalence and incidence of the three major neurodegenerative disorders - Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis is expected to increase dramatically in the near future. Two review articles on neurodegenerative diseases seek to bring a better understanding of the pathways that lead to these diseases and their symptoms to enhance an awareness of a few warning signs for early recognition of the malaise.

The next two review articles focus on novel treatment strategies. No standard treatment is available for autism spectrum disorders as the etiology is complex and involves an interplay of multiple factors including genetic and environmental influences. Current treatment strategies focus on reducing symptoms that interfere with daily functioning and improving the quality of life. In October 2023, the Central Drugs Standard Control Organization (CDSCO) approved India's first CAR-T cell therapy. In a review article, we bring you an exhaustive account of how T cells can be engineered to specifically target and destroy cancer cells.

As in previous issues, the current issue too includes articles on the Nobel Prizes in the Sciences for the year 2022. The Nobel prize section briefly outlines the works and discoveries of the prize winners, Carolyn R Bertozzi, Morten Meldal and K Barry Sharpless and their work on the relevance of the click chemistry reaction of CuAAC, and its biocompatible counterpart, SPAAC, *Svante Pääbo* and his work concerning the genomes of extinct hominins and human evolution was awarded the Nobel prize in Physiology or Medicine and Alain Aspect, John F. Clauser and Anton Zeilinger's award of the Nobel in Physics for experiments on entangled photons, establishing the violation of Bell inequalities and pioneering quantum information science.

To conclude we have yet another article authored by the second year Undergraduate students of the Department of Life Sciences devoted to the international year of millets. The inspiration for the article was the department's Mill-Sci fest 2023. The event included a talk by Dr. Amrita Hazra, Associate Professor, IISER, Pune, titled (MIL)LET'S DO IT!, a rangoli competetion depicting various scientific concepts studied by the first year Undergraduate students using different millets. Dr. Hazra has tagged this event on Twitter.* A food fest centered on millet based recipes was also a part of the event. The students presented posters featuring the geographical distribution and nutritional value of various millets. The article outlines the millet cultivation statistics and gives an overview of the nutritional value as well.

We wish you a happy reading!

*(https://mobile.twitter.com/Whytamin/status/1620043277314711552)

Invited Article

Smelly Gases Can Leave a Good Taste

Harinath Chakrapani
Correspondence: Professor, Department of Chemistry, Indian Institute of Science Education and Research, Pune.
Email: harinath@iiserpune.ac.in

All of us know that gases are a digestive signal – and a subject of many a comedic situation in cinema - we are not here to discuss that. Gases such as oxygen and carbon dioxide are well recognized by humans as being essential to life. Most other gases were considered unwanted byproducts of metabolism or waste that needs to be discarded. If there is one gas that many of us associate with smelly chemistry labs that drives some way from this lovely subject, it is hydrogen sulfide (H_2S) – you may recall the Kipp's apparatus standing in one corner of your lab. It was well known that this gas was produced in cells - by microbial breakdown of sulfur-containing compounds in the absence of oxygen (anaerobic) and the strong smell associated with sewer is largely due to the production of this gas. But not just that, other natural sources include volcanoes and natural gas deposits. Many of you are familiar with the oxygen version of H_2S, which is H_2O water, and although lighter, water is a liquid at room temperature while H_2S is a gas. You may recall that the concept of hydrogen bonding was introduced to explain this difference.

Nevertheless, there are other differences - being a mild acid, H_2S exists in equilibrium with its mono-anion and is a significant contributor to its properties in cells. This species interacts strongly with metal centers, it is an excellent reducing agent, and a much better nucleophile (displaces groups from a carbon center).

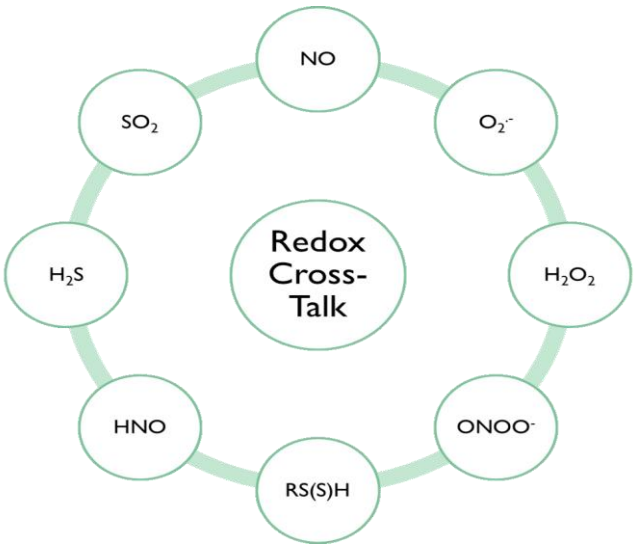


Figure 1. Some gas-derived species that are important mediators of cellular processes

At elevated levels, H_2S can cause respiratory arrest, and lead to severe cellular and physiological damage, and possibly death. Another example is carbon monoxide (CO) – a gas that needs no introduction – and is poisonous in large doses. It is a product of incomplete combustion in automobiles and other industrial processes, but is also used in many industrial processes as a raw material. One direct cause for toxicity is the ability of CO to coordinate rather strongly with heme of hemoglobin (Hb), and much stronger than oxygen. The resulting CO-Hb complex can over time accumulate and cause respiratory distress and possibly death. CO is produced during breakdown of hemoglobin by an enzyme heme oxygenase and is considered as an unintended byproduct. Similarly, nitric oxide (NO), which is another gas and is a toxic pollutant that is a byproduct of industrial processes and other natural phenomena such as lightning during thunder storms. NO is produced in cells as a part of the normal nitrogen metabolism. Being a free radical, NO has a wide chemical reactivity space. Like CO, NO can bind to metal centres to produce nitrosyl complexes, but it can get oxidized by oxygen to produce higher nitrogen oxides, which are highly reactive. Elevated NO levels cause respiratory problems, damage to cellular components such as lipids, DNA and proteins.

These toxic gases are produced in the cell?

As alluded to earlier, it was known that these gases were produced in microbes and plants and in some cases, humans as well. They were considered to be toxic byproducts and the cell wanted to get rid of them. However, when interesting properties of these gases began to emerge, a lot of attention was focused on them. For example, NO was found to activate neurons and hence could act as a signaling agent – this was a bit unexpected – and when several enzymes that deliberately produced them were discovered, it all made sense.

A gas is highly diffusible and barriers to penetration may not be very high when compared with other organic molecules that are typically involved in signaling. The organic molecules are sometimes deliberately poorly cell permeable as they interact with receptors outside the cell. Nevertheless, using a gas such as NO gives diversity to cells in transmitting signals. Similarly, the cell also deliberately produces CO and H_2S. Both these gases have now well-established signaling cascades.

Why do we want to deliberately supplement the cell with a toxic gas?

My lab has been interested in these gases for a while – more specifically, in generating them within cells on demand and at will. As a case in point, let me illustrate H_2S. This gas is biosynthesized or made in the cell using several enzymes, which are catalysts in cells, and some non-enzymatic ways as well. Together, the levels of H_2S appear to be maintained and this may be connected to the ability of H_2S to act as a reducing agent. Build-up of oxidants – frequently those derived from molecular oxygen – superoxide, hydrogen peroxide and hydroxyl radical – collectively referred to as reactive oxygen species (ROS) - is toxic and contributes to many degenerative diseases and conditions. Although ROS have signaling properties, one line of active research has been to bring down

levels of ROS through what are known as antioxidants that may be helpful in protecting cells from damage.

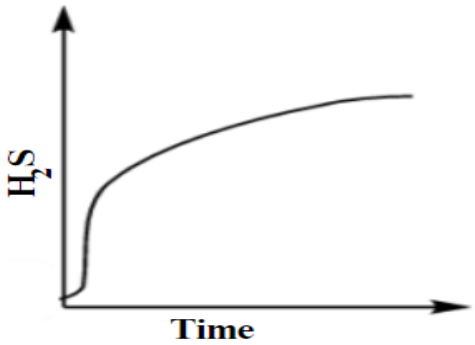


Figure 2. Typical release profile for inorganic salts.

Now, the question that many labs have been trying to address is – can compounds be developed that can enhance or augment H_2S so that we can exploit the antioxidant properties? One can think about using H_2S cylinders but one will easily dismiss this as a viable method for developing a therapeutic agent. Inorganic salt (Na_2S or NaHS) is another alternative but the generation of H_2S is very difficult to control and these salts can be difficult to store intact for extended durations (Figure 2). The alternative is to use organic compounds, which make a bulk of the drugs that people consume, as precursors of H_2S. The challenge is to use an organic compound to get into cells and generate H_2S. Now, of course, the compound that we use must have sulfur in it – and must be designed so that it is broken down to produce H_2S. This has been achieved and one derivative is in clinical trials as an antioxidant. Our lab has used a few approaches to generate H_2S in a controlled manner (Figure 3).

We initially developed protective groups that can be cleaved under certain favorable conditions. Imagine, if these compounds would produce H_2S only when needed, say for example, when oxidant levels are high. This requires us to design compounds that are otherwise stable but when exposed to oxidizing conditions, get cleaved to produce H_2S. This strategy can be extended to other stimuli as well including light, which is also known as photocleavage. It is now well known that the signaling initiated by H2S dominates its antioxidant properties and the cross-talk of sulfur dominates its effects.

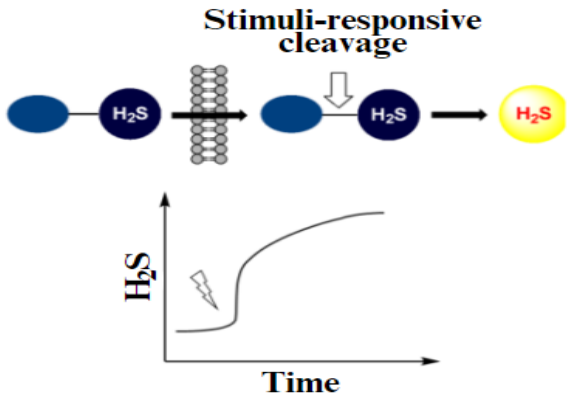


Figure 3. (Top) Strategy for generating H_2S in cells. The organic compound should permeate cells to be cleaved by a stimulus (ROS, for example) to produce H_2S. (Below) Expected H_2S release profile for the above strategy.

Summary and Future Directions

Since we have already discussed the importance of how one gas interacts with the other, it is not surprising that the cross-talk (Figure 1) among these species is also important. Persulfides (RS-SH) are produced when hydrogen sulfide interacts with ROS and these have powerful antioxidant properties and also can more efficiently

initiate antioxidant signaling cascades. A lot of focus has shifted to directly generating persulfides in cells. This is even more challenging since these species are less stable. Some compounds from our lab have shown promise in antioxidant models in both cells and in animals. We're now evaluating them in advanced models and also making derivatives with better properties. Besides the aforementioned gases, nitroxyl (HNO) and sulfur dioxide (SO_2) also have physiological relevance and these are also of interest in the overall scheme of redox biology (Figure 1). In summary, this is an exciting and challenging field of study and although some of these gases are smelly, they can leave a good taste in your mouth.

References

Gasotransmitter. (2024, March 26). *In Wikipedia.*https://en.wikipedia.org/wiki/Gasotransmitter

Fukuto, J. M., & Hobbs, A. J. (2021). A comparison of the chemical biology of hydropersulfides (RSSH) with other protective biological antioxidants and nucleophiles. *Nitric Oxide*, *107*, 46-57.

Mustafa, A. K., Gadalla, M. M., & Snyder, S. H. (2009). Signaling by gasotransmitters. *Science signaling*, *2(68)*, re2.

Wareham, L. K., Southam, H. M., & Poole, R. K. (2018). Do nitric oxide, carbon monoxide and hydrogen sulfide really qualify as 'gasotransmitters' in bacteria? *Biochemical Society Transactions*, *46*(5), 1107-1118.

Some relevant publications from our lab:

Bora, P., Chauhan, P., Manna, S., & Chakrapani, H. (2018). A vinyl-boronate ester-based persulfide donor controllable by hydrogen peroxide, a reactive oxygen species (ROS). *Organic letters*, *20*(24), 7916-7920.

Bora, P., Manna, S., Nair, M. A., Sathe, R. R., Singh, S., Adury, V. S. S., *et al.* (2021). Leveraging an enzyme/artificial substrate system to enhance cellular persulfides and mitigate neuroinflammation. *Chemical Science*, *12*(39), 12939-12949.

Bora, P., Sathian, M. B., & Chakrapani, H. (2022). Enhancing cellular sulfane sulfur through β-glycosidase-activated persulfide donors: Mechanistic insights and oxidative stress mitigation. *Chemical Communications*, *58*(18), 2987-2990.

Chauhan, P., Bora, P., Ravikumar, G., Jos, S., & Chakrapani, H. (2017). Esterase activated carbonyl sulfide/hydrogen sulfide (H2S) donors. *Organic letters*, *19*(1), 62-65.

Chauhan, P., Jos, S., & Chakrapani, H. (2018). Reactive oxygen species-triggered tunable hydrogen sulfide release. *Organic letters*, *20*(13), 3766-3770.

Chauhan, P., Gupta, K., Ravikumar, G., Saini, D. K., & Chakrapani, H. (2019). Carbonyl sulfide (COS) donor induced protein persulfidation protects against oxidative stress. *Chemistry–An Asian Journal*, *14*(24), 4717-4724.

Choudhary, B. S., Kumar, T. A., Vashishtha, A., Tejasri, S., Kumar, A. S., Agarwal, R., & Chakrapani, H. (2024). An esterase-cleavable persulfide donor with no electrophilic byproducts and a fluorescence reporter. *Chemical Communications*, *60*(13), 1727-1730.

Sharma, A. K., Nair, M., Chauhan, P., Gupta, K., Saini, D. K., & Chakrapani, H. (2017). Visible-light-triggered uncaging of carbonyl sulfide for hydrogen sulfide (H2S) release. *Organic letters*, *19*(18), 4822-4825.

Shukla, P., Khodade, V. S., SharathChandra, M., Chauhan, P., Mishra, S., *et al.* (2017). "On demand" redox buffering by H 2 S contributes to antibiotic resistance revealed by a bacteria-specific H 2 S donor. *Chemical science*, *8*(7), 4967-4972.

Cite this Article:

Chakrapani H. (2023). Smelly Gases Can Leave a Good Taste. S*CRIBE*, *4*(1), 7-11.

Research Article

Evaluation of Antimicrobial Effect of Biologically & Chemically Synthesized Silver Nanoparticles on Dental Biofilm-Forming Microorganisms (*Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa*)

Pranali Joshi, Aishah Dabir, Ayesha Parkar,
Zareen Shaikh, Aneska D'mello, Tuba Memon, Ayesha Shaikh
Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Ayesha Shaikh, Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai. Email: ayeshass786@gmail.com

Abstract

Dental microflora plays an important role in the etiology of oral diseases and the growth of dental biofilms that can cause tooth decay. The impending requirement of unearthing new strategies to circumvent antimicrobial resistance (AMR) of microorganisms has spurred numerous promising breakthroughs and their innovative applications - including the use of metal nanoparticles. Both silver nanoparticles and neem plant (*Azadirachta indica*) have been widely studied for their antimicrobial properties. As such, using Neem plant extract to synthesize Silver Nanoparticles (AgNPs) has been shown to have enhanced efficacy.

Introduction

The human dental microflora houses over 700 different types of bacteria which are crucial for both sustaining oral health and contributing to the development of oral diseases (Hojo *et al.*, 2009). This biofilm structure, composed of Extracellular Polymeric Substances (EPS) comprising polysaccharides, proteins, nucleic acids, and lipids, serves not only as a structural scaffold but also confers heightened resistance to antimicrobial agents such as detergents and antibiotics (Stewart & Costerton, 2001; Flemming & Wingender, 2010). Bacteria in the oral cavity form biofilms either by attaching directly to oral surfaces or by binding indirectly to already colonized bacterial cells (Kolenbrander *et al.*, 2002).

Dental caries, the primary cause of oral pain and tooth loss, is often caused by the acidic by-products from bacterial fermentation of dietary carbohydrates that destroy susceptible dental hard tissues (Selwitz *et al.*, 2007), gingivitis, and periodontitis (Takenaka *et al.*, 2018). Fungal opportunistic pathogen, *Candida albicans* (Metwalli. *et al.*, 2013) and bacteria *Staphylococcus aureus* (Wang & Ren, 2017) and *Pseudomonas aeruginosa* (Wu *et al.*, 2020) are associated with formation of dental biofilms leading to dental caries. Common non-surgical methods to control and prevent dental biofilms include tooth brushing and flossing. In cases where biofilm hardens into plaque, professional debridement may be necessary (Takenaka *et al.*, 2018).

As bacteria and bacterial biofilms develop increasing resistance to antimicrobial substances like antibiotics, nanoparticles are emerging as a promising research area. They hold the potential to become a valuable tool for antimicrobial purposes (Shkodenko *et al.*, 2020). Metal nanoparticles are under extensive investigation for their potential to inhibit and control dental biofilms. Silver nanoparticles, in particular, are highly favored for their antimicrobial properties. Silver nanoparticles (AgNPs) are currently used in many fields, including medicine, food, healthcare, and consumer and industrial applications. (Chernousova & Epple, 2012).

Over time, various methods have been developed to synthesize silver nanoparticles. Initially, chemical methods were used but their high cost and environmental hazards led to the development of safer and more environmentally friendly biological methods (Zhang *et al.*, 2016). Biological synthesis of silver nanoparticles shows high yield, solubility, and high stability (Chernousova & Epple, 2012). Plant extracts, such as those from *Azadirachta indica* (Neem) leaves, are a promising "green" biological method for synthesizing various nanoparticles like gold, zinc oxide, silver etc. It has been used for decades as a traditional remedy for a variety of human ailments, thanks to its astringent, antiseptic, insecticidal, antiulcer, and medicinal properties. (Naveed *et al.*, 2014). Throughout history, Neem twigs have been employed for various oral health purposes, including as a natural deodorant, a remedy for toothaches, and for dental hygiene practices such as teeth cleaning (Lakshmi *et al.*, 2015). The use of neem plant extract to synthesize metal nanoparticles is a recent development. Phytochemicals like terpenoids and flavanones reduce and stabilize the nanoparticles. When reacted with silver salt, it produces AgNPs. (Verma and Mehata, 2016). AgNPs possess excellent antimicrobial properties due to their small particle size and greater surface-to-volume ratio (Bapat *et al.*, 2018). They are potentially ideal fillers in biomaterials, including dental cements used to restore cavities caused by tooth decay.

Materials and Methods

Preparation of Neem Extract: Fresh Neem leaves (10g) were thoroughly washed with distilled water and then finely chopped. These chopped leaves were then boiled in 100mL of distilled water for a duration of five minutes. Subsequently, the resulting solution was filtered and stored at 4°C until required for subsequent procedures.

Chemical Synthesis of silver nanoparticles: 100mL of a 0.001M silver nitrate solution was gently heated and 5mL of a 1% Trisodium citrate solution was added dropwise with continuous stirring. The solution was heated until it attained a pale-yellow hue, indicating the successful synthesis of silver nanoparticles. Finally, the solution was allowed to cool and stored in the dark at room temperature for subsequent use.

Biological Synthesis of silver nanoparticles: 9mL of 0.001M silver nitrate solution was gently heated and 1mL of Neem leaf extract was added drop wise with constant stirring. The solution was heated until a pale-yellow hue appeared, indicating the synthesis of silver nanoparticles. The solution was then

cooled and stored in the dark at room temperature for further use.

Characterization of silver nanoparticles: Absorbance readings of both chemically and biologically synthesized AgNPs were recorded using a UV-spectrophotometer at various wavelengths (250-560 nm). The wavelength with the highest absorbance reading (490 nm) was compared to the expected UV-Spec absorption peak for silver nanoparticles (i.e. 380-430 nm) to verify successful synthesis.

Antimicrobial activity test against S. aureus, P. aeruginosa, C. albicans: To investigate the antimicrobial effect of Chemically synthesized AgNPs and Biologically synthesized AgNPs, a standard disc diffusion assay was performed. Briefly, sterile cotton swabs were used to inoculate three separate nutrient agar plates with saline suspensions of *S. aureus, P. aeruginosa, and C. albicans*, respectively. Three wells were then created in each plate using an alcohol flame-sterilized cork borer. Next, 2-3 drops of chemically synthesized AgNPs, biologically synthesized AgNPs, and Sterile Saline were pipetted into one well each on all three plates. The plates were incubated at 37°C for 24 hours. The zone of inhibition around each well was measured to determine the antimicrobial activity of the synthesized AgNPs.

Evaluation of inhibitory effect of AgNPs on biofilm formation of S. aureus, P. aeruginosa, C. albicans: To evaluate the inhibitory effect of AgNPs on biofilm formation of *S. aureus*, *P. aeruginosa*, and *C. albicans*, 10 mL sterile Nutrient Broth was dispensed into nine sterile large glass tubes. Subsequently, 1 mL of saline suspension of *S. aureus, P. aeruginosa*, and *C. albicans* was inoculated into three tubes each. For each bacterium, 1 mL of biologically synthesized AgNPs, chemically synthesized AgNPs, and sterile saline (control) were added to one tube each. The tubes were then incubated at 37°C for 24 hours. After incubation, the contents of each tube were decanted, and the tubes were allowed to air dry. 2 mL of 1:50 diluted Crystal Violet was added to each tube to stain the cells adhered to the tube. Then, 2 mL of 70% Ethanol was added to each tube to release the stain from the cells. The contents were decanted into separate clean and dry suspension tubes, and their absorbance readings were recorded at 530 nm using a UV-spectrophotometer.

Evaluation of antimicrobial effect of AgNP-infused dental cement on S. aureus, P. aeruginosa, C. albicans: To evaluate the antimicrobial effect of AgNP-infused dental cement on *S. aureus*, *P. aeruginosa*, and *C. albicans*, sterile Nutrient Agar plates were swabbed with each bacterial culture (OD at 530nm: 0.1). Biologically and chemically synthesized AgNPs were mixed and homogenized with crushed composite dental cement. Sterile filter paper discs were then dipped into the AgNP-infused cement mixture and placed onto the nutrient agar plates. The plates were subsequently incubated at 37°C for 24 hours to evaluate the inhibitory effect of the AgNP-infused dental cement on bacterial growth.

Results

Chemical Synthesis of silver nanoparticles and Characterization: The chemical

synthesis of silver nanoparticles was successfully performed as evidenced by the gradual development of a pale yellow hue upon incorporation of trisodium citrate solution into the silver nitrate solution with constant heating and stirring. The resulting silver nanoparticles were characterized using a UV spectrophotometer, and the maximum absorbance was recorded at 410 nm, slightly above the range of 300-400 nm reported by Sastry *et al.* (1997) in their study on the optical properties of silver colloidal particles (Sastry *et al.*, 1997). These results confirm the successful chemical synthesis and characterization of silver nanoparticles.

Biological Synthesis of silver nanoparticles and Characterization: As the neem extract was added to the silver nitrate solution, a pale yellow color slowly appeared while the mixture was continuously heated and stirred. The results indicate the successful biological synthesis of silver nanoparticles using neem leaf extract.

Characterization of silver nanoparticles: The synthesized nanoparticles were characterized using a UV spectrophotometer, which recorded a maximum absorbance of 490nm. This value is slightly above the range of 300-400nm reported by Sastry *et al.*, 1997 for the optical properties of silver colloidal particles. This indicates successful characterization of silver nanoparticles using UV-visible spectroscopy.

Antimicrobial activity test against S. aureus, P. aeruginosa, C. albicans: After 24 hours of incubation at 37°C, both chemically and biologically synthesized AgNPs demonstrated inhibitory effects against *S. aureus, P. aeruginosa*, and *C. albicans*. In all three plates, a clear zone of inhibition was observed around the wells containing AgNP solutions. The largest inhibition zone was observed for *C. albicans*, with a diameter of 4.6 cm for chemically synthesized AgNPs and 4.4 cm for biologically synthesized AgNPs. For *S. aureus*, both chemically and biologically synthesized AgNPs resulted in a zone of inhibition with a diameter of 2.8cm. Similarly, for *P. aeruginosa*, chemically and biologically synthesized AgNPs resulted in a zone of inhibition with diameters of 2.5cm and 2.6 cm, respectively. No zone of inhibition was observed around the wells inoculated with sterile saline. These findings demonstrate that both chemically and biologically synthesized AgNPs were effective in inhibiting the growth of the tested bacteria, with the greatest potency observed against *C. albicans*.

After 24 hours of incubation at 37°C, the inhibitory effect of AgNPs on biofilm formation was evaluated for *S. aureus, P. aeruginosa*, and *C. albicans*. The results showed that the control tubes (nutrient broth mixed with saline) for all three test bacteria had a conspicuous biofilm layer. However, the tubes containing nutrient broth mixed with biologically synthesized AgNPs showed visibly lower turbidity and absence of a biofilm layer for all three bacteria. On the other hand, chemically synthesized AgNPs inhibited biofilm formation only for *S. aureus*. The tubes containing nutrient broth mixed with chemically synthesized AgNPs inoculated with *P. aeruginosa* and *C. albicans*, showed higher turbidity and presence of a biofilm layer.

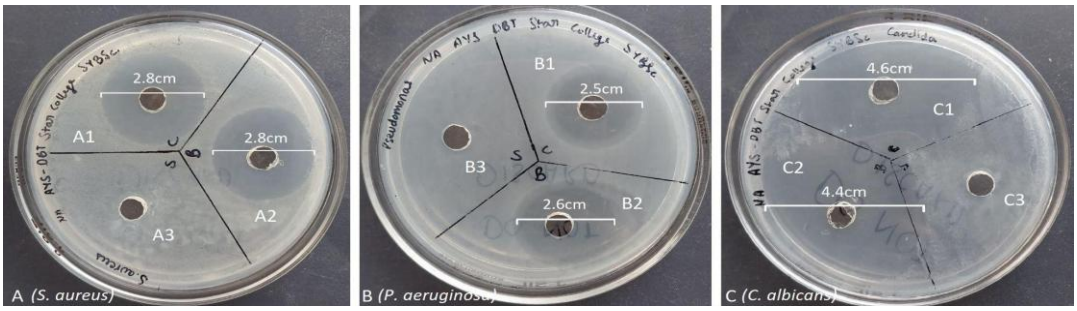


Figure 1. A1, B1, C1: Chemically synthesized AgNPs; A2, B2, C2: Biologically synthesized AgNPs; A3, B3, C3: Saline (control)

-	Control	Biologically synthesized AgNPs	Chemically synthesized AgNPs
S. aureus	1.72	1.11	1.28
P. aeruginosa	0.96	0.53	0.76
C. albicans	1.23	0.96	1.02

Table 1. Evaluation of inhibitory effect of AgNPs on biofilm formation (Absorbance recorded at 530 nm)

To quantitatively determine the biofilm formation in each tube, colorimetric methods were used, where the absorbance readings (taken at 530 nm) are directly proportional to the number of cells adhered to the walls of the tubes. As shown in the table above, biologically synthesized AgNPs exhibited maximum inhibition of bacterial growth and biofilm formation for all three test bacteria, while chemically synthesized AgNPs showed comparatively lower inhibition. Therefore, biologically synthesized AgNPs appear to be more effective against biofilm formation of *S. aureus*, *P. aeruginosa*, and *C. albicans*.

Evaluation of antimicrobial effect of AgNP-infused dental cement on S. aureus, P. aeruginosa, C. albicans: After incubation, zones of inhibition were observed around filter paper discs dipped in dental composite cement mixed with sterile saline (control), biologically synthesized AgNPs and chemically synthesized AgNPs, on Nutrient agar plates swabbed with pure bacterial cultures of *S. aureus*, *P. aeruginosa*, and *C. albicans*. A zone of inhibition was observed around the filter paper discs dipped in dental composite cement mixed with biologically synthesized AgNPs and chemically synthesized AgNPs on the plate inoculated with *P. aeruginosa*, with a diameter of 1.5cm. No inhibition was observed in the case of *C. albicans*. For *S. aureus*, a very small "halo" zone of inhibition was observed around the filter paper discs dipped in dental composite cement mixed with biologically synthesized

AgNPs and chemically synthesized AgNPs, with a diameter of 9mm.

Conclusion

Both chemically synthesized and biologically synthesized silver nanoparticles showed significant antimicrobial activity against *S. aureus*, *P. aeruginosa*, and *C. albicans* in their planktonic state. The nanoparticles were most effective in inhibiting the growth of *C. albicans* as compared to *S. aureus* and *P. aeruginosa*. The antimicrobial activity of dental composite cement infused with AgNPs was significantly lower, possibly due to physicochemical reactions between the AgNPs and the cement. This study highlights the potential of biologically synthesized AgNPs as effective antimicrobial agents for dental applications.

Acknowledgements

This study was supported by the DBT Star College grant received for undergraduate research projects.

Conflict of interest

The authors declare no conflict of interest.

References

Bapat, R. A., Chaubal, T. V., Joshi, C. P., Bapat, P. R., Choudhury, H., *et al.* (2018). An overview of application of silver nanoparticles for biomaterials in dentistry. *Materials Science and Engineering: C, 91*, 881-898. https://doi.org/10.1016/j.msec.2018.05.069

Chernousova, S., & Epple, M. (2013). Silver as antibacterial agent: ion, nanoparticle, and metal. *Angewandte Chemie International Edition*, *52*(6), 1636-1653. https://doi.org/10.1002/anie.201205923

Flemming, H. C., & Wingender, J. (2010). The biofilm matrix. *Nature reviews microbiology*, *8*(9), 623-633. https://doi.org/10.1038/nrmicro2415

Hojo, K., Nagaoka, S., Ohshima, T., & Maeda, N. (2009). Bacterial interactions in dental biofilm development. *Journal of dental research*, *88*(11), 982-990. https://doi.org/10.1177/0022034509346811

Kolenbrander, P. E., Andersen, R. N., Blehert, D. S., Egland, P. G., Foster, J. S., *et al.* (2002). Communication among oral bacteria. *Microbiology and molecular biology reviews*, *66*(3), 486-505. https://doi.org/10.1128/mmbr.66.3.486-505.2002

Lakshmi, T., Krishnan, V., Rajendran, R., & Madhusudhanan, N. (2015). Azadirachta indica: A herbal panacea in dentistry–An update. *Pharmacognosy reviews*, *9*(17), 41. https://doi.org/10.4103%2F0973-7847.156337

Metwalli, K. H., Khan, S. A., Krom, B. P., & Jabra-Rizk, M. A. (2013). Streptococcus mutans, Candida albicans, and the human mouth: a sticky situation. *PLoS pathogens*, *9*(10), e1003616. https://doi.org/10.1371/journal.ppat.1003616

Naveed, N., Murthykumar, K., Soundarajan, S., & Srinivasan, S. (2014). The use of neem in oral health. *Research Journal of Pharmacy and Technology*, *7*(9), 1060-1064.

Sakaue, Y., Takenaka, S., Ohsumi, T., Domon, H., Terao, Y., *et al.* (2018). The effect of chlorhexidine on dental calculus formation: an in vitro study. *BMC Oral Health, 18*(1), 1-7. https://doi.org/10.1186/s12903-018-0517-3

Sastry, M., Mayya, K. S., & Bandyopadhyay, K. (1997). pH dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 127*(1-3), 221-228. https://doi.org/10.1016/S0927-7757(97)00087-3

Selwitz, R. H., Ismail, A. I., & Pitts, N. B. (2007). Dental caries. *The Lancet, 369*(9555), 51-59. https://doi.org/10.1016/S0140-6736(07)60031-2

Shkodenko, L., Kassirov, I., & Koshel, E. (2020). Metal oxide nanoparticles against bacterial biofilms: Perspectives and limitations. *Microorganisms, 8*(10), 1545. https://doi.org/10.3390/microorganisms8101545

Stewart, P. S., & Costerton, J. W. (2001). Antibiotic resistance of bacteria in biofilms. *The Lancet, 358*(9276), 135-138. https://doi.org/10.1016/S0140-6736(01)05321-1

Verma, A., & Mehata, M. S. (2016). Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. *Journal of radiation Research and applied sciences, 9*(1), 109-115. https://doi.org/10.1016/j.jrras.2015.11.001

Wang, H., & Ren, D. (2017). Controlling *Streptococcus mutans* and *Staphylococcus aureus* biofilms with direct current and chlorhexidine. *Amb Express, 7*(1), 1-9. https://doi.org/10.1186/s13568-017-0505-z

Wu, X., Al Farraj, D. A., Rajaselvam, J., Alkufeidy, R. M., Vijayaraghavan, *et al.*, (2020). Characterization of biofilm formed by multidrug resistant *Pseudomonas aeruginosa* DC-17 isolated from dental caries. *Saudi Journal of Biological Sciences, 27*(11), 2955-2960. https://doi.org/10.1016/j.sjbs.2020.07.020

Zhang, X. F., Liu, Z. G., Shen, W., & Gurunathan, S. (2016). Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *International journal of molecular sciences, 17*(9), 1534. https://doi.org/10.3390/ijms17091534

Cite this Article:

Joshi, P., Dabir, A., Parkar, A., Shaikh, Z., D'mello. A., *et al.* (2023). Evaluation of Antimicrobial Effect of Biologically & Chemically Synthesized Silver Nanoparticles on Dental Biofilm-forming Microorganisms. *SCRIBE, 4*(1), 12-18.

Review Articles

The International Year of Millets: Food for Thought

Samika Pandey, Vijay Vig

Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Vijay Vig, Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai. Email: vijay.vig@sophiacollege.edu.in

Introduction

It is a popular notion that rice, wheat and maize have been the staple grains of human civilization since its very inception. However, one of the most unsung, yet most significant families of grains, are the millets.

Overlooked in the aftermath of the Green Revolution and increased production of paddy, wheat and other grains, the world is now realizing the overarching importance of millets in food production. To mark this growing recognition, the year 2023 was designated as "the International Year of the Millets" by a resolution of the UN General Assembly (FAO 2023).

What are millets?

Millets are a family of grasses that produce small grains, annual, and thrive in warmer climates. Taxonomically, all millets belong to the family *Poaceae,* spanning diverse genera such as *Echinochloa*, *Digitaria*, and *Panicum.* The most widely grown millet in India is the pearl millet - bajra - scientifically known as *Pennisetum glaucum*, which accounts for two-thirds of all millet production in the country; finger millet (ragi), *Eleusine coracana*, is also widely cultivated. Other important varieties of millet grown globally include foxtail millets (*Setaria italica*) in China, and proso millets (*Panicum miliaceum*) in Russia, and Ukraine (Vincent, 2023; FAO 2023).

History of Millets

Millets are amongst the oldest cereals cultivated by man, with evidence of millet cultivation being found as far back as 5,000 years ago. These virtually ubiquitous grains have found mentions in the Yajurveda of ancient India, the Old Testament of the Bible, Roman, Greek and Korean texts. They have also spanned a vast geographical area; being significant crops in Southeast Asia, Africa, and the Indian subcontinent (Upadhyay, 2023). They have played a vital role in global food production for years; as recently as 50 years ago, millets were the major crop grown in India.

Certain Indian states also have regional cuisines centers around a certain characteristic millet crop grown in the region: Rajasthan is famous for its bajra rotis, jowar forms the base ingredient for the famed bhakhris of Maharashtrian and Gujarati cuisines, while ragi predominates in dosa, idli and porridge batters in parts of South India.

Historically, millets were amongst the most widely cultivated crops. However, the advent of the green revolution led to an inadvertent decline in millet production; with the more widely available genetically modified varieties, global rice and wheat production increased to unforeseen levels, and millets came to be regarded as "coarse" and were distasteful to the increasingly urbane public sensibilities.

Are millets supergrains?

An increasing number of studies are piling on the evidence that millets are, in fact, potent "Nutri-cereals" with a host of nutritional benefits. Being gluten free, these are non-allergic in nature and rich in dietary fibers, and act as detoxification agents. Millets have been shown to reduce risk of cardiovascular disease – millet consumption has been conclusively linked to decreased total cholesterol, triacylglycerol and LDL levels (Anitha *et al.*, 2021). They've also been linked to increased hemoglobin levels, and reduced chances of Inflammatory Bowel Syndrome (IBS). Millet components, such as polyphenols, have also displayed downregulation of inflammatory factors (Shi *et al.,* 2017).

They are rich in micronutrients, especially iron, phosphorus, magnesium, and folate; pearl millet (bajra) is rich in vitamin B6 (pyridoxine), potassium and magnesium, while sorghum (jowar) is known to contain calcium. Finger millet (ragi) is similarly known to contain ergocalciferol, vitamins C and E, antioxidants and iron.

Another major upside to millets is their low glycemic index; since they are gluten-free, they are safer for consumption by diabetic individuals than regular rice or wheat flour. They stand at 52.7 on the glycemic index, which is a medium value, and considerably lower than maize, refined flour and rice (Richards, 2022).

Since they are rich in fibers, millets are also great for managing and preventing obesity. Their water absorption capacity promotes a feeling of satiation for longer durations, while not containing as many calories. Studies have also pointed to the anti-fungal, and anti-oxidative benefits of foxtail millets (Chen *et. al.*, 2022) and finger millets (Singh *et. al.*, 2022). Millets have hypoglycemic, hypercholesterolemic, and anti-ulcerative properties; and hence, the epithets of 'nutri cereals' and 'supergrains' are well-earnt.

Millets and Microflora - role in gut health

In recent years, a vast majority of research on the subject concurs that holistic health begins from gut health. Right from the robustness of the immune system, to brain health, it all starts with a healthy gut; and by extension, with healthy gut microflora.

Here too, millets come with myriad benefits, with studies indicating that they promote healthy gut microflora, and discourage gut pathogens.

Natural fermentation of millets revealed prevalence of *Leuconostoc, Pediococcus* and *Lactobacillus* strains, naturally occurring in conjunction with the crop (Antony & Chandra, 1997). All of these are known to act as probiotics within the gut, enhancing

digestion, reducing inflammation and competing with pathogenic bacteria to prevent their colonization.

A study by Singh *et. al.*, in 2022, found that the polyphenols contained in finger millet (*Eleusine coracana*), have good bioavailability, and hence, consumption enhances normal flora of the gut. It was also found to have prebiotic properties, which were utilized by gut bacteria, such as *Faecalibacterium, Eubacterium,* and *Roseburia*.

Millets also contain short chain fatty acids (SCFAs), which are indirectly responsible for inhibition of pathogenic gut flora, and for maintaining anaerobic conditions that assist the prevalence of bacteria such as *Bifidobacterium.* Tannin and β-glucan present in finger millets are known to have an antimicrobial effect, and prevent formation of biofilms, inhibiting pathogens and opportunistic pathogens like *Enterococcus faecalis, Shigella sonnei, Proteus vulgaris*, and *Lysinibacillus fusiformis*. (Singh *et. al.*, 2022).

Foxtail millets have also been proved to improve counts of *Bifidobacteria,* and *Lactobacillus*. In a study conducted with mice, suspensions of cecal content were plated on various selective media, and an improvement of natural flora after feeding the mice foxtail millet porridge was observed (Chen *et al.*, 2022).

Pearl millets have been found to contain non-digestible oligosaccharides that act as prebiotics. These non-adsorptive prebiotic molecules are utilized by probiotic gut microbes to produce SCFAs. SCFAs have been associated with not only gut health, but also play a role in neuro-endocrine regulation. They also have preventive action against colorectal cancers. The prebiotic oligosaccharides are characterized by their resistance to digestion by gastric juices, and selective utilization by probiotic microorganisms. They also play a role in maintaining the integrity of the intestinal mucosa. As many as 20 companies have begun purifying and marketing these prebiotics as dietary supplements and food additives (Mondal *et al.*, 2022).

Since the fermentation of millets is a heterolactic fermentation process, utilizing lactic acid bacteria, fermented millet drinks and food items also have a probiotic function. Hence, millet consumption has tremendous benefits for the wellbeing of the gut microbiome, which is in turn, crucial for cardiovascular health, immune homeostasis, IBS, management of metabolic diseases, and more (Shreiner *et al.*, 2015). Thus, by enhancing the gut microflora, millets can have a positive effect on all of these aspects.

Millets: Future of Food Production

Apart from the obvious nutritional benefits to be derived from these super-grains, as millets are now being dubbed in popular consciousness, they may also just hold the key to food security amidst the changing global climate. Since millet cultivation is organic by default, they are less dependent on chemical fertilizers; they tend to be hardy, drought tolerant and don't require nearly as much water as conventional food grain crops.

Another major benefit of millets, with respect to climate change, is their ability to be stored for longer durations, effectively making them famine reserves (Saxena *et al.*, 2018b).

It is due to these properties that millets are being looked at as the future of cereal production. The growing climate crisis means that more delicate, climate-sensitive crops, and crops that thrive in lower temperature conditions will cease to be a reliable source of food. As the global staple crop trifecta of rice, maize and wheat are all vulnerable to the weather and temperature imbalances caused by climate fluctuations. Millets thrive in tropical to subtropical climates, with wide adaptability towards soils ranging from poor to fertile soils, and are capable of tolerating some alkalinity as well. Some varieties, such as Kodo millet, also grow in rocky and gravelly soils of hilly regions. Pearl millets, in particular are highly tolerant of higher temperatures.

They have much lower impact on the environment, since they require fewer resources, and do not have as much requirement for chemical fertilizers. Millet cultivation, in fact, may just be the key to attaining the UN's Sustainable Development goals in a changing world. As per SGD# 2, “No Hunger”, one of the main objectives is to ensure food security for all. However, traditional agricultural practices are laced with use of chemical fertilizers, pesticides, weedicides and more – millets are the exception to this. Millets and their seeds are known to be less susceptible to attack by insects. Millet cultivation, thus, subverts the immense ecological damage inflicted by traditional agricultural practice, and channels cultivation practices towards a more sustainable outlook (Ceasar & Maharajan, 2022).

Myriad Applications of Millets

The food industry has been quick to adopt millets, in view of their several advantages. Millet flour has gained traction in recent years, with studies showing that millet-derived resistant starch (RS) flour has superior properties to traditionally sourced dietary fibers (DF) in industry (Kaimal *et al.*, 2021).

Apart from their traditional use as animal feed, millets are now increasingly used in beer and biscuit production, and for other food products like flaked millet; puffed millet; extruded and roller-dried millet products; fermented, malted and composite millet flours (Mathad *et al.*, 2022). Millets are also being used in fermented drinks that have a host of health benefits; ambli and ragi ambali are popular in Karnataka, as non-alcoholic fermented drinks. Various other fermented drinks made using millets are widely prevalent in cultures across Asia and Africa (Madalageri, 2012). Several millet-based supplements have increasingly become prevalent in the market, as the consumers become aware of the immense benefits of these. Health drinks, malt powders and flavored milk are just a few examples of products made using millets.

Cultivation of millets has also been known to convert dry lands to agriculturally productive lands.

CSIR-CFTRI have worked on a vast variety of millets-based technologies, many of which

have translated into the MSME (micro, small and medium enterprises) market. These include ragi and bajra based cookies, ragi and bajra bread, ragi flour, ready to eat ragi and sorghum based flaked snacks, millets-based pastas, muffins, papads, rusks, semolina and more. Many of these products are finding increasing popularity in rural areas as well.

Some research has also been conducted with the view of using millets as biofuels – back in 2001, ICRISAT and Indian Institute of Millets Research (IIMR) worked on producing higher biomass sorghum (jowar) that can act as a lignocellulosic second-generation biofuel. Since they can be grown in semi-arid regions, and require much less water for production than traditional biofuel feedstock like sugarcane, they are a promising alternative (ICRISAT, 2001).

In particular, bioethanol production using *E. coracana* (Finger millets) has been of particular interest in recent years, as a high-efficiency feedstock for 2nd generation bioethanol. The standard process of bioethanol production involves extraction of sugar by malting, and subsequent fermentation of the sugar by yeasts and fungi. Finger millets and sorghum have been found to possess the highest alpha-amylase activity in the malting process; higher than both maize and wheat. Use of *Zymomonas mobilis* with hydrolysed finger millet flour yields significant amounts of ethanol; in even more quantities than corn (Yemets *et al.*, 2020).

Studies have also been conducted with the view of using millets in basal media for various industrial fermentation processes; Daniel and Oboh conducted studies on the use of sweet potato and millets as the base for cultivation of commercially important fungi and bacteria, and found the medium to be a viable, cheaper alternative to traditional prepared media in industry (Daniel & Oboh, 2021).

In terms of biotechnology, *Setaria italica* (foxtail millet) is being studied as a potential engineering frontier, for incorporating the more efficient C4 metabolism into staple crops like rice and wheat. The C4 metabolic pathway has been proved to be more efficient in terms of water, carbon and nitrogen utilization, and thus is better for food productivity; by genetically engineering the diploid genes for the C4 system, alongside the genes for climate resilience, it may be possible to incorporate the desirable properties of millets into other crops as well. Further research is required to make this possible.

Conclusion

Owing to their vast range of often under-appreciated, but very vital properties, and their array of applications in a world with a brewing climate crisis, the International Year of Millets was a necessary step towards creating awareness about these indispensable crops. The initiative, first proposed by the government of India, has sparked interest globally and served as a catalyst for research and assimilation of millets into various agricultural and industrial applications.

References

ICRISAT (2001). A short history of millets and how we are recognising their importance in the modern context.

FAO (2023) About | International Year of Millets 2023 | Food and Agriculture Organization of the United Nations.

Anitha, S., Botha, R., Kane-Potaka, J., Givens, D., Ananthan, R., *et al.* (2021). Can millet consumption help manage hyperlipidemia and obesity?: A Systematic Review and Meta-Analysis. *Frontiers in Nutrition, 8*, 700778

Antony, U., & Chandra, T. (1997). Microbial population and biochemical changes in fermenting finger millet (eleusine coracana). *World Journal of Microbiology & Biotechnology*, *13*(5), 533–537.

Caesar, S. A., & Maharajan, T. (2022). The role of millets in attaining United Nations sustainable developmental goals. *Plants, People, Planet*, 4(4), 345–349.

Chen, Y., Zhang, R., Xu, J., & Ren, Q. K. (2022). Alteration of intestinal microflora by the intake of millet porridge improves gastrointestinal motility. *Frontiers in Nutrition, 9*.

Daniel, A., & Oboh, E. J. (2021). Formulation of Microbial Growth Using Millet and Sweet Potato. *Pharmaceutica Analytica Acta 6(7):53-57*

Introduction to Millets : FAO International Year of Millets. FAO. (2023). Food and Agriculture Organization.

IIT-B (2022) International Year of Millets 2022-23 | Indian Institute of Technology Bombay.

Kaimal, A. M., Mujumdar, A. S., & Thorat, B. N. (2021). Resistant starch from millets: Recent developments and applications in food industries. Trends in Food Science and Technology, 111, 563–580.

Mathad, P. F., Ganachari, A., Nidoni, U., & G, S. D. (2022). Millet Industry Scenario. In Handbook of Millets (pp. 343–366).

Madalageri, D. M. (2012). Biochemical Changes and Microbial Population In Fermented Beverages of Millets. *ResearchGate*.

Mondal, S., Balasubramanian, A., Biswas, P., Agrawal, S., Ghosh, S., *et al.* (2022). Characterization of pearl millet oligosaccharides and evaluation of their prebiotic potential. *Bioactive Carbohydrates and Dietary Fibre, 28*, 100324.

Newly developed high biomass sorghum and pearl millet a boon to 2G biofuel production in India – ICRISAT. (2001).

Richards, L. (2022, March 28). Can a person with diabetes eat millets?

Saxena, R., Vanga, S. K., Jin, W., Orsat, V., & Raghavan, V. (2018b). Millets for Food Security in the context of Climate Change: A review. Sustainability, 10(7), 2228.

Singh, V. K., Lee, G., Son, H., Amani, S., Baunthiyal, M., *et al.* (2022). Anti-diabetic prospects of dietary bio-actives of millets and the significance of the gut microbiota: A case of finger millet. *Frontiers in Nutrition, 9*.

Shi, J., Shan, S., Li, H., Song, G., & Li, Z. (2017). Anti-inflammatory effects of millet bran derived-bound polyphenols in LPS-induced HT-29 cell via ROS/miR-149/Akt/NF-κB signaling pathway. Oncotarget, 8(43), 74582–74594.

Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and in disease. *Current Opinion in Gastroenterology*, *31*(1), 69–75.

Upadhyay, S. (2023). Millets History | Millets Information. S & V Millets. Retrieved from https://svmillets.com/millets/

Vincent, M. (2023). Types of Millets in India 2023 – Type, botanical names, facts and benefits. Entri Blog.

Yemets, A. I., Blume, R. Y., Rakhmetov, D., & Blume, Y. B. (2020). Finger millet as a sustainable feedstock for bioethanol production. The Open Agriculture Journal, 14(1), 257–272.

Cite this article:

Pandey, S., & Vig, V. (2023). International year of millets: food for thought. *SCRIBE*, *4*(1), 19-25.

Evaluation of Curcumin as a Protective Agent for *Caenorhabditis elegans* Exposed to High Glucose Diet: An Insight

Mrunmayee Rade, Sukaina Abbas, Sree Nair
Department of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Sree Nair, Department of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai. Email: sree.nair@sophiacollege.edu.in

Abstract

Hyperglycemia, a hallmark of diabetes, generates reactive oxygen species (ROS), which cause oxidative stress leading to detrimental consequences. *Caenorhabditis elegans*, a powerful model for metabolic studies, exhibits elevated ROS levels under chronically high glucose conditions. Curcumin, a natural compound, offers potential protection against hyperglycemia-induced stress. This study investigates the protective efficacy of curcumin in *C. elegans* exposed to a high glucose diet. Curcumin's unique membrane interaction can mitigate oxidative stress, reduce ROS levels, and potentially prevent stress-induced mutations. Studies utilizing *C. elegans* as a model, will offer significant insights into the potential of curcumin as a shield against hyperglycemia-induced stress. These insights could lay the foundation for further research exploring curcumin-based interventions as potential therapeutic strategies for managing diabetic complications. Employing *C. elegans* as a powerful tool for understanding metabolic stress, contributes to the anticipation in the field of diabetic management and opens doors for future investigations focusing on the translation of these findings to human applications.

Curcumin, a polyphenolic compound derived from turmeric, has remarkable antioxidant properties, which are crucial in combating oxidative stress and related pathologies (Menon & Sudheer, 2007). Curcumin has a significant antioxidant potential since it may scavenge free radicals and strengthen the body's own antioxidant enzymes, making it a potential candidate for therapeutic interventions in oxidative stress-related diseases (Aggarwal & Harikumar, 2009). In the context of high glucose conditions, *C. elegans* serves as an effectual model, reproducing the molecular and metabolic disruptions observed in diabetes-related hyperglycemia (Lee *et al.*, 2009). This model enables the investigation of the impact of high glucose on biological systems and the possible mitigating effects of antioxidants like curcumin. In this setting, the effectiveness of curcumin as a protective mediator against oxidative ravage influenced by high glucose levels can be judiciously assessed, providing insights into its therapeutic potential. The subsequent sections plan to induct and delineate the link between curcumin’s antioxidant facility and its shielding role in high glucose environments as modeled by *C. elegans*.

Caenorhabditis elegans

This review surveys *Caenorhabditis elegans* as a key model organism in biological research. Established as the first multicellular organism to have its genome sequenced, *C. elegans* offers substantial acumen into molecular, cellular, developmental, and behavioral biology. It is a non-parasitic nematode and has transformed biotic research due to its genomic entirety and biological predictability (Stiernagle, 2018).

The sequencing of the *C. elegans* genome marked an essential instance in biological research, setting a base for genomic analyses in multicellular organisms (Stiernagle, 2018). The acuity of molecular and cellular biology research on *C. elegans* is wide-ranging, proposing insights into gene functions and cellular pathways relevant across species (Stiernagle, 2018). The developmental and behavioral studies of *C. elegans* have been significant in elucidating these processes in more complex organisms (Stiernagle, 2018). Investigations into the immune system of *C. elegans* have yielded vital understanding of immunological processes, with conjectures for human immune studies (Stiernagle, 2018).

The natural history and biology of *C. elegans* are crucial in understanding fundamental scientific concepts. Its function as a prototype creature in various fields of biology underlines its importance in scientific advancement.

Hyperglycaemia and *C. elegans*

C. elegans can be used to investigate molecular targets affected by both normal and abnormal glucose concentrations because of its short life span, ease of genome modification, and straightforward insulin receptor system (DAF-2). Extensive studies have shown that nematodes' longevity is negatively impacted by elevated, or millimolar, amounts of glucose (in addition to the typical *E. coli* OP50 food supply). Various studies were done in *C. elegans* to study the effects of a glucose diet on their morphology and physiology. In a study wherein *C. elegans* were given glucose ranging from 10 to 50 mM concentration, there was an increase in total body glucose content as seen in diabetic patients' non-controlled diets. The conclusion from these studies is that a high-glucose diet increases total internal glucose levels in the body. L4 larvae were subjected to 100 mM glucose concentrations that led to a reduction in egg laying and the number of eggs in utero (Bonomini *et al.*, 2015). The oxidative damage brought on by glucose stress may have contributed to the life expectancy decline that was observed in direct proportion to the level of glucose stress. Glucose restriction promotes lipid storage, transport, and oxidation (Bonomini *et al.*, 2015).

C. elegans lives longer when glucose is restricted (Schulz *et al.*, 2007). Recent work using a model of defective glucose metabolism indicates that an increase in ROS triggers a subsequent teratogenic increase in stress defense, which ultimately lowers net stress levels (Schulz *et al.*, 2007). High glucose concentrations, on the other hand, shortened the life span of *C. elegans*.

It was demonstrated that adding glucose to a diet reduced the anoxia survival of wild type

animals and that sensory neuron mutants were able to eliminate the worms' chemotaxis sensitivity to glucose. In examining the impact of dietary glucose on egg production in anoxic conditions, Garcia from the Padilla Lab observed a notable decrease in egg-laying rates (Garcia, 2015). This finding aligns with the 2016 study which presented a distinct phenotype characterized by reduced egg-laying in response to hyperglycemia, an effect that could be mitigated through the administration of buformin (Teshiba *et al.*, 2016). The research focussed attention on the role of tph-1, the gene encoding tryptophan hydroxylase vital for serotonin (5-HT) synthesis, in this process. The inhibition of the glucose-induced egg-laying phenotype by lowered tph-1 activity involves serotonin as a key moderator in glucose sensing (Teshiba *et al.*, 2016). Further, it was exhibited that the manifestation or lack of glucose did not really amend the reduced egg-laying rates in tph-1 mutants, suggesting that external serotonin supplementation could counteract the glucose-associated decline in egg production (Teshiba *et al.*, 2016). These discoveries denote that a paucity in serotonin reflects the reproductive phenotype generated by glucose, although this decreased egg-laying could also be endorsed to food deprivation.

Curcumin

Turmeric (*Curcuma longa*) is a plant in the ginger family (Zingiberaceae) that has its source in India and is now cultivated around the globe, embracing China, Southeast Asia, and Latin America. Turmeric, because of its savor and hues, is a staple food (Amalraj *et al.*, 2017). India is the world's foremost manufacturer and exporter of turmeric. Tendering to various reports, the international demand for turmeric is projected to be worth 1.7 million metric tons and is estimated to escalate by 2027 (Ireson *et al.*, 2001). Besides its culinary application as a curry spice, turmeric features an extensive history of scientific applications including natural coloration (food, cosmetics, and textiles), insect repellency, and antibacterial properties. Turmeric is used for wound healing, lung difficulties, liver problems, and dermatological conditions in Ayurveda medicine (Goel *et al.*, 2008; Ireson *et al.*, 2001).

Curcumin, also known as 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, is a lipophilic chemical that quickly enters cell membranes (Jaruga *et al.*, 1998). It mimics typical apoptosis events and alters the structure and function of the cell membrane; however, the cellular response to curcumin is different from the typical apoptosis process in that it causes an immediate and partially reversible loss of membrane integrity, from which cells can recover rather quickly (Jaruga *et al.*, 1998). It was also proposed that curcumin's modifications to the membrane might account for some of its actions (curcumin may influence protein kinase C activity, for example, by altering access to phosphatidylserine).

Curcumin is an ideal option for structure-activity interactions and leads optimization studies due to its uncomplicated chemical structure and the relative density of functional groups.

Numerous investigations on the pharmacologic and metabolic properties of

curcumin have been reported (Ireson *et al.*, 2001). Curcumin's poor bioavailability and selectivity have led to the introduction and testing of several analogues of this material to assess their activities against established biological targets and to enhance the pharmacological profile (bioavailability, selectivity, and stability) of the natural product. Curcumin has emerged as a promising lead component for the creation of innovative chemotherapy drugs intended to treat cancers including prostate and colon cancer as well as other diseases that require chemotherapy (Tomeh *et al.*, 2019). Curcumin mimics a number of antiangiogenic effects by asymmetrically replacing the phenolic groups with substituted phenyls and other aromatics, and by replacing the diketone group with an a- or b-unsaturated ketone (Lee *et al.*, 2013).

Curcumin and Diabetes

Owing to its antioxidant and anti-inflammatory qualities in STZ-induced pancreatic impaired models, curcumin has exhibited promise in reducing hyperglycemia and hypercholesterolemia in type 2 diabetes and stipulates protective gains against pancreatic injury, exceptionally in insulin-releasing cells. Numerous reports have revealed that curcumin not only tends to reduce the complications wholly caused by diabetes but also radically improves the indirect consequences of this illness (Zhang *et al.*, 2013). Curcumin treatment, for example, has been shown to alleviate diabetic neuropathy, a microvascular disorder primarily caused by oxidative damage and inflammation (Daugherty *et al.*, 2018). Furthermore, the use of curcumin also improved diabetic retinopathy, nephropathy, and cardiomyopathy, which are frequent complications of long-term diabetes (Reusch, 2003).

Curcumin Studies in *C. elegans*

Curcumin-Mediated Lifespan Extension in *C. elegans*

In traditional Indian medicine, curcumin has been used for many years to treat a variety of illnesses by promoting wound healing. Due to its nonsteroidal anti-inflammatory and chemo-preventive properties, it has recently gained recognition in Western medicine. The Zingiberaceae family of plants, which includes the spice turmeric (*Curcuma longa*), has the pharmacologically useful compound curcumin, which is used as a yellow coloring pigment.

Traditional Indian medicine regards curcumin as a useful medication for treating a variety of conditions, including diarrhea, ulcers, jaundice, flatulence, wounds, sprains, arthritis, acne, skin infections, and eye infections. Given its ability to inhibit tumor development, propagation, succession, and spread in animal models, curcumin is a potentially useful tool for cancer therapy (Duvoix *et al.*, 2005). Recent preclinical and clinical investigations have shown that curcumin has potential therapeutic efficacy against a number of chronic illnesses, including neoplastic, neurological, cardiovascular, pulmonary, metabolic, and psychiatric disorders (Goel *et al.*, 2008). Curcumin's pleiotropic activity notably influences several key signaling pathways, including those regulated by nuclear factors

(NF)-κB and Akt, and it also impacts Nrf2-mediated cytoprotective pathways, which are vital for cell viability and protection against cellular stress. (Hatcher *et al.*, 2008). Several genetic variables, such as those controlling antioxidant qualities, metabolic processes, DNA repair, cell senescence, and cell death, affect an organism's lifetime. Central to aging is the role of inflammation and oxidative stress, which can exacerbate age-associated cellular damage primarily caused by the production of reactive oxygen species (ROS). These factors are increasingly recognized as key contributors to the aging process (Jaruga *et al.*, 1998). In addition to its potent antioxidant and anti-inflammatory properties, there is compelling evidence that curcumin can prevent or postpone the onset and progression of numerous age-related illnesses as well as the process of senescence.

C. elegans is a prominent model organism for researching aging and longevity because of its relatively short lifetime, quick reproduction period, and well-defined genetic and environmental variables that impact lifespan. Mammals and *C. elegans* appear to age similarly in that both exhibit Sarcopenia, or the loss of muscle mass that occurs with aging; however, it is unclear how the lifetime extension observed in *C. elegans* translates to humans (Herndon *et al.*, 2002). Adding to this, consumption of less calories increases the lifespan of mammals as well as *C. elegans.* According to several experimental reports, medications used to extend the lifetime of C. elegans can be beneficially employed to treat age-related disorders including cancer and neurological diseases (Pinkston *et al.*, 2006).

Curcumin Impelled Behaviour of *C. elegans*

Growing bacteria produces detrimental metabolites which could be harmful to the *C. elegans.* A recent study stated that curcumin treated *C. elegans* emanated in reduced pathogenicity, as curcumin is a powerful antimicrobial agent. Hence, the antimicrobial attributes of curcumin could offer insights into the mechanism of extending lifespan by reducing pathogens and safeguarding C. elegans from impairment, thereby maintaining their integrity.

Reduction of Reactive Oxygen Species Production Due to Curcumin

Under conditions of oxidative stress and high temperature, an extended longevity is directly related to improved survivability. A robust antioxidant, curcumin may mitigate cellular damage brought on by aging by preventing the production of reactive oxygen species (ROS). (Queen & Tollefsbol, 2010). Curcumin has the ability to cause apoptosis and senescence, which is analogous to one of the roles of the tumor suppressor p53. Since curcumin is a powerful redox scavenger, it makes sense that it functions as a pro-oxidative mediator to produce checkpoints. Curcumin appears to act as a cellular regulator by targeting various enzymes in the ROS pathway, modulating internal ROS levels and potentially triggering cell death or senescence through p53-dependent and independent mechanisms (Trachootham *et al.*, 2009).

Studies in *C. elegans* suggest curcumin's interaction with multiple ROS-managing

enzymes and its surprising ability to increase internal ROS levels might hold promise as a future anti-cancer treatment due to a mechanism involving ROS (Larasati *et al.*, 2018). Interestingly, adding glutathione (GSH), a cellular antioxidant, weakens curcumin's ability to slow tumor growth, hinting that the level of ROS inside cells might be linked to curcumin's anti-cancer effects. While the amount of ROS within cells seems to be important, it might not be the sole factor in curcumin's anti-cancer activity, suggesting that other reactive molecules generated from ROS, like carbonyls and aldehydes, could be the key culprits in killing tumour cells (Larasati *et al.*, 2018). Pinpointing these ROS-derived molecules could be a game-changer for improving curcumin's effectiveness and developing more targeted therapies, particularly in *C. elegans* research.

Curcumin Enhances the Stress Resistance of Wild-Type *C. elegans*

Greater stress tolerance in *C. elegans* is associated with longer lifespans; higher survival in harsh environments, such as heat or oxidative stress, is clearly linked to longer lifespans (Lithgow *et al.*, 1995; Munoz and Riddle, 2003). By administering curcumin to wild-type N2 worms prior to subjecting them to oxidative stress and heat shocks, researchers examined whether curcumin may improve stress tolerance in *C. elegans.* Synchronized L1 larvae were treated with curcumin for 72 hours in order to evaluate its effect on oxidative stress resistance. Afterwards, they were exposed to juglone (a compound causing internalized peroxide buildup) (de Castro *et al.*, 2004) and to further incubation. The results of the study demonstrated that pretreating *C. elegans* worms with curcumin greatly increased their survival rate when they were subjected to oxidative stress caused by juglone, indicating a possible function for curcumin in boosting stress resistance. A substantial improvement in worm survival was seen after curcumin administration when synchronized L1 larvae were pretreated with curcumin for 72 hours before being exposed to a fatal heat shock (38°C for 7 hours). This was done to assess the effect of curcumin on heat stress. (de Castro *et al.*, 2004).

Effects of Curcumin on Hyperglycemic *C. elegans*

In a model of STZ-induced pancreatic damage, curcumin demonstrated anti-hyperglycemic and hypocholesterolaemia properties in type 2 diabetes in addition to its protective effect against pancreatic injury (primarily on cells which produces insulin) via its antioxidant as well as anti-inflammatory effect. Numerous studies have demonstrated that curcumin significantly improves the unexpected consequences of diabetes in addition to its tendency to minimize the difficulties that this illness directly causes. Caenorhabditis elegans is a useful model organism to research molecular targets changed by normal and pathological glucose concentrations because of its short lifespan, simple genome modification process, and simple insulin receptor system (daf-2). For instance, curcumin treatment was shown to alleviate diabetic neuropathy, a micro-vascular disorder primarily brought on by oxidative damage and inflammation. *C. elegans* are currently being used to study this

condition by looking at their morphology and physiology. When curcumin was utilized, the negative consequences of long-term diabetes, such as diabetic retinopathy, nephropathy, and cardiomyopathy, decreased considerably. Using a model of *C. elegans* with impaired glucose metabolism, it was demonstrated that curcumin may prevent and alleviate these consequences, which lower net stress levels, A subsequent mutagenesis increase in stress defense is also induced by an increase in ROS. *C. elegans* given curcumin (10, 25, and 50 μM) had less fat deposition and a lower body size (width) than the control, without changing feeding behavior (Yue *et al.*, 2021)

Conclusion

Diabetes-related chronic hyperglycemia produces harmful reactive oxygen species (ROS), which can result in oxidative stress and a variety of problems. Because of its preserved metabolic pathways, *C. elegans* becomes an invaluable model for researching the problems of diabetes. Elevated ROS levels in glucose-exposed worms mimic diabetic neuropathy. Studies demonstrated that curcumin treatment significantly reduces ROS levels and neuronal degeneration in glucose-stressed *C. elegans,* mirroring improvements in lifespan and movement. Compared to established drugs like metformin or resveratrol, curcumin exhibits a superior safety profile with additional anti-inflammatory benefits. This positions it as a potentially safer and more well-rounded therapeutic option. *C. elegans* based studies pave the way for further investigation of curcumin's efficacy in human diabetic models. Its safety, affordability, and promising antioxidant properties make it a compelling candidate for managing diabetes and its associated complications.

References

Aggarwal, B. B., & Harikumar, K. B. (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *International Journal of Biochemistry and Cell Biology, 41*(1), 40–59. https://doi.org/10.1016/j.biocel.2008.06.010

Amalraj, A., Varma, K., Jacob, J., Divya, C., Kunnumakkara, A. B., *et al*. (2017). A Novel Highly Bioavailable Curcumin Formulation Improves Symptoms and Diagnostic Indicators in Rheumatoid Arthritis Patients: A Randomized, Double-Blind, Placebo-Controlled, Two-Dose, Three-Arm, and Parallel-Group Study. *Journal of Medicinal Food*, *20*(10), 1022–1030. https://doi.org/10.1089/jmf.2017.3930

Bonomini, F., Rodella, L. F., & Rezzani, R. (2015). Metabolic syndrome, aging and involvement of oxidative stress. *Aging and Disease*, *6*(2), 109–120. https://doi.org/10.14336/AD.2014.0305

Daugherty, D. J., Marquez, A., Calcutt, N.A., & Schubert, D. (2018). A novel curcumin derivative for the treatment of diabetic neuropathy. *Neuropharmacology*, *129*, 26–35. https://doi.org/10.1016/j.neuropharm.2017.11.007

De Castro, J. M. (2004). The Time of Day of Food Intake Influences Overall Intake in

Humans. *Journal of Nutrition, 134*(1), 104–111. https://doi.org/10.1093/jn/134.1.104

Duvoix, A., Blasius, R., Delhalle, S., Schnekenburger, M., Morceau, F., *et al.* (2005). Chemopreventive and therapeutic effects of curcumin. *Cancer Letters, 223*(2), 181–190. https://doi.org/10.1016/j.canlet.2004.09.041

Garcia, A. M., Ladage, M. L., Dumesnil, D. R., Zaman, K., Shulaev, V., *et al.* (2015). Glucose induces sensitivity to oxygen deprivation and modulates insulin/IGF-1 signaling and lipid biosynthesis in *Caenorhabditis elegans*. *Genetics, 200*(1), 167–184. https://doi.org/10.1534/genetics.115.174631

Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. (2008). Curcumin as "Curecumin": From kitchen to clinic. *Biochemical Pharmacology, 75*(4), 787–809. https://doi.org/10.1016/j.bcp.2007.08.016

González, P., Lozano, P., Ros, G., & Solano, F. (2023). Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections. *International Journal of Molecular Sciences, 24*(11). https://doi.org/10.3390/ijms24119352

Hatcher, H., Planalp, R., Cho, J., Torti, F. M., *et al.* (2008). Curcumin: From ancient medicine to current clinical trials. *Cellular and Molecular Life Sciences, 65*(11), 1631–1652. https://doi.org/10.1007/s00018-008-7452-4

Herndon, L. A., Schmeissner, P. J., Dudaronek, J. M., Brown, P. A., Listner, K. M., *et al.* (2002). Stochastic and genetic factors influence tissue-specific decline in aging *C. elegans. Nature, 419*(6909), 808–814. https://doi.org/10.1038/nature01135

Ireson, C., Orr, S., Jones, D. J. L., Verschoyle, R., Lim, C. K., *et al.* (2001). Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Research, 61*(3), 1058–1064.

Jaruga, E., Salvioli, S., Dobrucki, J., Chrul, S., Bandorowicz-Pikuła, J., *et al.* (1998). Apoptosis-like, reversible changes in plasma membrane asymmetry and permeability, and transient modifications in mitochondrial membrane potential induced by curcumin in rat thymocytes. *FEBS Letters, 433*(3), 287–293. https://doi.org/10.1016/S0014-5793(98)00919-3

Larasati, Y. A., Yoneda-Kato, N., Nakamae, I., Yokoyama, T., Meiyanto, E., *et al.* (2018). Curcumin targets multiple enzymes involved in the ROS metabolic pathway to suppress tumor cell growth. *Scientific Reports, 8*(1). https://doi.org/10.1038/s41598-018-20179-6

Lee, S. J., Murphy, C. T., & Kenyon, C. (2009). Glucose Shortens the Life Span of *C. elegans* by Downregulating DAF-16/FOXO Activity and Aquaporin Gene Expression. *Cell Metabolism, 10*(5), 379–391. https://doi.org/10.1016/j.cmet.2009.10.003

Lee, W.-H., Loo, C.-Y., Bebawy, M., Luk, F., Mason, R., *et al.* (2013). Curcumin and its Derivatives: Their Application in Neuropharmacology and Neuroscience in the

21st Century. *Current Neuropharmacology, 11*(4), 338–378. https://doi.org/10.2174/1570159x11311040002

Lithgow, G. J., White, T. M., Melov, S., & Johnson, T. E. (1995). Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proceedings of the National Academy of Sciences of the United States of America, 92*(16), 7540–7544. https://doi.org/10.1073/pnas.92.16.7540

Menon, V. P., & Sudheer, A. R. (2007). Antioxidant and anti-inflammatory properties of curcumin. *Advances in Experimental Medicine and Biology, 595*, 105–125. https://doi.org/10.1007/978-0-387-46401-5_3

Muñoz, M. J., & Riddle, D. L. (2003). Positive selection of *Caenorhabditis elegans* mutants with increased stress resistance and longevity. *Genetics, 163*(1), 171–180. https://doi.org/10.1093/genetics/163.1.171

Pinkston, J. M., Garigan, D., Hansen, M., & Kenyon, C. (2006). Mutations that increase the life span of *C. elegans* inhibit tumor growth. *Science, 313*(5789), 971–975. https://doi.org/10.1126/science.1121908

Queen, B., & Tollefsbol, T. (2010). Polyphenols and Aging. *Current Aging Science, 3*(1), 34–42. https://doi.org/10.2174/1874609811003010034

Reusch, J. E. B. (2003). Diabetes, microvascular complications, and cardiovascular complications: what is it about glucose? *Journal of Clinical Investigation, 112*(7), 986–988. https://doi.org/10.1172/jci19902

Schulz, T. J., Zarse, K., Voigt, A., Urban, N., Birringer, M., *et al.* (2007). Glucose Restriction Extends *Caenorhabditis elegans* Life Span by Inducing Mitochondrial Respiration and Increasing Oxidative Stress. *Cell Metabolism, 6*(4), 280–293. https://doi.org/10.1016/j.cmet.2007.08.011

Stiernagle. (2018). WormBook. *WormBook*. Retrieved January 6, 2024, from http://www.wormbook.org/

Teshiba, E., Miyahara, K., & Takeya, H. (2016). Glucose-induced abnormal egg-laying rate in Caenorhabditis elegans. *Bioscience, Biotechnology and Biochemistry, 80*(7), 1436–1439. https://doi.org/10.1080/09168451.2016.1158634

Tomeh, M. A., Hadianamrei, R., & Zhao, X. (2019). A review of curcumin and its derivatives as anticancer agents. *International Journal of Molecular Sciences, 20*(5). https://doi.org/10.3390/ijms20051033

Trachootham, D., Zhang, W., & Huang, P. (2009). Oxidative stress and drug resistance in cancer. *Drug Resistance in Cancer Cells*, 137–175. https://doi.org/10.1007/978-0-387-89445-4_7

Yue, Y., Hao, G., Cho, J., & Park, Y. (2021). Curcumin reduced fat accumulation in *Caenorhabditis elegans*. *Current Research in Food Science, 4*, 551–556. https://doi.org/10.1016/j.crfs.2021.08.005

Zhang, D. W., Fu, M., Gao, S. H., & Liu, J.L. (2013). Curcumin and diabetes: A systematic review. *Evidence-Based Complementary and Alternative Medicine*, *2013*(1), 636053 https://doi.org/10.1155/2013/636053

Cite this Article:

Rade, M., Abbas, S., Nair, S. (2023). Evaluation of curcumin as a protective agent for *Caenorhabditis elegans* exposed to high glucose diet: an insight. *SCRIBE*, *4*(1), 26-35.

Swimming with Anxiety: Bridging the Gap in Anxiety Research through Animal Models

Irshikaa Sharma, Navin Chawathe, Tressa Jacob
Departmemt of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding authors: Tressa Jacob, Departmemt of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai.
Email: tressa.jacob@sophiacollege.edu.in (Current email id: tressa@somaiya.edu)

Abstract

Anxiety is a common psychological condition characterized by excessive fear in everyday situations. Understanding the neurobiology of anxiety is crucial for developing effective diagnostic tools and treatments. Animal models offer valuable insights into the underlying mechanisms of anxiety and provide a platform for drug discovery and development. There are various behavioral tests that have been used to evaluate the effects of chronic stressors. Few of the physiological changes associated with anxiety are restlessness and altered exploratory behavior. These parameters are exhibited by zebrafish in response to Schrekstoff exposure. Schreckstoff (German for “scary stuff”) - is released by the skin cells of wounded fish upon predatory attack. It induces anxiety-like responses in the rest of the fish in the shoal, triggering a heightened state of vigilance and anti-predatory behavior. Schreckstoff has majorly been studied in the Ostariophysi order of fishes but it has also been documented in other animals such as sea urchins and sea slugs. Here we present an overview of the tests used to study anxiety in model organisms and further explain the known pathway of Schreckstoff perception in zebrafish.

Anxiety: Its Perception and Response

In humans, intense, excessive and persistent worry and fear about normal everyday situations for no obvious reasons is known as anxiety. Anxiety is better termed as GAD - Generalized anxiety disorder which is a spectrum of disorders. Anxiety is the emotional response to the prospect of a future threat. It is commonly related with muscular tension and alertness in preparation for future danger, as well as cautious or avoidant behaviors. Anxiety can be of various types such as, visual anxiety which is caused when the anxiety causing stimulus is visible, odor anxiety which is caused when the anxiety causing stimulus is sensed by the olfactory system, mechanical anxiety where the anxiety causing stimulus is felt by the organism.

Approximately one billion people worldwide suffer from anxiety and much more go undiagnosed. Various diagnoses and treatments for anxiety exist, many of which are not globally available and economically affordable. We need better ways to diagnose anxiety and make anxiety treatments and medications accessible. Therefore, rigorous research in the field of neuropsychopharmacology is necessary. Due

to restrictions in conducting research in humans, animal models are used for this purpose. These are non-man species that are used in medical research to stimulate aspects of a certain disease that affects humans. Model organisms have the benefit of being responsive to changes that are impossible in experiments with humans. Animal models with high genetic similarities with humans like rats, mice, zebrafish and even monkeys are used to study anxiety.

It's not only humans that experience anxiety and fear. Like joy and grief, anxiety is another feeling that we share with other animals. According to scientists, animal models have been extremely helpful in contributing to scientific research studying multiple facets of behavior. Assays for screening anxiolytics utilizing various model organisms are in use now. The symptoms of anxiety reflect various coping mechanisms and physiological responses to anxiety-provoking stimuli (Chand & Marwaha, 2023). For instance, in mice anxiety is characterized by increased vigilance, immobility or hypoactivity, elevated heart rate, and abated feeding behavior. These responses serve as adaptive coping mechanisms with potential threats in their surroundings (Lezak *et al.,* 2017). Similar patterns can be observed in an anxious or fear-ridden zebrafish. Increased shoal cohesion: where zebrafish exhibit a stronger tendency to stay close to their social group for safety and reassurance. Faster swimming (darting) with spontaneous rapid turns: indicating heightened arousal and vigilance. Freezing bouts: heightened frequency and duration are observed as a defensive reaction to perceived threats. Anxious zebrafish also display decreased aggression, remarkably increased bottom dwelling, and seek refuge in areas perceived as safe. Understanding the similarities in behavioral reactions across species empower us with the knowledge to study and explore the underlying mechanisms of anxiety and fear (Egan *et al.,* 2009). As an example of the various approaches to studying anxiety in other model species, several behavioral tests have been devised to evaluate anxiety in rodents. Some of the commonly used tests are the elevated plus maze test: rodents are exposed to two open and two closed mazes to assess the time they spend in both. The forced swim test: rodents are put in a cylinder of water and observed for their escaping instinct.

A variety of tests are used to assess behavioral anxiety levels in rodents; specifically in rats and mice. One such test is the Geller-Seifter test: electrical stimulation is employed to induce anxiety, such as increased lever pressing frequency (Geller & Seifter, 1962). Elevated mazes: involve visual stimuli, where anxiogenic responses are indicated by increased time spent in enclosed or dark spaces (Lister, 1987). The Four-Plate Test: analyzes the frequency of transitions between plates via electrical stimulation; increased activity is indicative of anxiolytic effects (Boissier *et al.,* 1968). The Forced Swim Test: conducted in a water-filled cylinder, the evaluation assesses spatial behaviors such as striving and swimming, with anxiolytic effects observed as heightened striving and neutral swimming behaviors, contrasting with increased floating (Ferre *et al.,* 1994). These tests provide insights into anxiogenic factors and potential anxiolytic effects in rodent models.

Animals of various species exhibit multiple reactions, as conventional behavior that are referred to as the anxiety-like response (ALR). Moreover, there is a greater propensity to perceive ambiguous cues as dangerous. The ALR's role is to recognise and respond to dangers, notably those posed by predators. The ALR's physiological, cognitive, and behavioral alterations are ways to carry out this purpose. ALR prepares the body to respond by hyperventilating to oxygenate the blood, directing blood to the muscles, and sweating to cool the skin (Burman *et al.*, 2009).

While there is a plethora of model organisms available to study anxiety, choosing a model organism that is best for a certain study is of the utmost importance. Zebrafish is one such model organism in which anxiety can be studied, as it shows clear behavioral changes when exposed to anxiogenic or anxiolytic stimulus.

Anxiety in Zebrafish

Danio rerio, a fish native to the Indian subcontinent, commonly known as zebrafish, is a teleost of the family Cyprinidae. Eighty-two percent of genes associated with human diseases have a zebrafish counterpart. This helps to study and correlates the results as seen in the fish to those expected in humans (Howe *et al.*, 2013). The zebrafish central nervous system (CNS) has a comparable overall architecture, neuroanatomical characteristics, and cellular morphology to those of mammals (Kalueff *et al.*, 2014). Both in humans and zebrafish, the ventral telencephalon and lateral habenula are responsible for anxiety behavior suggesting that the regional function is conserved

There are varied behavioral responses to different stimuli causing anxiety, such as freezing, bottom dwelling, and irregular movements. Zebrafish have well-defined behavioral responses, as well as robust anxiety-like behaviors that are highly sensitive to acute or chronic stress (Khan *et al.*, 2017). Various tests help provide insights in anxiety behavior in zebrafish and the effects of anxiolytic interventions and underlying mechanisms.

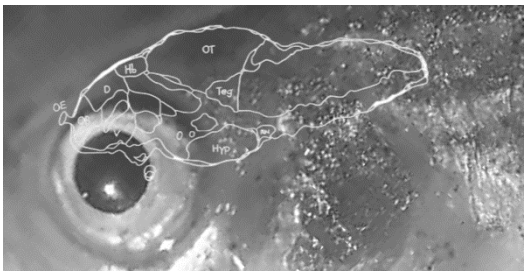
A few common tests that are used to study anxiety related behaviors in zebrafish are stated. The novel tank test assay and the scototaxis assay can together be used to analyze anxiety behavior. The former uses positioning in the tank whereas the latter uses light/dark preferences as a measure. Upon anxiety stimulus the fish spend most of their time showing freezing or erratic movements at the bottom of the tank (which could be a relatively darker area as compared to the upper brighter areas of the tank), which is reversed upon addition of an anxiolytic stimulus (Egan *et al.*, 2009 and Khan *et al.*, 2017). The other assays that can be used are open field test and shoaling/social preference test. The open field test assesses the time spent in the periphery of the field (more anxiolytic) as compared to the center of the field (less anxiolytic) (Stewart *et al.*, 2015). The shoaling/social test analyzes the shoal area, average interfish distance, and nearest/farthest neighbor distances. The closer the distances between two fish in the shoal indicate a more anxious behavior which

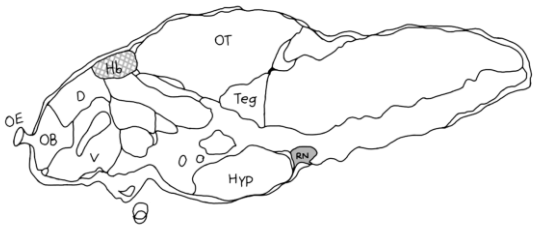
is reversed upon introduction of an anxiolytic (Kalueff *et al.,* 2014).

In zebrafish, Schreckstoff is an anxiety inducer. The zebrafish behavioral response to this inducer can be tested using a novel tank test assay. Schreckstoff was first reported in 1938 by Karl von Frisch. Schreckstoff is a German word that literally translates to “scary stuff”. Biochemically a major component of Schreckstoff is chondroitin sulfate, a type of glycosaminoglycan (Mathuru *et al.,* 2012). Biochemically a major component of Schreckstoff is chondroitin sulfate, a type of glycosaminoglycan (Mathuru *et al.*, 2012).

Schreckstoff - A Gripping Anxiety Inducer in Zebrafish

Once a fish in a shoal is wounded after a predatory attack, there is a release of Schreckstoff from the wounded skin (club cells of the epithelium) which serves as an alarm substance. This alerts the other fish in the shoal about potential danger around. As a response to this alarm substance, the other fish in the shoal show anxiety behaviors like darting movements, freezing of movement and dwelling at the bottom of the tank. A danger cue is a response produced by the "sender" (the wounded fish), which reacts to a threat and warns the “receivers” (the unwounded shoal fish) of the threat.

(a) 

(b) 

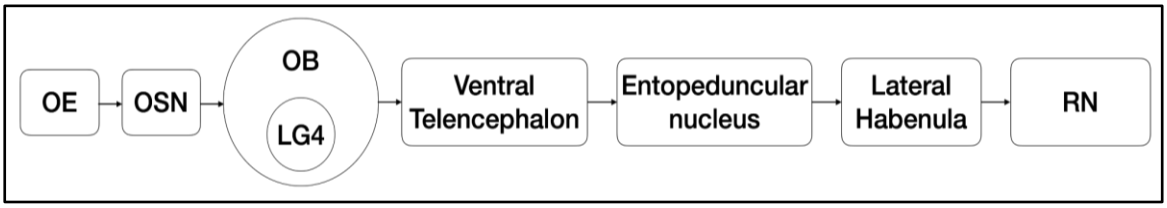
(c) 

Figure 1. Zebrafish brain regions and path involved in anxiety perception.

(a) Superimposition of the sagittal section of the brain in zebrafish.
(b) Sketch of sagittal section of the zebrafish brain. Image adapted and modified from http://zebrafishucl.org/forebrain-regions/habenulae.
(c) Schematic pathway representing the sensing and relay of Schreckstoff in the zebrafish brain.
OE: Olfactory epithelium, OSN: Olfactory Sensory Neurons, RN: Raphe Nucleus OB: Olfactory bulb, D: Dorsal, V: Ventral, Hb: Habenula, PTh: Parathyroid, PG: Pineal gland, Th: Thyroid, RN: Raphe nucleus, OT: Optic nerve, Hyp: Hypothalamus, Teg: Tegmentum, Ce: Cerebellum.

This chemical alarm signal is perceived by the olfactory system (Braubach *et al.*, 2012). When this is sensed by the olfactory epithelium receptors, they recognize the threat and display an anxiety response. Two glomeruli in the lateral and middle olfactory bulbs are known to respond to this disturbance (Mathuru *et al.*, 2012). Activity in the lateral olfactory bulb corresponds with activity in the lateral pallium and ventral telencephalon. Based on this information, it is proposed that telencephalic neurons participate in the anxiety circuit.

The lateral habenula, as indicated by a gray striped region in the brain sketch (figure 1 (a) and (b)) which projects to the raphe as indicated by a gray striped region in the brain sketch (figure 1 (a) and (b)), posterior tuberculum, and locus coeruleus, is most likely in charge of controlling these regions. Abrasion of the lateral habenula limits the reaction to Schreckstoff in adult zebrafish, underlining its significance as a hub in the response network. It is known that the entopeduncular nucleus provides input to the lateral habenula which in turn receives input from the telencephalon (Lal *et al.*, 2018). The figure 1 (c) shows a schematic representation of this pathway and the figure 1 (a) shows a superimposition of the brain sketch on the adult zebrafish.

Schreckstoff is also reported as an alarm substance in cephalopods, Ostariophysi (second-largest superorder of fish), such as minnows, catfish and quail fish (piranhas and tetras), and has also been documented in other species such as salmon (Actinopterygii) (Mathuru *et al.*, 2012). Along with various fishes, this chemical has also been found in insects such as the damselfly, sea urchins and sea slugs. More than just an alarm signal, Schreckstoff also acts as an immune response to the injured fish, repelling parasites and pathogens such as molds, trematodes and sunlight (Ángeles, 2012). This substance is one of a kind because it induces anxiety in the fish through chemical signals instead of mechanical ones. Initially, this chemical was proposed to be hypoxanthine-3-N-oxide (H_3NO) but further research revealed that H_3NO is not reliably found in the skin. This compound is a mixture of various components out of which the glycosaminoglycan - chondroitin sulfate is the major component which was identified using liquid chromatography/mass spectrometry (LC/MS).

Although while studying the behavior, Mathuru *et al.* (2012), showed that using only chondroitin sulfate was not enough to elicit the same intensity of the anxiety response. Recent studies have additionally identified and shown that the skin extract may also comprise of 24-methyl-5α-cholestane-3α,7α,12α,24,28-pentahydroxy 28-sulfate, a novel oxysterol sulfate, and 5α-cyprinol sulfate, in very small amounts which can induce the anxiety behavior (Li *et al.*, 2023).

Anxiety caused by such substances can be treated using anxiolytics. Anxiolytics are drugs that help loosen the effects of anxiety. These drugs usually work by acting on neurotransmitter systems and altering their functions. However, these existing anxiolytics are non-specific and generally cause a lot of side effects. Overtime, anxiolytics cause acclimatization that reduces its effects. Using the assays

mentioned, we can test novel anxiolytics before they are ready for the market, aiding us in creating better and more specific drugs to treat disorders like anxiety. The pathway for anxiety perception in zebrafish now having been mapped by Jesuthasan *et al.* (2021), can be used to better study anxiety. However, the neurotransmitters involved in this anxiety pathway are still unknown and should be established for an even better understanding.

References

Ángeles Esteban, M. (2012). An overview of the immunological defenses in fish skin. *International scholarly research notices*, *2012*(1), 853470. http://doi.org/10.5402/2012/853470

Boissier, J. R., Simon, P., & Aron, C. (1968). A new method for rapid screening of minor tranquillizers in mice. *European journal of pharmacology*, *4*(2), 145-151. http://doi.org/10.1016/0014-2999(68)90170-2

Braubach, O. R., Fine, A., & Croll, R. P. (2012). Distribution and functional organization of glomeruli in the olfactory bulbs of zebrafish (*Danio rerio*). *Journal of comparative neurology*, *520*(11), 2317 2339. http://doi.org/10.1002/cne.23075

Burman, O. H. P., Parker, R. M. A., Paul, E. S., & Mendl, M., T. (2009). Anxiety-induced cognitive bias in non-human animals. *Physiology & Behavior*, *98*(3), 345-350. http://doi.org/10.1016/j.physbeh.2009.06.012

Chand, S. P., & Marwaha, R. (2023). Anxiety. In StatPearls. StatPearls Publishing.Bookshelf ID: NBK470361 PMID: 29262212

Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J., Canavello, P. R., *et al.* (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*, *205*(1), 38–44. http://doi.org/10.1016/j.bbr.2009.06.022

Ferré, P., Teruel, F., Escorihuela, R. M., Garcia, E., Zapata, A., *et al.* (1994). Struggling and flumazenil effects in the swimming test are related to the level of anxiety in mice. *Neuropsychobiology*, *29*(1), 23-27.Geller, I., & Seifter, J. (1962). The effects of mono-urethans, di-urethans and barbiturates on a punishment discrimination. *Journal of Pharmacology and Experimental Therapeutics*, *136*(3), 284-288.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., *et al.* (2013). The Zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*(7446), 498–503. http://doi.org/10.1038/nature12111

Jesuthasan, S., Krishnan, S., Cheng, R. K., & Mathuru, A. (2021). Neural correlates of state transitions elicited by a chemosensory danger cue. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *111*, 110110. http://doi.org/10.1016/j.pnpbp.2020.110110

Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in*

pharmacological sciences, *35*(2), 63–75. http://doi.org/10.1016/j.tips.2013.12.002

Khan, K. M., Collier, A. D., Meshalkina, D. A., Kysil, E. V., Khatsko, S. L., *et al.* (2017). Zebrafish models in neuropsychopharmacology and CNS drug discovery. *British journal of pharmacology*, *174*(13), 1925–1944. http://doi.org/10.1111/bph.13754

Lal, P., Tanabe, H., Suster, M. L., Ailani, D., Kotani, Y., *et al.* (2018). Identification of a neuronal population in the telencephalon essential for fear conditioning in zebrafish. *BMC biology*, *16*, 1-18. http://doi.org/10.1186/s12915-018-0502-y

Lezak, K. R., Missig, G., & Carlezon, W. A. (2017). Behavioral methods to study anxiety in rodents. *Dialogues in Clinical Neuroscience*, *19*(2), 181–191. https://doi.org/10.31887/dcns.2017.19.2/wcarlezon

Li, Y., Yan, Z., Lin, A., Yang, X., Li, X., *et al.*, (2024). Epidermal oxysterols function as alarm substances in zebrafish. *Iscience*, *27*(5). http://doi.org/10.1016/j.isci.2024.109660

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology*, *92*, 180-185.

Mathuru, A. S., Kibat, C., Cheong, W. F., Shui, G., Wenk, M. R., *et al.* (2012). Chondroitin fragments are odorants that trigger fear behaviour in fish. *Current Biology*, *22*(6), 538-544. http://doi.org/10.1016/j.cub.2012.01.061

Panula, P., Chen, Y. C., Priyadarshini, M., Kudo, H., Semenova, S., *et al.* (2010). The comparative neuroanatomy and neurochemistry of Zebrafish CNS systems of relevance to human neuropsychiatric diseases. *Neurobiology of disease*, *40*(1), 46–57. http://doi.org/10.1016/j.nbd.2010.05.010

Stewart, A. M., Gerlai, R., & Kalueff, A. V. (2015). Developing highER-throughput Zebrafish screens for in-vivo CNS drug discovery. *Frontiers in behavioral neuroscience*, *9*, 14. http://doi.org/10.3389/fnbeh.2015.0001

Cite this Article:

Sharma, I., Chawathe, N., Jacob, T. (2023). Swimming with anxiety: bridging the gap in anxiety research through animal models. *SCRIBE*, *4*(1), 36-42.

Neurodegenerative pathways: A review

Sonia Joshi, Mayur Gaikwad
Department of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Mr. Mayur Gaikwad, Department of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai. Email: mayur.gaikwad@sophiacollege.edu.in

Abstract

Neurodegeneration is a process that involves the progressive damage and loss of nerve cells which can cause the loss of cognitive function, memory and decision making. This phenomenon is a key aspect of many neurodegenerative diseases such as dementia (Alzheimer's disease), Parkinson's disease and ALS, all sharing similar cellular-level processes such as programmed cell death and the build-up of toxic proteins. Unfortunately, there is no cure for these diseases, and the available medications can only alleviate symptoms. Therefore, research focuses on understanding the mechanisms of the disease to improve the development of treatments. Genetics, early symptoms, and tests for identifying the conditions are also core research objectives. Studying neurodegeneration is important because of the increasing occurrences of neurological disorders in the country. Neurodegenerative diseases are characterized by the degeneration of neurons, leading to impaired movement, memory loss, mood changes, and impaired talking, among other symptoms. These diseases have a significant social and financial burden and are predicted to affect a larger proportion of the population by the end of the current decade. Most of the current therapies only treat symptoms, and there are limited therapies available to treat neurodegenerative diseases. Identifying drug targets for these diseases is crucial, and novel assays that study neurodegeneration processes have made it possible to test multiple experimental conditions and drugs in a short time. Understanding the initiating factors that trigger these diseases is also important to develop disease-modifying therapies.

Neurodegeneration

Neurodegeneration is the loss of neuronal function over time and synaptic plasticity that cause cognitive decline as well as symptoms of dementia. It is an umbrella term to explain the deleterious physiological state of the brain during conditions like dementia, Alzheimer's disorder (AD), Parkinson's disorder (PD), Huntington's disease, Amyotrophic Lateral Sclerosis (ALS), Bell's Palsy, Spinal muscular atrophy and Ataxia. However, ALS, AD and PD are considered to be the major types of neurodegenerative disorders. Neurodegenerative disorders are characterized by increasing symptoms with progressing age and the occurrence of these disorders worldwide is expected to rise with the increasing life expectancy. Despite the severity of the issue, there are now no effective medicines that can stop or at least delay the growth of most neurodegenerative

disorders because it is still unclear what causes them. The pathogenesis of several neurodegenerative disorders may overlap at various molecular levels and pathways that eventually result in cell death, according to mounting evidence. It is well known that many contributing factors, such as inflammatory processes, oxidative damage, mitochondrial dysfunction, pathogenic proteins, apoptosis, and autophagy dysfunction, are not exclusively linked to any specific neurodegenerative disorder and have been identified in both sporadic and familial forms (Ramanan *et al.*, 2013; Ruffini *et al.*, 2020).

The pathophysiology of neurodegeneration is thought to be heavily influenced by the interdependence and close relationship between oxidative stress and inflammation. Because of this, it is essential for neuroprotection to maintain the redox balance, which is dependent on the production and removal of free radicals. Similarly, it is well recognized that exposure to many environmental variables, including pesticides, increases the incidence of age-related neurodegeneration (Cannon and Greenamyre, 2011; Baltazar *et al.*, 2014). The most common neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, can also be linked to alcohol dependence, as prolonged alcohol consumption helps to produce reactive oxygen species, which in turn triggers a neuroimmune response and neural cell death (Kamal *et al.*, 2020). While it is often feasible to avoid alcohol consumption over long periods of time and so prevent diseases linked with neuronal loss, it is far more challenging to do so when it comes to polluted air. This is especially true in light of recent scientific research. Inflammation along with free radical generation (ROS) are mentioned as potential mechanisms that can be linked to death of neurons. Few reviews have published evidence demonstrating the role of air pollution as a contributing factor to the onset and progression of neurodegeneration (Jankowska-Kietyka *et al.*, 2021).

Inflammation is a key element in the development of neurodegenerative illnesses, and more studies in recent years has made this increasingly clear. Aging, genetic, lifestyle, and environmental variables interact in complicated ways to cause neurodegenerative diseases, which impact a variety of cell types. For instance, Alzheimer's illness has been linked to spreading inflammation. Studies have demonstrated that "dampening inflammation," by hindering the activity of cathepsin, may lead to a good strategy in order to delay the onset of AD. This is because there is evidence that inflammatory crosstalk occurs between the periphery and the central nervous system via the blood-brain barrier (BBB) (Ni & Wu, 2021).

Role of inflammation in neurodegeneration - Inflammatory pathways in Alzheimer’s disease

One of the major players in the inflammatory response in neurodegeneration is microglia, they act as protective cells for the central nervous system. Microglia can be activated in response to injury or disease, and they can produce a wide range of cytokines and chemokines that can either promote or inhibit inflammation. In neurodegenerative diseases,

microglia activation is often prolonged and dysregulated, leading to chronic inflammation that can exacerbate neuronal damage and accelerate disease progression. Microglia are otherwise present in a deactivated state in order to produce an anti-inflammatory response. Microglia adopt an activated phenotype in response to the spread of pathogen or tissue damage, which promotes an inflammatory response that boosts the immune system and initiates tissue repair. When the infection gets better or the tissue damage is repaired, this reaction usually subsides on its own.

The absence of regular resolution mechanisms or consistent presence of an inflammatory stimulus are implied by persistent inflammation that leads to tissue disease. Environmental variables or the development of endogenous components (such as protein aggregates) that the immune system interprets as "foreign" or "danger" signals might provide a continuous stimulation. Normal resolution mechanisms may be overcome by inflammatory reactions that create input loops. Despite the fact that some inflammatory stimuli have positive effects, for instance elimination of waste, dead cells and wear and tear of tissues, uncontrolled inflammation can lead to the production of substances (toxic to the nervous system) that worsen underlying disease states. Role of inflammation in the pathogenesis of AD, PD, ALS, and MS is comparable however it is quite contrasting (Streit *et al.*, 2002; Glass *et al.*, 2010). Typically, pattern recognition receptors that bind to pathogen-associated molecular patterns start inflammatory responses to infectious pathogens. The Toll-like receptors (TLRs), which recognise a wide range of pathogen-associated chemicals that are not present in the host, are an example of one form of pattern recognition receptor.

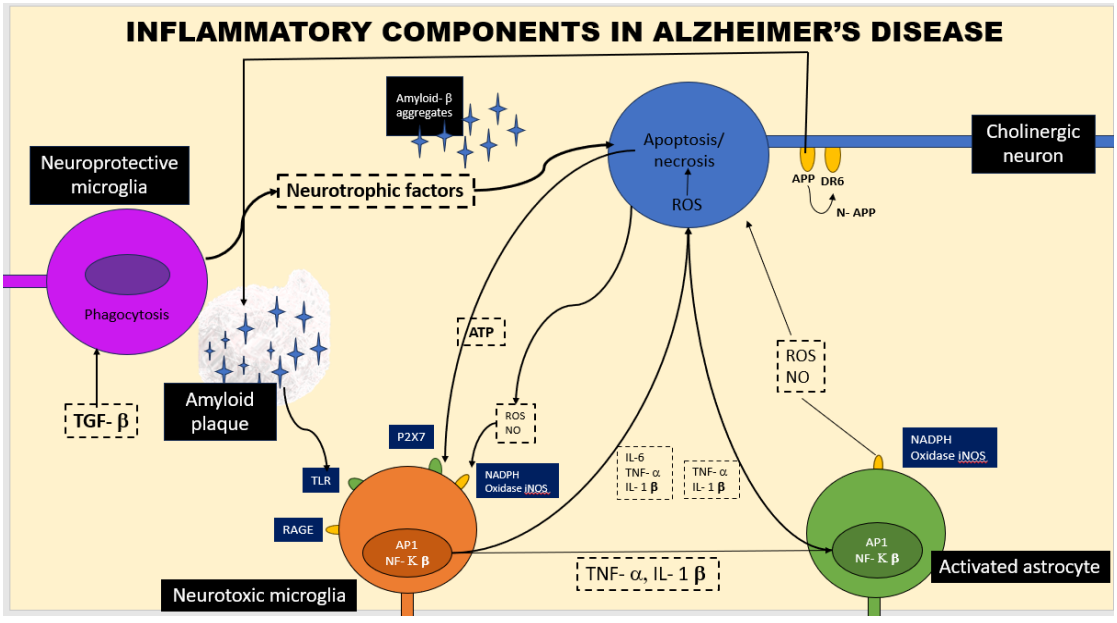


Figure 1. Factors affecting the inflammatory pathways in Alzheimer's Disease - Source: Glass *et al.*, 2010

Although these receptors are expressed on a wide variety of cell types, macrophages and microglia, which are key players in innate immune responses, have the highest levels of expression. More recently, it has been discovered that pattern recognition receptors can also react to endogenously produced chemicals, such as those generated by necrotic cells (danger signals) and those that could result from pathogenic pathways. TLR4 polymorphisms are linked to a number of age-related diseases in humans, such as atherosclerosis, type 2 diabetes, and rheumatoid arthritis. It is unclear from this if these receptors are involved in the inflammatory processes that are connected to neurodegenerative diseases. Roles of TLR2 and TLR4 have been published and studied in many animal models based on inflammatory responses (Balistreri *et al.*, 2009). Purinergic receptors, which are also present on microglia and astrocytes, have the ability to react to ATP released from cells after cell death, trauma, or ischemia, apart from pattern recognition receptors (Di Virgilio *et al.*, 2009). Furthermore, oxidized proteins, lipids, and apoptotic cells have been shown to be taken up by a number of "scavenger receptors" expressed by microglia and astrocytes. These receptors may also contribute to cell signaling (Husemann *et al.* 2002).

Binding of pattern recognition receptors causes the signaling pathways that control various transcriptional and posttranscriptional processes to be activated. It assists in NF-κB, AP-1, and interferon regulatory factor (IRF) family signal activation and transcription. Signals from microglia are recognized by TLR4 receptors, and inflammation inducing molecules such as TNF-α and IL-1β which stimulate astrocytes to induce further inflammation in surrounding tissues. The cleavage of APP produces an amyloid peptide, which combines with RAGE and Toll-like receptors (TLRs) to trigger microglia.

Reactive oxygen species (ROS) are produced as a result of the activation of these receptors, which also promotes the development of inflammatory mediators such as cytokines.

Inflammatory pathways in Parkinson's disease

Similar to AD, Parkinson's Disease (PD) is another disorder concerning the accumulation of proteinaceous lesions in the brain called Lewy bodies and Lewy neurites. These Lewy bodies are aggregates of misfolded α-synuclein proteins which causes defective motor as well as non motor symptoms in the affected individual due to functional loss in the dopaminergic neurons in the substantia nigra and few other parts of the brain. Apart from loss of dopaminergic neurons, the number of active microglia and astroglia contributing towards inflammation are allegedly on the rise. According to a number of lines of research, inflammatory mediators like ROS, NO, TNF-α, and interleukin (IL)-1 produced by non-neuronal cells like microglia affect how quickly neurons die in Parkinson's disease (PD) (Hirsch and Hunot, 2009). (Figure 2). Studies of LPS-mediated neurotoxicity give proof that inflammatory reactions originating from non-neuronal cells are sufficient to result in the death of dopaminergic neurons (Castano *et al.* 1998). Prior to the death of

dopaminergic neurons after LPS injection into the mouse brain, inflammatory mediator levels, such as COX-2 and iNOS, are elevated (Hunter *et al.* 2007). TLR4, the primary Lipopolysaccharide receptor, is preferentially expressed on microglia in comparison to astrocytes (Kim *et al.*, 2000), while it is barely detectable or nonexistent on neurons. This discovery is supported by the observation that when tested in a tissue culture medium, microglia respond to Lipopolysaccharide significantly more quickly than astrocytes while neurons exhibit almost no response (Saijo *et al.*, 2009). On the other hand, cultures of astrocytes and neurons are more susceptible to the neurotoxic effects of conditioned media derived from LPS-treated microglia. The conclusion that microglia are the main first responders to LPS and create intermediaries like TNF-α and IL-1 that trigger astrocytes is most consistent with the division of the various cell type elements in the mixed culture system.

The synthesis of substances by astrocytes and activated microglia may, in turn, enhance neurotoxicity. It's interesting that these factors are more toxic to dopaminergic neurons than to other types of neurons, which begs the question of whether the different sensitivity of neurons in PD is caused by factors that are relatively specific to dopaminergic neurons or whether dopaminergic neurons are more sensitive to all neurotoxic factors (Saijo *et al.*, 2009). The loss of dopaminergic neurons in the substantia nigra of the midbrain and the appearance of intracellular aggregates made up of masses of the α-synuclein protein, or Lewy bodies, are two significant neuropathological indications of Parkinson's disease (PD).

Aggregates of α-synuclein also create Lewy bodies and intermediate-state oligomers, which are discharged from neurons and activate microglia through pathways distinct from Toll-like receptor (TLR) signaling. As a result, NF-B is activated, and proinflammatory mediators and reactive oxygen species (ROS) are produced. The primary (but not the only) neurons that die in PD are dopaminergic neurons of the substantia nigra, which are directly impacted by these variables. In a positive feedback loop, these substances also stimulate microglia, which in turn stimulate more microglia, amplifying the inflammatory response. Compounds made by astrocytes and microglia work together to cause neurotoxicity. The death of dopaminergic neurons in the substantia nigra is caused by an inflammatory response brought on by bacterial lipopolysaccharide (LPS), which principally acts via TLR4 produced by microglia (Glass *et al.*, 2010). Through blocking NF-κB target genes, the transcription factor NURR1 reduces inflammatory reaction in microglia and astrocytes.

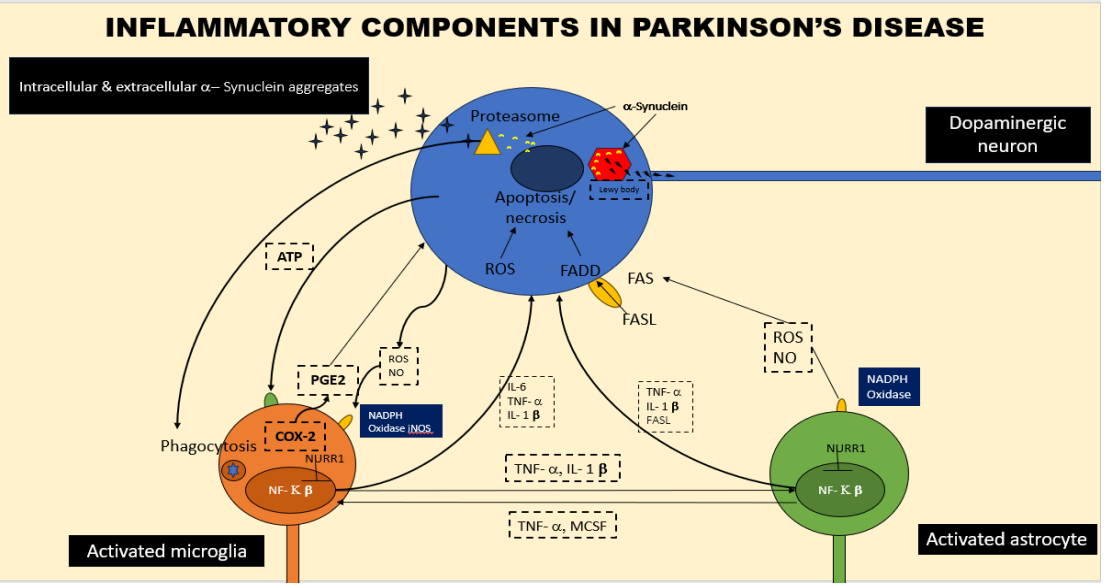


Figure 2. Factors affecting the inflammatory pathways in Parkinson's Disease - Source: Glass *et al.*, 2010

Inflammatory pathways in Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) or Lou Gehrig's disease is a neurodegenerative disorder associated with defective motor neurons majorly affecting the brainstem and spinal cord. It is characterized by loss of motor function, muscle wastage and increased spasticity. Respiratory complications due to deleterious effects on diaphragm muscles is often observed in ALS leading to fatality. Accumulation of microglia and astrocytes is a characteristic feature of ALS. Glial activation is associated with production of ROS, COX-2 and cytokines like IL-1β, TNF-α, and IL-6 along with elevated expression of major histocompatibility complex and complement receptors on microglia specifically in primary motor cortex (McGeer *et al.*, 2002). The main causes of motor neuron loss in ALS are yet unknown. Deletion of either *SOD1*, *TARBDP* or *FUS/TLS* genes which are associated with ALS, alone may not affect the course of the disease in SOD1 mutant animals, despite the fact that IL-1 and TNF- α are neurotoxic in vitro (Gowing *et al.*, 2006, Nguyen *et al.*, 2001). From a broader outlook, motor neuron degeneration in ALS is the result of a number of potentially confounding variables, but IL-1 and TNF-α do not materialize to contribute considerably to the disease. In this case, the absence of just one effector molecule is insufficient to change the disease's manifestation. Based on the observation that motor neurons isolated from transgenic SOD1 mutant mice were more susceptible to Fas- or NO-triggered cell death than wild-type motor neurons, a motor neuron-specific death pathway has been proposed for ALS (Raoul *et al.*, 2002).

The intracellular part of Fas attracts the adaptor protein FADD when FasL binds to the Fas receptor, which subsequently initiates a caspase reaction that results in motor neuronal death. ALS-dependent motor

neuron degeneration has also been linked to the p75 neurotrophin receptor, a constituent of the same receptor family. Particularly, motor neurons expressing p75 were killed by nerve growth factor released by SOD1 mutant astrocytes via a process involving the production of NO and peroxide (Pehar *et al.*, 2004). Although nearby glial cells are likely involved in the pathogenesis of ALS, even if motor neurons are the primary cells damaged (Clement *et al.*, 2003, Yamanaka *et al.*, 2008). Inflammation may be involved in the presymptomatic stage of the disease, according to gene expression profiling, which suggests that inflammatory cascades are initiated prior to the beginning of motor neuron degeneration (Vargas *et al.*, 2008).

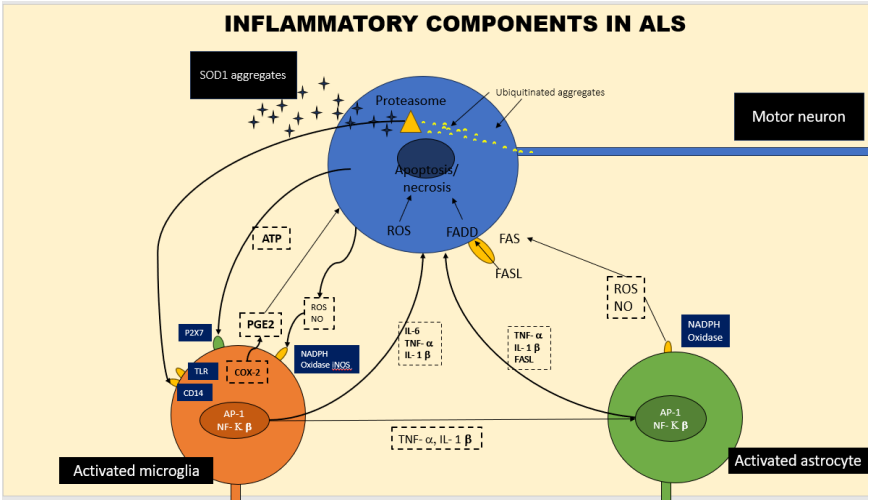


Figure 3. Factors affecting the inflammatory pathways in ALS - Source: Glass *et al.*, 2010

Role of oxidative stress in neurodegeneration

Neurodegeneration is quite often related to age-related oxidative stress on nucleic acids, especially RNA. Diseases like AD, PD as well as ALS are associated with RNA oxidative damage. It has been established that modification in RNA can be observed in coding as well as non-coding regions.

Thus, due to oxidative damage, abnormal production of microRNA and proteins has been observed. If this aberrant function is not lethal it may lead to occurrence of neurodegenerative disorders. Biomarkers of oxidative damage i.e., the production of aberrant products from the oxidative stress on the RNA/DNA play a role in not only the onset of neurodegenerative diseases but also the pathogenesis of the same. Products of oxidative stress, mainly reactive oxygen species (ROS) or reactive nitrogen species (RNS), can be detected in blood, plasma, serum, cerebrospinal fluid (CSF), urine, and saliva. Overproduction of ROS and/or a decline in antioxidant protection may be contributing factors to the neurodegeneration seen in AD.

When Aβ deposition interacts with mitochondria or microglial surface receptors, ROS levels can rise as a direct result. The major producer of ROS, mitochondria, are also a target of ROS and have been linked to

changes in energy metabolism in AD patients. ROS may also be thought of as a second messenger because it can activate several signaling channels or trigger the production of antioxidant enzymes (Nrf2). In the brain of AD patients, oxidative damage to proteins, lipids, and mitochondrial DNA and RNA has been found. Iron has been linked to ferroptosis, and elevated amounts of iron and other metals may be to blame for the generation of ROS. The role of altered Calcium ion influx and the ensuing mitochondrial dysfunction in oxidative damage is also significant. Cognitive impairment in AD is brought on by oxidative damage to synapses. Reduced levels of several lipids have been linked to cognitive impairment in AD, pointing to their potential use as biomarkers or in treatment interventions. Oxidative stress is characterized by the upset of the balance between reactive oxygen and/or nitrogen species (ROS/RNS) levels and antioxidant defenses.

Neighboring cells and tissues may become oxidatively damaged as a result of elevated amounts of ROS and/or RNS and low antioxidant levels. Numerous oxidative damage products are measured in the brains of people with AD and preclinical AD (that is, evidence of Amyloid plaque and tau-deposition in the brain with no clinical disease symptoms), as well as patients with MCI (a mild clinical impairment without evidence of underlying pathology with biomarkers), and numerous studies have pointed to OS as an early contributor to the pathogenesis of neurodegeneration in AD (Butterfield *et al.*, 2019; Nunomura *et al.*, 2001; Buccellato *et al.*, 2021).

Loss of melanin-pigmented nigral neurons, dopamine depletion in the striatum, and the development of Lewy bodies are pathological features of Parkinson's disease (PD). Lewy bodies are filamentous intraneuronal inclusions that are eosinophilic, detergent-insoluble, and commonly positive for ubiquitin and synuclein.

The precise molecular mechanism of the pathogenesis is still unknown, despite the fact that several pieces of evidence point to little difference between the uncommon familial variants of PD and the more common sporadic types. Both sporadic and familial PD may be caused by a combination of genetic predisposition, mitochondrial dysfunction, oxidative damage, environmental variables, and other neurodegenerative illnesses. Synuclein's physiological role in maintaining synapses and plasticity is normal, however mutations that result in overexpressed synuclein are neurotoxic and cause apoptosis. Further data suggests that the conventional tauopathies (such as NFT development in AD) and synucleinopathies frequently experience illness overlap.

There is increasing evidence linking the accumulation of genetic variations in mitochondrial DNA to complex I deficiency or mitochondrial dysfunction in Parkinson's disease (PD). It is well known that the mitochondrial DNA codes for 13 proteins, mitochondrial transfer RNA, and ribosomal RNA, which includes electron transport chain subunits. Patients with PD have been documented to have point mutations or deletions in the mitochondrial DNA that codes for the Complex I subunit. Post-

mortem PD patient tissues have provided evidence that a deficiency in complex I of the mitochondrial electron-transport chain in the substantia nigra, leading to a 30–40% reduction in activity, might be the fundamental etiology of sporadic PD. Complex 1 disintegration, underproduction of specific complex 1 subunits, or self-inflicted oxidative damage could all be contributing factors to the decreased activity (Galloway *et al.*, 1992; Kikuchi *et al.*, 2002; Jenner, 2003; Shukla *et al.*, 2011).

Huntington disease (HD) is an extremely rare neurodegenerative condition of the central nervous system characterized by irrational movements, behavioral abnormalities, and mental health problems. Long projection neurons are lost in pathological HD, which causes the caudate nucleus, putamen, and globus pallidus to atrophy over time. An increase in the number of CAG repeats in the HD gene results in HD, a "trinucleotide repeat" disease. Repeats of 26 or fewer are regarded as normal, while 40 or more are associated with the appearance of illness. Although intermediate repeat counts, which range from 27 to 35, are not associated with the expression of illness, they may rise during paternal transmission and cause the disease in progeny.

Repeats of 36–39 are synonymous with a less pathogenic condition wherein some individuals develop HD and others do not. Because of an enlarged polyglutamine region, mutant HTT provides a dominant "gain of function" to the protein, which causes neurodegeneration. Several lines of evidence show that mitochondrial malfunction resulting in poor energy metabolism as one of the main effects of the gene expansion. Possible causes of lowered mitochondrial energy include an increase in free radical generation and an increase in oxidative damage. HD patients have poor catalase activity in skin fibroblast cultures, even though oxidative stress has less of an impact on HD than it does on other serious neurodegenerative disorders. Studies using mutant HTT-knock-in mouse embryos revealed a severe deficit in ATP generation and mitochondrial respiration (Shukla *et al.*, 2011; Myers *et al.*, 2004).

Role of autophagic pathways in neurodegeneration

Misfolded protein intracellular aggregates are a typical symptom of many neurodegenerative disorders. Depending on the condition, these aggregates contain various proteins and can be found in various cell types. In other cases, it has been discovered that the aggregates contain mutations in a specific protein. For instance, Huntington's disease (HD) and Parkinson's disease (PD) are caused by increased polyglutamine tracts and α-synuclein mutations, respectively.

In other cases, the aggregates' main protein species are not altered. While there are a variety of ways that these misfolded proteins might induce pathology, recent research has focused on the involvement of autophagy in these disorders as both a pathologic pathway and a therapeutic target. Cytoplasmic materials are transported to the lysosome by the autophagic breakdown process occurring inside the cell. Despite its simplicity, autophagy is known to play a variety of

physiological and pathological roles, some of which are intricate, according to recent research. The phases that comprise autophagy are degradation, utilization of degradation products, transport to lysosomes, and sequestration. Each of these phases may have a unique function. In addition, a variety of extracellular and intracellular triggers, such as hunger, hormonal or pharmaceutical therapy, bacterial infection, aggregated and misfolded proteins, and damaged organelles, can also cause autophagic vacuole creation as an adaptive response. A preliminary observation suggesting the potential for abnormal autophagy in AD was the build-up of autophagic vesicles in damaged neurons. While previously assumed to signify enhanced autophagy, more recent research reveals that this build-up is owing to defective autophagosome clearance. In addition to causing amyloid accumulation, neuronal loss, and lysosome pathology, Presenilin-1 and Presenilin-2 mutations also cause familial AD.

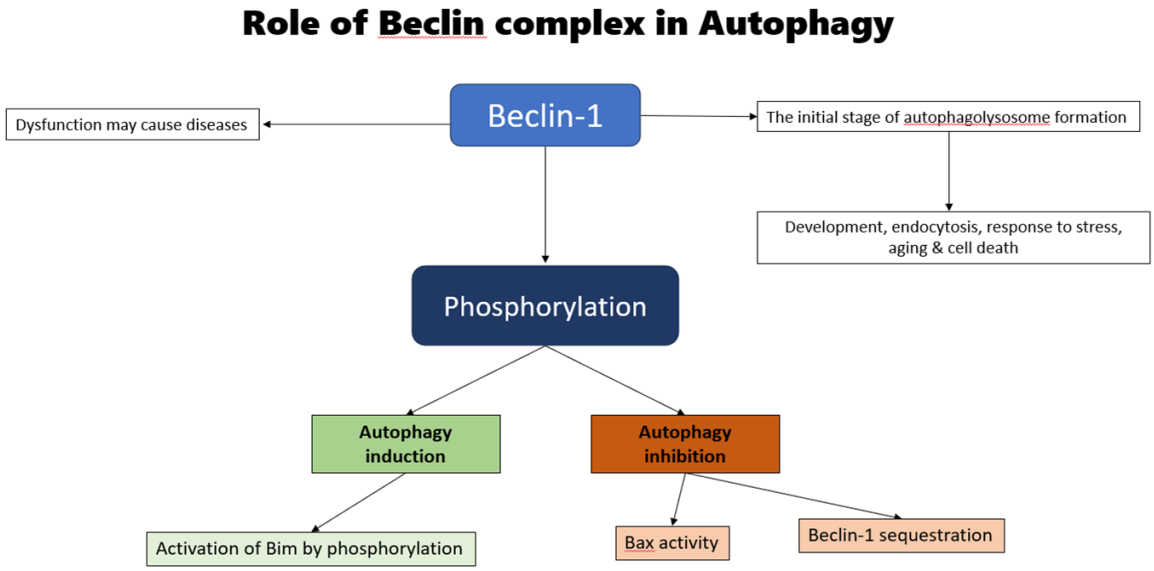


Figure 4. Role of Beclin complex in Autophagy; Source- Hu *et al.* 2020

In AD brain tissue, the autophagy gene BECN1, which codes for beclin 1, has lower mRNA levels. Caspase-3 can cleave beclin 1, which impairs the production of autophagosomes, and may be triggered in AD neurons. Mice overexpressing human APP showed autophagy disruption and increased pathology when bred with beclin 1 haploinsufficient mice. As a result of the lysosomal deficiencies, AD may be linked to errors in both autophagosome biogenesis and autophagosome degradation. This is because loss of beclin 1 activity decreases autophagosome formation (Pickford *et al.*, 2008; Lucin *et al.*, 2013; Frake *et al.*, 2013). The two important proteins that cluster in AD; tau and Amyloid beta, are autophagy substrates. Tau levels drop when autophagy is stimulated. On the other hand, mice's forebrains that lack autophagy due to provisional removal of the autophagy protein ATG7 develop phospho-tau accumulation that resembles a pre-tangle state. Although tau deletion does not stop the creation of inclusions, it does reverse neurodegeneration in such mice. It has been postulated that

autophagy is crucial for the metabolism of Amyloid beta, but it may also be crucial for Amyloid beta synthesis. It has been demonstrated that both APP and PS1 are present in the autophagic vesicles that build up in AD neurons (Luna-Muñoz *et al.*, 2007). Research on the roles played in mitophagy by Parkin RBR E3 ubiquitin protein ligase (PARK2, also known as Parkin) and PTEN-induced putative kinase 1 (PINK1, also known as PARK6) is arguably the most well-known connection between autophagy and familial types of Parkinson's disease (PD).

Parkin and PINK1 loss-of-function mutations cause juvenile-onset Parkinson's disease (PD) that is sporadic and autosomal-recessive, respectively. The breakdown of damaged mitochondria, known as mitophagy, is regulated by these proteins. When membrane potential is lost, PINK1 binds to injured mitochondria and activates Parkin, an E3 ubiquitin ligase that ultimately triggers mitophagy. The effects of these proteins' overexpression on mitochondrial clearance have been studied extensively in cell culture, but the implications of Parkin loss on mitophagy in living organisms have been debated.

In conclusion, a number of contributors play a role in neurodegenerative disorders. Though inflammation, autophagy and oxidative stress are observed as a common occurrence in most neurodegenerative disorders, the pathways that trigger these events and the cellular mechanisms involved for each disorder varies. In addition, the consequences of these occurrences have extremely varied pathological symptoms.

The importance of understanding the cascade of reactions that ultimately lead to the hallmarks presented in any neurodegenerative disorder would be the key to its prevention as well as treatment. Most neurodegenerative disorders stand to be incurable at this time and age, however the knowledge of the underlying mechanisms and understanding whether the mechanisms are the triggers or they are a result of some external detrimental stimuli would be paramount in learning about neurodegeneration as a whole. Till a decade ago, the number of neurological issues have been very limited in the country, however with the increasing number of neurodegenerative disorders globally as well, it is of great relevance to study about neurodegeneration in order to understand the etiology of widely occurring neurological disorders.

References

Balistreri, C. R., Colonna-Romano, G., Lio, D., Candore, G., Caruso, C. (2009). TLR4 polymorphisms and ageing: implications for the pathophysiology of age-related diseases. *Journal of clinical immunology, 29*, 406-415. http://doi.org/10.1007/s10875-009-9297-5

Baltazar, M. T., Dinis-Oliveira, R. J., de Lourdes Bastos, M., Tsatsakis, A. M., Duarte, J. A., *et al*. (2014). Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases-a mechanistic approach. *Toxicologyletters*, *230*(2), 85–103. https://doi.org/10.1016/j.toxlet.2014.01.039

Buccellato, F. R., D'Anca, M., Fenoglio, C., Scarpini, E., Galimberti, *et al.* (2021). Role of Oxidative Damage in Alzheimer's Disease and Neurodegeneration: From Pathogenic Mechanisms to Biomarker Discovery. *Antioxidants*, *10*(9), 1353. MDPI AG. Retrieved from http://dx.doi.org/10.3390/antiox10091353

Butterfield, D. A., & Halliwell, B. (2019). Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nature reviews. Neuroscience*, *20*(3), 148–160. https://doi.org/10.1038/s41583-019-0132-6

Cannon, J. R., & Greenamyre, J. T. (2011). The role of environmental exposures in neurodegeneration and neurodegenerative diseases. *Toxicological sciences: an official journal of the Society of Toxicology*, *124*(2), 225–250. https://doi.org/10.1093/toxsci/kfr239

Castano, A., Herrera, A. J., Cano, J., & Machado, A. (1998). Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *Journal of neurochemistry*, *70*(4), 1584-1592. http://doi.org/10.1046/j.1471-4159.1998.70041584.x

Clement, A. M., Nguyen, M. D., Roberts, E. A., Garcia, M. L., Boillee, S., *et al.* (2003). Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science*, *302*(5642), 113-117. http://doi.org/10.1126/science.1086071

Di Virgilio, F., Ceruti, S., Bramanti, P., & Abbracchio, M. P. (2009). Purinergic signalling in inflammation of the central nervous system. *Trends in neurosciences*, *32*(2), 79-87. http://doi.org/10.1016/j.tins.2008.11.003

Frake, R. A., Ricketts, T., Menzies, F. M., & Rubinsztein, D. C. (2015). Autophagy and neurodegeneration. *The Journal of clinical investigation*, *125*(1), 65-74. http://doi.org/10.1172/JCI73944

Galloway, P. G., Mulvihill, P., & Perry, G. (1992). Filaments of Lewy bodies contain insoluble cytoskeletal elements. *The American journal of pathology*, *140*(4), 809.

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell*, *140*(6), 918-934. https://doi.org/10.1016/j.cell.2010.02.016

Gowing, G., Dequen, F., Soucy, G., & Julien, J. P. (2006). Absence of tumor necrosis factor-α does not affect motor neuron disease caused by superoxide dismutase 1 mutations. *Journal of Neuroscience*, *26*(44), 11397-11402. http://doi.org/10.1523/JNEUROSCI.0602-06.2006

Hirsch, E. C., & Hunot, S. (2009). Neuroinflammation in Parkinson's disease: a target for neuroprotection? *The Lancet Neurology*, *8*(4), 382-397. https://doi.org/10.1002/ana.10481

Hu, Y. J., Zhong, J. T., Gong, L., Zhang, S. C., Zhou, S. H. (2020). Autophagy-Related Beclin 1 and Head and Neck Cancers.

OncoTargets and therapy, *13*, 6213–6227. https://doi.org/10.2147/OTT.S256072

Hunter, R. G., Bellani, R., Bloss, E., Costa, A., Romeo, R. D., *et al.* (2007). Regulation of CART mRNA by stress and corticosteroids in the hippocampus and amygdala. *Brain research*, *1152*, 234–240. https://doi.org/10.1016/j.brainres.2007.03.042

Husemann, J., Loike, J. D., Anankov, R., Febbraio, M., & Silverstein, S. C. (2002). Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. *Glia*, *40*(2), 195-205. http://doi.org/10.1002/glia.10148

Jankowska-Kieltyka, M., Roman, A., & Nalepa, I. (2021). The air we breathe: air pollution as a prevalent proinflammatory stimulus contributing to neurodegeneration. *Frontiers in Cellular Neuroscience*, *15*, 647643. https://doi.org/10.3389/fncel.2021.647643

Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *53*(S3), S26-S38. http://doi.org/10.1002/ana.10483

Roch, J. M., Masliah, E., Roch-Levecq, A. C., Sundsmo, M. P., Otero, D. A., *et al.* (1994). Increase of synaptic density and memory retention by a peptide representing the trophic domain of the amyloid beta/A4 protein precursor. *Proceedings of the National Academy of Sciences*, *91*(16), 7450-7454. http://doi.org/10.1073/pnas.91.16.7450

Kamal, H., Tan, G. C., Ibrahim, S. F., Shaikh, M. F., Mohamed, I. N., *et al.* (2020). Alcohol use disorder, neurodegeneration, Alzheimer's and Parkinson's disease: Interplay between oxidative stress, neuroimmune response and excitotoxicity. *Frontiers in Cellular Neuroscience*, *14*, 282. http://doi.org/10.3389/fncel.2020.00282

Kikuchi, A., Takeda, A., Onodera, H., Kimpara, T., Hisanaga, K., *et al.* (2002). Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiology of disease*, *9*(2), 244-248. http://doi.org/10.1006/nbdi.2002.0466

Kim, W. G., Mohney, R. P., Wilson, B., Jeohn, G. H., Liu, B., *et al.* (2000). Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *Journal of Neuroscience*, *20*(16), 6309-6316. https://doi.org/10.1523/JNEUROSCI.4849-06.2007.

Lucin, K. M., O'Brien, C. E., Bieri, G., Czirr, E., Mosher, K. I., *et al*. (2013). Microglial beclin 1 regulates retromer trafficking and phagocytosis and is impaired in Alzheimer's disease. *Neuron*, *79*(5), 873–886. https://doi.org/10.1016/j.neuron.2013.06.046

Luna-Muñoz, J., Chávez-Macías, L., García-Sierra, F., & Mena, R. (2007). Earliest stages of tau conformational changes are related to the appearance of a sequence of specific phospho-dependent tau epitopes in

Alzheimer's disease. *Journal of Alzheimer's disease*, *12*(4), 365–375. https://doi.org/10.3233/jad-2007-12410

McGeer, P. L., & McGeer, E. (2002). Inflammatory processes in amyotrophic lateral sclerosis. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*, *26*(4), 459-470. http://doi.org/10.1002/mus.10191

Myers R. H. (2004). Huntington's disease genetics. *NeuroRx: the journal of the American Society for Experimental NeuroTherapeutics*, *1*(2), 255–262. https://doi.org/10.1602/neurorx.1.2.255

Nguyen, L., Rigo, J. M., Rocher, V., Belachew, S., Malgrange, B., *et al.* (2001). Neurotransmitters as early signals for central nervous system development. *Cell and tissue research*, *305*, 187-202. http://doi.org/10.1007/s004410000343

Ni, J., & Wu, Z. (2021). Inflammation spreading: negative spiral linking systemic inflammatory disorders and Alzheimer's disease. *Frontiers in Cellular Neuroscience*, *15*. https://doi.org/10.3389/fncel.2021.638686

Nunomura, A., Perry, G., Aliev, G., Hirai, K., Takeda, A., *et al.* (2001). Oxidative damage is the earliest event in Alzheimer disease. *Journal of Neuropathology & Experimental Neurology*, *60*(8), 759-767. http://doi.org/10.1093/jnen/60.8.759

Pehar, M., Cassina, P., Vargas, M. R., Castellanos, R., Viera, L., *et al.* (2004). Astrocytic production of nerve growth factor in motor neuron apoptosis: implications for amyotrophic lateral sclerosis. *Journal of neurochemistry*, *89*(2), 464-473. http://doi.org/10.1111/j.1471-4159.2004.02357.x

Pickford, F., Masliah, E., Britschgi, M., Lucin, K., Narasimhan, R., *et al.* (2008). The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *The Journal of clinical investigation*, *118*(6), 2190–2199. https://doi.org/10.1172/JCI33585

Ramanan, V. K., & Saykin, A. J. (2013). Pathways to neurodegeneration: mechanistic insights from GWAS in Alzheimer's disease, Parkinson's disease, and related disorders. *American journal of neurodegenerative disease*, *2*(3), 145–175.

Raoul, C., Estévez, A. G., Nishimune, H., Cleveland, D. W., delapeyriere, O., *et al.* (2002). Motoneuron death triggered by a specific pathway downstream of Fas: potentiation by ALS-linked SOD1 mutations. *Neuron*, *35*(6), 1067-1083.

Ruffini, N., Klingenberg, S., Schweiger, S., & Gerber, S. (2020). Common Factors in Neurodegeneration: A Meta-Study Revealing Shared Patterns on a Multi-Omics Scale. *Cells*, *9*(12), 2642. https://doi.org/10.3390/cells9122642

Saijo, K., Winner, B., Carson, C. T., Collier, J. G., Boyer, L., *et al.* (2009). A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell*, *137*(1), 47-59. http://doi.org/10.1016/j.cell.2009.01.038

Shukla, V., Mishra, S. K., Pant, H. C. (2011). Oxidative stress in neurodegeneration. *Advances in pharmacological sciences*, *2011*. http://doi.org/10.1155/2011/572634

Streit, W. J. (2002). Microglia as neuroprotective, immunocompetent cells of the CNS. *Glia*, *40*(2), 133-139. http://doi.org/10.1002/glia.10154

Vargas, M. R., Johnson, D. A., Sirkis, D. W., Messing, A., & Johnson, J. A. (2008). Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. *Journal of Neuroscience*, *28*(50), 13574-13581. http://doi.org/10.1523/JNEUROSCI.4099-08.2008

Yamanaka, K., Chun, S. J., Boillee, S., Fujimori-Tonou, N., Yamashita, H., *et al*. (2008). Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nature neuroscience*, *11*(3), 251-253. http://doi.org/10.1038/nn2047

Cite this Article:

Joshi, S., Gaikwad, M., (2023). Neurodegenerative pathways: A review. *SCRIBE, 4*(1), 43-57.

Non-Motor Symptoms of Parkinson's Disease

Deepanjali Ghadge, Mayur Gaikwad
Department of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Mayur Gaikwad, Department of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai. Email: mayur.gaikwad@sophiacollege.edu.in

Abstract

Multiple conditions that can lead to Parkinson's disease are exacerbated by motor and nonmotor symptoms. The onset and progression of both motor and nonmotor deficits are characteristic of Parkinson's disease (PD), a complex and persistent condition.

People's quality of life is significantly affected by the complexity and progressive neurodegeneration in Parkinson's disease. The burden placed on family members and informal caretakers increases with the person's deteriorating physical, mental, and emotional health. Managing specific requirements in a community, conducting routine assessments of non-motor symptoms, and exercising caution during every stage of Parkinson's disease progression are essential to increasing patient satisfaction as well as minimizing hospitalizations during a crisis.

Non-motor symptoms (NMS) in PD are presently perceived as a fundamental part of multisystem issues since they are firmly connected with motor symptoms. In the later stages of PD, NMS frequently have a greater impact than motor symptoms. Even though levodopa cannot control many NMS, dopaminergic therapies can help with some Parkinson's disease-disabled NMS. Recognition and treatment of NMS are becoming increasingly important in treating PD patients.

Introduction

Parkinson's disease is a motor defect caused by the loss of dopaminergic neurons containing a pigment called neuromelanin. Neuromelanin is a dark-coloured compound present in the presynaptic terminal of dopaminergic neurons, which can be considered a primary player in the etiology of neurodegenerative disorders such as Parkinson's disease (Carballo-Carbajal *et al.*, 2019).

These dopaminergic neurons are lost from the pars compacta, which is a region of the substantia nigra. Other pathological hallmarks are the increased accumulation of proteins in the surviving neurons known as Lewy bodies and Lewy neurites. The Lewy bodies are intra-cytoplasmic eosinophilic deposits of α-synuclein, which is a misfolded protein that accumulates in different parts of the brain in a prion-like fashion, causing motor as well as non-motor symptoms (Gómez-Benito *et al.*, 2020).

Degeneration of these neurons leads to motor defects that lead to unintended and uncontrollable movements, which is an

identifying feature of PD. Since PD is a progressive disease, the symptoms are developed slowly over the years, and the development of symptoms may differ from person to person. Motor symptoms of PD include tremors, slowness and paucity of movement, also known as bradykinesia and hypokinesia, limb stiffness, gait, balance problems, etc. In Parkinson's disease, patient nerve endings that produce norepinephrine are degraded, which regulates a variety of body functions like heart rate and blood pressure (Mazzoni *et al.*, 2012). The loss of norepinephrine may be the cause of some Parkinson's non-motor symptoms, such as fatigue, irregular blood pressure, dropped food movement through the digestive tract, and an unexpected drop in blood pressure when a person changes orientation (Delaville *et al.*, 2011).

Over the last decade, it has been proven that PD patients suffer from symptoms that go beyond classic motor symptoms. In the case of PD, along with motor symptoms, nonmotor symptoms are also seen, which may arise due to the loss of neurons from dopaminergic, non-dopaminergic, or a combination of both pathways. The side effects of levodopa and some other conditions also contribute to NMS, and it has also been found that NMS does not respond to levodopa treatment, suggesting PD to be a multisystem brain neurodegenerative disorder and that reaching beyond the nigrostriatal system is needed (Lee & Koh, 2015).

Non-motor symptoms

Nonmotor symptoms (NMS) of Parkinson’s disease are crucial for diagnosing motor symptoms since they appear before motor symptoms in patients. NMS can also be used to easily distinguish idiopathic Parkinson's disease (PD) from several other Parkinsonian disorders. (Lee & Koh, 2015). Motor symptoms develop at later stages, but nonmotor symptoms are seen in the early stages of disease development. Nonmotor symptoms of Parkinson's disease were reported by James Parkinson in 1817 (Todorova *et al.*, 2014). Due to the development of motor symptoms at a later stage, patients face severe issues in many areas, including a decline in their health and an increase in hospitalization costs for treatment because they are unable to move freely and must rely on others. So diagnosing non-motor symptoms at an early stage and keeping a check on their development can help patients have a better life.

Non-motor symptoms seen in patients suffering from Parkinson's disease can be listed as sleep disorder, olfactory dysfunction, dysautonomia, cognitive and neuropsychiatric issues, depression, anxiety, and fatigue (Todorova *et al.*, 2014; Gupta & Shukla, 2021). NMS may arise due to the loss of dopaminergic neurons, non-dopaminergic neurons, or a combination of both pathways (Gupta & Shukla, 2021; Todorova *et al.*, 2014).

Many times, these non-motor symptoms are overlooked by patients as well as clinicians, when it is very important to keep an eye on these symptoms to track the development of PD. From the patient's side, this negligence can be due to various reasons, such as self-consciousness, embarrassment when speaking about the symptoms, or being

unaware that these symptoms can have a link with Parkinson's disease. The clinician may believe that treating these symptoms is not necessary. Some of the NMS are considered premotor symptoms, thus opening a new potential therapeutic approach. PD is diagnosed in very late stages, until almost 70% of dopaminergic neurons are lost (Gupta & Shukla, 2021).

Non – Motor Symptoms of Parkinson's Disease are-

Neurobehavioral changes or neuropsychiatric symptoms

Neurobehavioral changes or neuropsychiatric symptoms have a huge impact on the quality of life of patients. This includes symptoms like depression, anxiety, cognitive impairment, dementia, psychosis, and apathy.

Cognitive Problems and Dementia

Cognitive impairment in Parkinson's disease has a direct connection to dopaminergic and non-dopaminergic systems.

In people with PD, memory and thinking issues can be moderate to severe. According to some studies, these issues affect at least 40% of people with Parkinson's disease over time. Taking more time to process information, having trouble making decisions, multitasking, remembering recent events, and judging distances are all common cognitive symptoms (Fang *et al.*, 2020).

Psychosis and Hallucinations

A psychological disorder called psychosis causes a person to lose contact with reality. Twenty percent to forty percent of patients taking PD medicines experience psychotic symptoms. Despite the fact that many PD drugs can have psychosis as a side effect, especially in patients who already have cognitive problems, psychosis is not well understood. The most prevalent signs of psychosis in patients with Parkinson's disease are visual hallucinations or seeing things that aren't real. Typically, these occurrences worsen and occur more frequently as PD progresses.

Hallucinations in PD can affect any of the five senses, i.e., vision, smell, auditory, taste, and touch, among which visual is most common, and thus pose a challenge to PD patients as a treatment for motor symptoms can trigger and worsen these hallucination events (Weil & Reeves, 2020).

Hallucination can be defined as when someone sees, hears, or feels something that is not present. In contrast, delusion can be defined as illogical, dysfunctional views or persistent thoughts that are not reality-based.

Finding effective and safe treatment for hallucinations and delusions is an urgent need, as antipsychotic drugs have significant side effects such as sedation, worsening motor symptoms and symptoms associated with cognitive impairment, and further increasing the risk of stroke and death. Serotonergic agents have opened new doors for cognitive impairment treatment, but more study needs to be done (Weil & Reeves, 2020).

Mood disorders

People with Parkinson's disease frequently suffer from mood disorders such as depression, anxiety, and apathy. All of these conditions have the potential to make motor symptoms worse and significantly lower a person's quality of life.

Sadness, a loss of pleasure, and diminished interest in activities are typically signs of depression in PD. Anxiety can be characterized by panic attacks, excessive worry, or fear. The loss of motivation that results in diminished speech, movement, and emotional expression is known as apathy.

Autonomic failure

According to recent research, Parkinson's disease "starts in the gut" because the enteric nervous system exhibits Lewy bodies and aberrant alpha synuclein aggregates, and the gut microbiome via inflammatory pathways causes neurodegeneration (Braak *et al.*, 2006). Dysfunction of the blood pressure, the gastrointestinal system, the sexual system, the thermoregulatory system, and the urine system are all examples of autonomic failure. These symptoms are more prevalent in PD patients and can develop at any stage.

Blood pressure abnormalities

Blood pressure abnormalities can be seen in the early stages of PD, which include orthostatic hypotension and postprandial hypertension, as well as nocturnal and supine hypertension, indicating impaired BP regulation. (Hou *et al.*, 2018). Among all these, orthostatic hypotension is the most prevalent and can be attributed to central as well as peripheral autonomic dysfunction (Allcock, 2010).

Gastrointestinal abnormalities

Weight loss, dental degradation, excessive salivation, dysphagia, gastroparesis, fewer bowel movements, anorectal dysfunction, drooling, dyspepsia, constipation, abdominal pain, and fecal incontinence are manifestations of gastrointestinal abnormalities.

The central and enteric nervous systems both contribute to these symptoms (Pfeiffer, 2011; Salat-Foix, 2012)

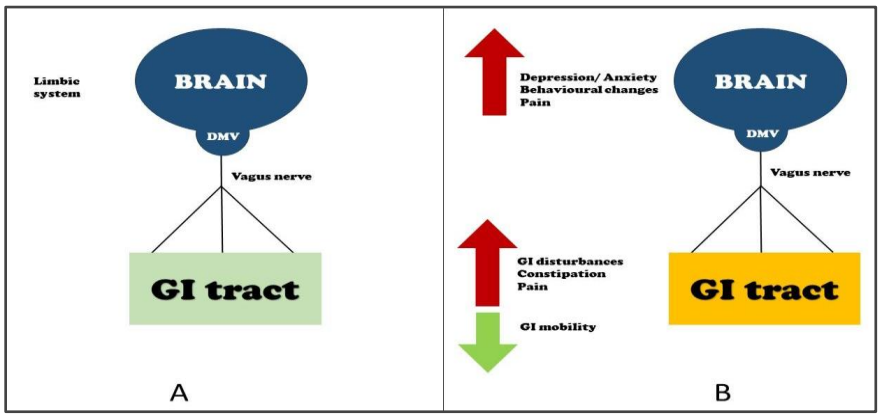


Figure 1. (A) Gut-Brain communication via vagus nerve
(B) Non-Motor symptoms of Parkinson's disease

Sexual Abnormalities

Sexual abnormalities are encountered in both genders. Male patients experience hypersexuality (HS), erectile dysfunction, and problems with ejaculation, while females experience loss of lubrication, vaginal tightness, and involuntary urination during sex (Bronner & Vodušek, 2011).

Thermoregulatory dysfunction

The brainstem and hypothalamus, with alpha-synuclein, influence thermoregulation, leading to a sweating deficit and vasomotor tone. A deficiency of dopamine combined with peripheral nervous system dysfunction leads to temperature imbalance; thus, thermoregulatory tests can be used to differentiate PD from other neurodegenerative diseases (Coloman & Levin., 2021; Coon & Low, 2018).

Urinary dysfunction

Urinary dysfunction is more prevalent and occurs earlier than other autonomic symptoms. Urinary symptoms can be classified into two classes: the first is irritative symptoms, which include frequency, urgency, and urge incontinence, and the second is obstructive symptoms, which include hesitancy and a weak urinary stream (Singer, 1998).

Sensory impairments

Sensory impairments include symptoms like olfactory dysfunction, visual impairment, and pain, among which olfactory dysfunction can be said to be more common since it occurs in approximately 90% of PD patients (Doty, 2012).

Visual impairment

Visual impairments include symptoms like dry eye, double vision, and impaired depth perception. In PD, impaired driving ability is linked to reduced spontaneous blinking and decreased tear film production. Color vision and contrast sensitivity in PD patients have been found to be lower than in controls in other studies. This is thought to be due to a combination of cortical visual dysfunction and intrinsic retinal pathology in PD. When PD patients have low dopamine levels, they may experience diplopia, among other visual disturbances, due to the significant role that dopamine plays in vision-related processes like adaptation to light, oculomotor control, contrast sensitivity, color vision, visuospatial construction, and spatial working memory (Weil & Reeves, 2016; Savitt and Aouchiche, 2020).

Olfactory dysfunction

Olfactory dysfunction in the case of PD may occur in earlier stages than the motor symptoms; thus, it can be used as a potential predicting marker for PD. It can be very important in assessing the development of a medical condition or cognitive deterioration.

Olfactory dysfunction occurs in PD as well as other neurodegenerative diseases like Alzheimer's disease. Though the exact pathophysiology of olfactory dysfunction is not known, it is believed that it begins in the olfactory bulb and the dorsal motor nucleus complex of the glossopharyngeal and vagus nerves. Damage to the non-dopaminergic

neurotransmitter system may cause olfactory damage (Doty, 2012). Olfactory impairment, which is prevalent in Parkinson's disease (PD), has been linked to the early deposition of synuclein pathology in olfactory areas.

Pain

Pain is one of the frequently occurring symptoms in PD patients. Pain can be divided into various types. Many scientists have proposed various classifications of pain.

Deuschl and Wassner divided pain into two main groups namely nociceptive and neuropathic. Nociceptive symptoms include musculoskeletal, visceral, and cutaneous pain, while neuropathic pain includes peripheral and central pain (Wasner *et al.*, 2012).

Chaudhuri and Schapira distinguished pain in types such as musculoskeletal, PD-related chronic pain, fluctuation-related pain, nocturnal pain, coat-hanger, orofacial, peripheral limb, and abdominal pain (Chaudhuri et al, 2009).

But the most cited and referred classification is given by Ford where he categorized pain into musculoskeletal pain, radicular or neuropathic pain, dystonia-related pain, akathitic discomfort, and primary, central parkinsonian pain. (Ford, 2010).

Sleep disorders

A sleep disorder can arise due to motor symptoms such as restless leg syndrome, tremors, and bradykinesia. Along with that, other reasons can also cause sleep disorders, which include insomnia, depression, excessive daytime sleepiness, nocturnal awakening, changes in the brainstem, medication side effects, etc. This can develop at any stage of the disease (Kurtis *et al.*, 2013). For diagnostic purposes, polysomnography and multiple sleep latency tests can be used.

Consequences of NMS

Age is a major component in the development of symptoms in PD, which is regarded as an age-dependent disease. However, the development of symptoms along with age depends on a number of factors, including the severity of the disease, environmental factors, hereditary factors, lifestyle choices, etc. NMS can develop at any stage of life; in some cases, it might be dominant to motor symptoms and occur before the motor symptoms appear (Chaudhuri *et al.*, 2016).

Since non-motor symptoms are overlooked in many cases by patients and, in some cases, by doctors, over time, they deteriorate the quality of life and further increase the economic burden on patients due to the increased cost of hospitalization.

Treating the NMS of PD has become a major need nowadays to cure PD and improve the quality of life by treating NMS.

Management of NMS

Motor symptoms can be referred to as the "tip of the iceberg" of symptoms of PD. Concentrating just on motor symptoms has a negative impact on patients because it leaves out the chance to address non-motor symptoms that may be negatively affecting

the person's quality of life and possibly accelerating the loss of function. In order to effectively manage Parkinson's disease, it is crucial to assess and treat both motor and non-motor symptoms. This can be accomplished by promoting a healthy lifestyle, assisting and guiding the patient, caretaker, and family in understanding the disease, and managing symptoms over the course of the disease.

The intricacy and gradual neurodegeneration of Parkinson's disease have a negative impact on people's quality of life and place a strain on patients' families as well as society. To guarantee that sufficient help is set up, it is fundamental to give close consideration to the individual's insight and to speak with the person's casual parental figure and family routinely. Access to additional consumer-focused information will be made easier, and social isolation will be reduced if the patient and their caregiver are connected to the local support group. Motor symptoms can be treated with levodopa, but most NMS do not respond to the same treatment, so developing a therapy aimed at the betterment of NMS is a waste of time.

References

Allcock, L., 2010. Orthostatic hypotension in Parkinson's disease. *ACNR* (10) 51–52. https://doi.org/10.2217/nmt.13.30

Braak, H., de, Vos, R.A., Bohl, J., Del, Tredici, K. (2006). Gastric α-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neuroscience Letters, 396,* 67–72. https://doi.org/10.1016/j.neulet.2005.11.012

Bronner, G., & Vodušek, D. B. (2011). Management of sexual dysfunction in Parkinson's disease. *Therapeutic advances in neurological disorders, 4*(6), 375–383. https://doi.org/10.1177/1756285611411504

Carballo-Carbajal, I., Laguna, A., Romero-Giménez, J., Cuadros, T., Bové, J., *et al.* (2019). Brain tyrosinase overexpression implicates age-dependent neuromelanin production in Parkinson's disease pathogenesis. *Nature communications, 10*(1), 973. https://doi.org/10.1038/s41467-019-08858-y

Chaudhuri, K. R., & Schapira, A. H. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *The Lancet Neurology, 8*(5), 464-474. https://doi.org/10.1016/S1474-4422(09)70068-7

Coloman, I. I., & Levin, O. S. (2021). Thermoregulatory dysfunction in Parkinson's disease. *Zhurnal Nevrologii i Psikhiatrii Imeni SS Korsakova, 121*(10. Vyp. 2), 69-75. https://doi.org/10.17116/jnevro202112110269

Coon, E. A., & Low, P. A. (2018). Thermoregulation in Parkinson's disease. *Handbook of clinical neurology, 157*, 715–725. https://doi.org/10.1016/B978-0-444-64074-1.00043-4

Cusso, M. E., Donald, K. J., & Khoo, T. K. (2016). The Impact of Physical Activity on Non-Motor Symptoms

in Parkinson's Disease: A Systematic Review. *Frontiers in medicine, 3,* 35. https://doi.org/10.3389/fmed.2016.00035

Delaville, C., Deurwaerdère, P. D., Benazzouz, A. (2011). Noradrenaline and Parkinson's disease. *Frontiers in systems neuroscience, 5,* 31. https://doi.org/10.3389/fnsys.2011.00031

Doty, R. L. (2012). Olfactory dysfunction in Parkinson disease. *Nature Reviews Neurology, 8*(6), 329-339. https://doi.org/10.1038/nrneurol.2012.80

Fang, C., Lv, L., Mao, S., Dong, H., Liu, B. (2020). Cognition Deficits in Parkinson's Disease: Mechanisms and Treatment. *Parkinson's disease, 2020*(1), 2076942. https://doi.org/10.1155/2020/2076942

Ford, B. (2010). Pain in Parkinson's disease. *Movement Disorders, 25*(S1), S98-S103. https://doi.org/10.1002/mds.22716

Gómez-Benito, M., Granado, N., García-Sanz, P., Michel, A., Dumoulin, M., *et al.* (2020). Modeling Parkinson's Disease with the Alpha-Synuclein Protein. *Frontiers in pharmacology*, *11*, 356. https://doi.org/10.3389/fphar.2020.00356

Gupta, S., & Shukla, S. (2021). Non-motor symptoms in Parkinson's disease: opening new avenues in treatment. *Current Research in Behavioral Sciences, 2,* 100049.https://doi.org/10.1016/j.crbeha.2021.100049

Haining, R. L., & Achat-Mendes, C. (2017). Neuromelanin, one of the most overlooked molecules in modern medicine, is not a spectator. *Neural regeneration research, 12*(3), 372–375. https://doi.org/10.4103/1673-5374.202928

Hou, L., Li, Q., Jiang, L., Qiu, H., Geng, C., *et al.* (2018). Hypertension and Diagnosis of Parkinson's Disease: A Meta-Analysis of Cohort Studies. *Frontiers in neurology, 9,* 162. https://doi.org/10.3389/fneur.2018.00162

Kumaresan, M., & Khan, S. (2021). Spectrum of non-motor symptoms in Parkinson's disease. *Cureus, 13*(2). https://doi.org/10.7759/cureus.13275

Kurtis, M. M., Rodriguez-Blazquez, C., Martinez-Martin, P., & ELEP Group. (2013). Relationship between sleep disorders and other non-motor symptoms in Parkinson's disease. *Parkinsonism & related disorders, 19*(12), 1152-1155. http://doi.org/10.1016/j.parkreldis.2013.07.026

Lee, H. M., & Koh, S. B. (2015). Many Faces of Parkinson's Disease: Non-Motor Symptoms of Parkinson's Disease. *Journal of movement disorders, 8*(2), 92–97. https://doi.org/10.14802/jmd.15003

Mazzoni, P., Shabbott, B., Cortés, J. C. (2012). Motor control abnormalities in Parkinson's disease. *Cold Spring Harbor perspectives in medicine*, *2*(6), a009282. https://doi.org/10.1101/cshperspect.a009282

Pfeiffer, R. F. (2011). Gastrointestinal dysfunction in Parkinson's disease. *Parkinsonism & related disorders*, *17*(1), 10-15.

https://doi.org/10.1016/j.parkreldis.2010.08.003

Salat-Foix, D., & Suchowersky, O. (2012). The management of gastrointestinal symptoms in Parkinson's disease. *Expert review of neurotherapeutics, 12*(2), 239–248. https://doi.org/10.1586/ern.11.192

Savitt, J., & Aouchiche, R. (2020). Management of visual dysfunction in patients with Parkinson's disease. *Journal of Parkinson's disease, 10*(s1), S49-S56. http://doi.org/10.3233/JPD-202103

Singer C. (1998). Urinary dysfunction in Parkinson's disease. *Clinical neuroscience (New York, N.Y.), 5*(2), 78–86. http://doi.org/10.1201/9780203508596.ch26

Thanvi, B. R., Munshi, S. K., Vijaykumar, N., & Lo, T. C. N. (2003). Neuropsychiatric non-motor aspects of Parkinson's disease. *Postgraduate Medical Journal, 79*(936), 561-565. http://doi.org/10.1136/pmj.79.936.561

Tibar, H., El Bayad, K., Bouhouche, A., Ait Ben Haddou, E. H., Benomar, A., *et al.* (2018). Non-Motor Symptoms of Parkinson's Disease and Their Impact on Quality of Life in a Cohort of Moroccan Patients. *Frontiers in neurology, 9,* 170. https://doi.org/10.3389/fneur.2018.00170

Todorova, A., Jenner, P., Chaudhuri, K. R. (2014). Non-motor Parkinson's: Integral to motor Parkinson's, yet often neglected. *Pract. Neurol., 14, 310-322.* 10.1136/practneurol-2013-000741

Wasner, G., & Deuschl, G. (2012). Pains in Parkinson disease—many syndromes under one umbrella. *Nature Reviews Neurology, 8*(5), 284-294. https://doi.org/10.1038/nrneurol.2012.54

Weil, R. S., & Reeves, S. (2020). Hallucinations in Parkinson's disease: new insights into mechanisms and treatments. *Advances in clinical neuroscience & rehabilitation, 19*(4), ONNS5189. https://doi.org/10.47795/ONNS5189

Cite this Article:

Ghadge, D., Gaikwad, M. (2023). Non-motor symptoms of Parkinson's Disease. *SCRIBE, 4*(1), 58-66.

Is Stem Cell Therapy an Effective Treatment for Autism?

Pranali Joshi, Arjumanara Surti
Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Dr. Arjumanara Surti, Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai. Email: arjumanara.surti@sophiacollege.edu.in

Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder marked by characteristic symptoms of impairment in development of language, social interaction and behavior. As a heterogeneous disorder caused by a complex interplay of genetic and environmental factors, ASD does not have a single underlying cause. Therefore, evidence-based therapies are focused on improving symptoms rather than curing the condition. While Autism is considered to be associated with several immune abnormalities stemming from the many genetic mutations that cause it, additional research is necessary to elucidate the link between these genetic abnormalities and their impact on neurological processes. The use of stem cell therapy has emerged as a possible treatment option for disorders that were previously considered untreatable. As a result, many institutions worldwide have begun to market and provide stem cell therapy to patients diagnosed with Autism. However, as no clinical trials have provided valid results to support these practices, they remain controversial and unethical.

Understanding Autism

Classification of Autism - A Brief History: Swiss psychiatrist Eugen Bleuler coined the term "Autism" in 1911 as a milder symptom of Schizophrenia (Bender 1953, 1971). Autism was only recognized as a condition in the 1980s and was defined in the DSM I (1952) and DSM II (1968) as having 3 cardinal signs: failure or lack of social development, delayed or deviant development of language, and ritualistic activities (Rutter & Bartak., 1971). DSM III (1987) and IV (2000) provided a more detailed description of the diagnosis and categorized it as one of the Pervasive Developmental Disorders (PDD). With the introduction of DSM-V in 2013, Autism, Aspergers and other PDDs were recognized as separate disorders under the Autism Spectrum (APA - DSM IV, 2000) (WHO - ICD-10, 1992). Presently, Autism Spectrum Disorder (ASD) is defined as a neurodevelopmental disability arising from the delay in nervous system development during infancy or childhood (APA - DSM-V, 2013).

Autism and Genetics: Autism is a complex neurodevelopmental disorder with no single root cause. It is considered to have a strong genetic liability along with non-genetic factors such as environmental influences (Geschwind, 2009). Twin studies have shown that monozygotic twins (who share 100% of their genome) have a higher rate of co-

Edition	Note	Reference
DSM I (1952)	The initial 1952 DSM edition mentioned autism once in relation to schizophrenic reactions in young children, aligning with the term's 1911 coining by Eugen Bleuler.	American Psychiatric Association. (1952). Diagnostic and statistical manual of mental disorders (DSM-1). Washington, DC
DSM II (1968)	DSM II listed Autistic behaviour as a sympton under Childhood Schizophrenia and Schizoid personality.	American Psychiatric Association. (1968). Diagnostic and statistical manual of mental disorders (2nd ed., DSM-II). Washington, DC
DSM III (1980) DSM III-R (1987)	DSM III listed Infantile Autism as a standalone disorder and described the diagnostic criteria as: A. Onset before 30 months of age B. Pervasive lack of responsiveness to other people (autism) C. Gross deficits in language development D. If speech is present, peculiar speech patterns such as immediate and delayed echolalia, metaphorical language, pronominal reversal. E. Bizarre responses to various aspects of the environment, e.g., resistance to change, peculiar interest in or attachments to animate or inanimate objects. F. Absence of delusions, hallucinations, loosening of associations, and incoherence as in Schizophrenia. The revised version of DSM III provided a more detailed set list of diagnostic criteria for Autism across 3 main categories of symptoms with examples: Qualitative impairment in reciprocal social interaction, Qualitative impairment in verbal and nonverbal communication and in imaginative activity, Markedly restricted repertoire of activities and interests; with the onset occurring during infancy or early childhood	American Psychiatric Association. (1980). Diagnostic and statistical manual of mental disorders (3rd ed., DSM-III). Washington American Psychiatric Association. (1987). Diagnostic and statistical manual of mental disorders (3rd ed., rev., DSM-III-R). Washington, DC
DSM IV (1994) DSM IV-R (2000)	DSM IV and IV-R recognized the Autistic Disorder with diagnostic criteria similar to that in DSM III-R spanning across: Qualitative impairment in social interaction, Qualitative impairments in communication, Restricted, repetitive, and stereotyped patterns of behavior, interests, and activities, Delays or abnormal functioning, with onset prior to age 3 years, in (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play. It also listed Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS), Asperger's Disorder, Rett's Disorder and Childhood Disintegrative Disorder under the spectrum.	American Psychiatric Association. (1994). Diagnostic and statistical manual of mental disorders (4th ed., DSM-IV). Washington, DC American Psychiatric Association. (2000). Diagnostic and statistical manual of mental disorders (4th ed., text rev., DSM-IV-TR). Washington, DC
DSM V (2013)	DSM V consolidated Autistic Disorder, Asperger's disorder, and PDD-NOS into autism spectrum disorder under Neurodevelopmental Disorders. The diagnostic criteria focused on the severity of symptoms across deficits in social communication, restricted or repetitive patterns of behaviour which present in early stages of development, cause clinically significant impairment in social, occupational, or other important areas of functioning and cannot be adequately explained by intellectual disabilities. It also lay emphasis on the comorbidities that occur frequently with ASD.	American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed., DSM-5). Washington, DCal disorders (4th ed., DSM-IV). Washington, DC

Table 1: Classification of Autism in Diagnostic and Statistical Manual (DSM), 1952-2013

occurrence of ASD than dizygotic twins (who share 50% of their genome, similar to non-twin siblings) (Rosenberg *et al.*, 2009).

Overall, heritability estimates suggest that genetic factors account for about 70–80% of the variation in risk for ASD (Rosenberg *et al.*, 2009). The genetics of ASD are complex, involving multiple mutations that interact with each other and with environmental factors to lead to Autism (Abrahams & Geschwind, 2008). Certain copy number variants (CNVs - non-heritable de novo mutations), which account for up to 10% of ASD cases, have effects large enough to cause ASD independently (Geschwind, 2011). However, some CNVs associated with Autism have been observed to be present in a small percentage (1-2%) of unaffected individuals as well (Beaudet, 2007). Several of these CNVs are pleiotropic and are associated with comorbidities alongside Autism - such as schizophrenia, epilepsy, and ADHD, among others (Geschwind, 2011). It is important to note that no specific gene is responsible for the majority of ASD cases and even the most common genetic mutations are present in only about 1-2% of the cases (Abrahams & Geschwind, 2008).

Evidence-based therapies for Autism: ASD has no definitive cure due to its unknown etiology. Focused Intervention Practices and Comprehensive Program Models are the two primary intervention classes used in the literature for children and youth with Autism (Smith, 2013). Psychopharmacological treatments, which integrate medication with therapy, are often used in severe cases, including antiseizure drugs, neuroleptics, stimulants, antianxiety drugs, and antidepressants (Juane Heflin & Simpson, 1998). However, medications are not a cure for Autism but rather support other therapies. Even today, Autism remains a mysterious disorder considering that even the most promising treatment strategies are based on insufficient research (Herbert *et al.*, 2002). Young patients and especially their parents are often left vulnerable to pseudoscience out of desperation. One such alarming case is the trend of employing stem cell therapy and claiming it to be a cure for Autism.

Stem Cell Therapy as a Prospective Treatment for Autism

Stem cells, characterized by their capacity for proliferation and differentiation into specialized cellular types, are classified based on their differentiation potential and derivation method. Understanding the distinct characteristics of various stem cell types is crucial for exploring potential applications of stem cell technology. These cells exhibit plasticity, allowing them to differentiate into diverse cellular types. Pluripotent stem cells, capable of generating specialized cells from all three germ layers, and multipotent stem cells, which give rise to more limited lineages based on their germ layer of origin, are two broad categories (Bongso *et al.*, 2008).

Experimental studies have investigated the potential of stem cell-based therapies for a range of neurological disorders, including Parkinson's, Huntington's, ALS, Alzheimer's, multiple sclerosis, stroke, spinal cord injury, brain tumors, and lysosomal storage diseases (Kim & De Vellis, 2009). The ability of stem cells to divide and develop in different cell

types makes stem cell therapy a promising option for treating various disorders.

Mesenchymal stem cells (MSCs), which are obtained from adipose tissue and bone marrow, are being looked into as a potential treatment for Autism (Short *et al.*, 2003). MSCs are characterized by their ability to adhere to plastic surfaces in cell culture, expression of specific surface antigen, and the potential of multilineage differentiation in vitro - including neural lineage cells such as cholinergic or dopamine neurons (Zhang *et al.*, 2012). The relatively non-invasive and simple procedure to obtain MSCs, rapid in vitro growth and efficient storage make these stem cells extremely valuable in clinical trials. They have also been reported to possess strong anti-inflammatory and immunosuppressive activity which further minimizes the need for pharmacological immunosuppression (Petrie Aronin & Tuan, 2010).

Autism Spectrum Disorder (ASD) has been linked to certain immune abnormalities, like heightened immune function and neuroinflammation (Gupta *et al.*, 2010). Although our understanding of the immunological connections to neuropathological conditions in ASD is still developing, there's growing interest in mesenchymal stem cell therapy because of its potential to modulate the immune system (Sotiropoulou and Papamichail, 2007).

Previous Clinical Trials using Stem Cell Therapy as a Treatment for Autism

Many clinical trials (Table 2) have been conducted in the past to evaluate the clinical potential and application of mesenchymal stem cells (MSCs) derived from various sources. This review focused on nine completed trials, the first of which is a non-randomized open-label trial conducted in 2009 which aimed to examine the effect of cord blood mononuclear cells (CB-MNCs) and umbilical cord blood mesenchymal stem cell (UCB-MSCs) therapy on 37 autistic subjects aged 3 to 12 years. The participants were divided into three groups - Group 1 received intrathecal infusion of CB-MNCs and rehabilitation therapy, Group 2 received intrathecal infusion of CB-MNCs and IV infusion of UCB-MSCs (both allogeneic), Group 3 (control group) received only rehabilitation therapy. All three groups received weekly stem cell administrations and follow-ups every four weeks.

At the 24-week mark, progress was recorded using the Childhood Autism Rating Scale (CARS), Clinical Global Impression (CGI) Scale, and Aberrant Behavior Checklist (ABC) assessments. The findings revealed that Group 2 showed the most improvement, followed by Group 1, and finally, Group 3. The trial suggested that CB-MNCs and UCB-MSCs may have a positive impact on behavioral symptoms and functions in children with autism (Lv *et al.*, 2013). However, there were several limitations to this study: 1) The trial was non-randomized, which hinders proper comparability between the intervention and control groups; 2) The small sample size of the study renders the results unreliable; 3) Follow-ups were conducted only up to 24 weeks from baseline, hence any potential mid and long-term

Year	Type of study	Type of SCT	Sample size (age range)	No. of doses	Interval between each dose	Additional interventions	Follow up	Results	Source
2009	Nonrandomised, open label, controlled	Intrathecal infusion of CB-MNCs and IV infusion of UCBMSCs	37 (3 to 12 years)	4	1 week	Group 1: CB-MNC + Therapy Group 2: CB-MNC + UCB-MSCs + Therapy Group 3: Therapy (Control)	4, 8, 16, 24 weeks	Largest improvement showed by Group 2 followed by 1 and then 3 on the CARS, CGI and ABC scales.	Lv et al., 2013
2012	Randomised, blinded, placebo-controlled, crossover trial	IV infusion of autologous UCBMSCs	29 (2 to 7 years)	1 Control (saline), 1 Test, (UCB-MSCs crossed over at 24 weeks	24 weeks	NA	12 and 24 weeks after each infusion	No statistically significant improvements in OWPVT, CGI, Stanford-Binet Fluid Reasoning and Knowledge, and VABS .	Chez, M. et al., 2018
2013	Nonrandomised, open label, controlled	Intrathecal autologous BMMNC transplant	35 (3 to 33 years)	1	NA	Sensory integrative occupational therapy interventions, speech therapy, and specific dietary recommendations.	5 to 26 months	91% patients had improved ISAA scores; 62% showed decreased severity on CGI-I. Some reported increase in seizures.	Sharma, A. et al., 2013
2014	Randomised, open label, uncontrolled	IV Infusion of autologous UCBMSCs	25 (2 to 5 years)	1	NA	NA	6 and 12 months	Improvement on the VABS scales in the first 6 months, no improvements in 6-12 month period	Dawson, G. et al., 2017
2014	Randomised, open label, uncontrolled	IV Infusion of autologous UCBMSCs	20 (6 to 16 years)	4	3 months	NA	3 and 12 months	CARS and ATEC scores of 8 subjects decreased over the course of treatment, placing them in a lower ASD symptom category when compared with baseline	Riordan, N. H., et al, 2019
2017	Open label, uncontrolled	Intrathecal and IV infusion of BMMNCs	30 (3 to 7 years)	2	6 months	8 weeks of educational intervention based on the Early Start Denver Model.	18 months	Improvement in CARS& VABS scores. Repetitive behaviors and hyperactivity decreased remarkably.	Thanh, L. N. et al., 2021
2017	Open label, uncontrolled, Phase I	IV infusion of allogeneic hCT-MSCs	12 (4 to 9 years)	1 or 2 or 3	2 months	NA	6 and 12 months	Variable outcomes on Nonverbal DQ, VABS, PDDBI, CGI scales	Sun, J. M. et al., 2020a
2020	Randomised, placebo-controlled, double-blind, Phase II	IV infusion of autologous or allogeneic UCBMSCs	180 (2 to 7 years)	1	NA	NA	6 months	Analysis showed no evidence that CB was associated with improvements in VABS, PDDI and One-Word Picture Vocabulary Test scores.	Dawson, G. et al., 2020b
2020	Randomised, controlled, single-blind	Intrathecal injection of autologous BMMSCs	32 (5-15 years)	2	2 weeks	Group 1: BMMSCs + rehabilitation therapy Group 2: Rehabilitation therapy	6 and 12 months	No significant differences in improvement of CARS and GARS scores after 12 months from baseline.	Sharifzadeh, N. et al., 2021

Table 2: Previous Clinical Trials using Stem Cell Therapy as a Treatment for Autism

effects of stem cell therapy could not be evaluated; 4) The trial had an open-label design which introduces bias into the evaluation

A randomized, double-blinded, placebo-controlled trial with a cross-over design was conducted in 2019. 29 autistic participants (aged 2 to 7) were administered autologous UCB-MSCs and the evaluation was carried out using the CGI, One Word Picture Vocabulary (OWPV) Test, and Stanford-Binet Fluid Reasoning & Knowledge Scale (VABS) tests. No significant improvements in the scores of these tests were observed and the authors therefore concluded that the efficacy of autologous UCB-MSCs as a treatment for autism required stricter investigation to draw reliable conclusions. (Chez *et al.*, 2018).

In a 2014 study, an open-label, randomized trial was conducted involving 25 autistic children aged 2 to 5 years. All participants received a one-time intravenous infusion dose of autologous umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs). Significant improvements were noted on the Vineland Adaptive Behavior Scales (VABS) communication and socialization scales within the first 6 months. Follow-up sessions were conducted at 6 and 12 months from baseline. However, no further improvements were observed between the 6 to 12-month period. Despite these findings, a more reliable evaluation of the effect of UCB-MSC therapy as a treatment for Autism is warranted. Furthermore, the study lacked a control arm, had a small sample size of subjects which puts constraints on the validity of the results. The open-label design could also potentially influence the outcome of the study (Dawson *et al.*, 2017).

In the same year, another open-label clinical trial testing the effect of autologous UCB-MSCs on 20 autistic children (aged 6 to 16 years) was conducted. The participants received 4 doses of allogeneic UCB-MSCs via IV at 3-month intervals, and follow-up sessions were held at 3 and 12 months.

Although CARS and Autism Treatment Evaluation Checklist (ATEC) scores improved for 8 subjects over the course of treatment, the small sample size and lack of a control group leave room for ambiguity regarding the effectiveness of SCT in treating autism (Riordan *et al.*, 2019).

In Mumbai, India, a clinical trial was conducted using an open-label, non-randomised approach. In this trial, a single intrathecal dose of autologous bone marrow mononuclear cells (BMMNCs) was administered to 35 participants ranging in age from 3 to 33 years. The participants were then followed up for varying durations, ranging from 5 to 26 months.

During this period, participants also received occupational therapy interventions based on a sensory integrative approach, activities of daily living training, psychological interventions based on behavior modification techniques, speech therapy, and specific dietary recommendations. Ninety-one percent of patients showed improved Indian Scale for Assessment of Autism (ISAA) scores, and 62% exhibited decreased severity on the CGI Scale. Some participants reported

an increase in the occurrence of seizures. However, due to the lack of a control arm, it is not possible to determine if the improvements are attributable to SCT intervention (Sharma, A. *et al.*, 2013).

A clinical trial conducted in Hanoi, Vietnam, involved 30 autistic subjects (aged 3 to 7 years) who received 2 doses of BMMNCs (intrathecally and via IV infusion) at 6-month intervals. All participants underwent an 8-week educational intervention based on the Early Start Denver Model. At the 18-week follow-up, improvements in CARS and VABS scores, enhanced social communication, language, and daily skills, along with a reduction in repetitive behaviors and hyperactivity, were reported. However, similar to some previous trials, the major drawbacks of this trial include a small sample size, open-label bias, and the absence of a control arm (Thanh *et al.*, 2021).

Duke University in the USA conducted Phase I and Phase II clinical trials at the Duke Center for Autism and Brain Development. Phase I trials involved 12 autistic subjects (aged 4 to 9 years) who received 1, 2, or 3 doses of allogeneic hCT-MSCs at 2-month intervals. Follow-up sessions at 6 and 12 months revealed variable outcomes on Nonverbal DQ, VABS, PDDBI, and CGI scales (Sun *et al.*, 2020a). Subsequently, the Phase II trial, a randomized, placebo-controlled, double-blind study, administered a single dose of autologous or allogeneic UCB-MSCs (via IV infusion) or saline (control) to 180 subjects (aged 2 to 7 years). A follow-up session was conducted for 6 months. Analysis showed no evidence that SCT was associated with improvements in VABS, PDDI, and OWPV Test scores (Dawson *et al.*, 2020b).

A randomized, controlled, single-blind study (with only researchers involved in data collection being blinded) included 32 autistic children aged 5-15 years. The intervention involved administering two doses of intrathecally injected autologous BM-MSCs over four weeks, with follow-ups at 6 and 12 months from baseline. The intervention group received stem cell infusions and rehabilitation therapy, while the control group received only rehabilitation therapy. Evaluation of CARS and GARS scores at 12 months revealed no significant differences between the two groups (Sharifzadeh *et al.*, 2021).

Thus, it is observed that due to repetitive flaws in trial designs (small sample size, lack of a control arm, short-term administration of subjects, open-label bias), no definitive consensus on whether SCT is effective in treating autism has been established yet. Although no adverse effects were reported in any of the trials, the most common side effects experienced by subjects included fatigue, headaches, transient fevers, gastrointestinal discomfort, etc.

The Scenario at Present

Learning that one's child has autism can be devastating news for parents. As they seek the best therapies and treatment for their children, they are left vulnerable to pseudoscientific theories and ineffective practices that may cause physical, emotional, and/or financial harm. Despite the deficit of solid proof supporting stem cell therapy as an effective treatment for autism, several

hospitals and clinics around the world have been offering this treatment to young autistic patients for years. The expenses incurred are high—over $450,000 USD (~₹37.3 lakh INR) in Western countries like the USA and upwards of $2,000 USD (~₹1.66 lakh INR) in India (Lyfboat, 2022). Besides, stem cell therapy is not recognized as an effective treatment for autism by the Indian Council of Medical Research (ICMR), raising legal and ethical questions concerning its practice. A hospital in Seawoods, Navi Mumbai, had its registration revoked by the Navi Mumbai Municipal Corporation (NMMC) on 24th February 2023 for violating ICMR guidelines, allegedly providing stem cell therapy to more than 12,000 autistic patients from 75 countries (Times of India, 2023). Although using stem cells to treat autism provides interesting avenues for research, the results of clinical trials held so far clearly indicate that the practice is still premature to become translational yet.

Conclusion

Ample research and literature exist connecting the link between genes that increase the risk factor of developing Autism and their abnormal immunological and neuropathological influences. However, more concrete data explaining their causative effects on the behavior of autistic patients are required to determine whether stem cell therapy is an effective treatment for autism and whether it is yet another tool for reducing symptoms or treating the disorder altogether.

References

Abrahams, B. S. & Geschwind, D. H. (2008). Advances in Autism genetics: on the threshold of a new neurobiology. *Nature reviews genetics, 9*(5), 341-355. https://doi.org/10.1038/nrg2346

American Psychiatric Association. (2000). Diagnostic and statistical manual of mental disorders. *Text revision.*

Bender, L. (1953). Childhood schizophrenia. *Psychiatric Quarterly, 27,* 663–681. https://doi.org/10.1007/BF01562517

Bender, L. (1971). Alpha and omega of childhood schizophrenia. *Journal of Autism & Childhood Schizophrenia*, *1*(2), 115–118. https://doi.org/10.1007/BF01537952

Beaudet, A. (2007). Autism: Highly heritable but not inherited. *Nature Medicine, 13,* 534-536. https://doi.org/10.1038/nm0507-534

Bongso, A., Fong, C. Y., & Gauthaman, K. (2008). Taking stem cells to the clinic: major challenges. *Journal of cellular biochemistry*, *105*(6), 1352-1360. https://doi.org/10.1002/jcb.21957

Chez, M., Lepage, C., Parise, C., Dang-Chu, A., Hankins, A., *et al.* (2018). Safety and observations from a placebo-controlled, crossover study to assess use of autologous umbilical cord blood stem cells to improve symptoms in children with autism. *Stem Cells Translational Medicine*, *7*(4), 333–341. https://doi.org/10.1002/sctm.17-0042

Dawson, G., Sun, J. M., Davlantis, K. S., Murias, M., Franz, L., *et al.* (2017). Autologous Cord Blood Infusions Are Safe and Feasible in Young Children with Autism Spectrum Disorder: Results of a Single-Center Phase I Open-Label Trial. *Stem cells*

translational medicine, 6(5), 1332–1339. https://doi.org/10.1002/sctm.16-0474

Dawson, G., Sun, J. M., Baker, J., Carpenter, K., Compton, *et al.* (2020b). A phase II randomized clinical trial of the safety and efficacy of intravenous umbilical cord blood infusion for treatment of children with autism spectrum disorder. *The Journal of pediatrics, 222*, 164-173. https://doi.org/10.1016/j.jpeds.2020.03.011

Diagnostic Criteria for Autistic Disorder through the years (2013) DSM-I - DSM-IV diagnostic criteria, University of Oregon.

Geschwind, D. H. (2009). Advances in Autism. *Annual review of medicine, 60*, 367-380.https://doi.org/10.1146/annurev.med.60.053107.121225

Geschwind, D. H. (2011). Genetics of Autism spectrum disorders. *Trends in cognitive sciences, 15*(9), 409-416. https://doi.org/10.1016/j.tics.2011.07.003

Gupta, S., Samra, D., & Agrawal, S. (2010). Adaptive and innate immune responses in Autism: rationale for therapeutic use of intravenous immunoglobulin. *Journal of Clinical Immunology, 30*(1), S90–S96. https://doi.org/10.1007/s10875-010-9402-9

Herbert, J. D., Sharp, I. R., & Gaudiano, B. A. (2002). Separating fact from fiction in the etiology and treatment of autism. A scientific review of the evidence. *The Scientific Review of Mental Health Practice, 1*(1), 23-43.

Juane Heflin, L., & Simpson, R. L. (1998). Interventions for children and youth with autism: Prudent choices in a world of exaggerated claims and empty promises. Part I: Intervention and treatment option review. *Focus on autism and other developmental disabilities, 13*(4), 194-211.

https://doi.org/10.1177/108835769801300401
Kim, S. U., & De Vellis, J. (2009). Stem cell-based cell therapy in neurological diseases: a review. *Journal of neuroscience research, 87*(10), 2183-2200. https://doi.org/10.1002/jnr.22054

Lv, Y. T., Zhang, Y., Liu, M., Qiuwaxi, J., Ashwood. P., *et al.* (2013). Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. *Journal of Translational Medicine, 11*, 196. https://doi.org/10.1186/1479-5876-11-196

Lyfboat. (2022). *Stem cell therapy for autism treatment*. Retrieved August 6, 2024, from https://www.lyfboat.com/stem-cell-therapy-for-autism-treatment/

Navi Mumbai hospital's registration revoked for using stem cell therapy for autism 'illegally'. (2023, February 26). *Times of India*, NA. https://link.gale.com/apps/doc/A738585731/HRCA?u=anon~96290ece&sid=sitemap&xid=0886c2a8[1]

Nguyen Thanh, L., Nguyen, H. P., Ngo, M. D., Bui, V. A., Dam, P. T., *et al.* (2021). Outcomes of bone marrow mononuclear cell transplantation combined with interventional education for autism spectrum disorder. *Stem cells translational medicine, 10*(1), 14-26. https://doi.org/10.1002/sctm.20-0102

Petrie Aronin, C. E., & Tuan, R. S. (2010). Therapeutic potential of the immunomodulatory activities of adult mesenchymal stem cells. *Birth Defects Research C, 90*(1), 67–74. https://doi.org/10.1002/bdrc.20174

Riordan, N. H., Hincapié, M. L., Morales, I., Fernández, G., Allen, N., *et al.* (2019). Allogeneic human umbilical cord mesenchymal stem cells for the treatment of autism spectrum disorder in children: safety profile and effect on cytokine levels. *Stem cells translational medicine, 8*(10), 1008-1016. https://doi.org/10.1002/sctm.19-0010

Rosenberg, R. E., Law, J. K., Yenokyan, G., McGready, J., Kaufmann, *et al.* (2009). Characteristics and concordance of autism spectrum disorders among 277 twin pairs. Archives of pediatrics & adolescent medicine, 163(10), 907-914. https://doi.org/10.1001/archpediatrics.2009.98

Rutter, M., & Bartak, L. (1971). Causes of infantile autism: Some considerations from recent research. Journal of autism and childhood schizophrenia, 1(1), 20-32. https://doi.org/10.1007/BF01537740

Sharifzadeh, N., Ghasemi, A., Tavakol Afshari, J., Moharari, F. *et al.* (2021). Intrathecal autologous bone marrow stem cell therapy in children with autism: a randomized controlled trial. Asia-Pacific psychiatry, 13(2), e12445. https://doi.org/10.1111/appy.12445

Sharma, A., Gokulchandran, N., Sane, H., Nagrajan, A., Paranjape, A., *et al.* (2013). Autologous bone marrow mononuclear cell therapy for autism:an open label proof of concept study. *Stem cells international, 2013*(1), 623875. https://doi.org/10.1155/2013/623875

Short, B., Brouard, N., Occhiodoro-Scott, T., Ramakrishnan, A., & Simmons, P. J. (2003). Mesenchymal stem cells. *Archivesof Medical Research, 34*(6), 565–571. https://doi.org/10.1016/j.arcmed.2003.09.007

Smith T. (2013). What is evidence-based behavior analysis? *The Behavior analyst, 36*(1), 7–33. https://doi.org/10.1007/BF03392290

Sotiropoulou, P. A., & Papamichail, M. (2007). Immune properties of mesenchymal stem cells. *Methods in Molecular Biology*, *407*, 225–243. https://doi.org/10.1007/978-1-59745-536-7_16

Sun, J. M., Dawson, G., Franz, L., Howard, J., McLaughlin, C., *et al.* (2020). Infusion of human umbilical cord tissue mesenchymal stromal cells in children with autism spectrum disorder. *Stem Cells Translational Medicine, 9*(10), 1137-1146. https://doi.org/10.1002/sctm.19-0434

World Health Organization. (1992). *The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines* (Vol. 1). World Health Organization.

Zhang, L., Tan, X., Dong, C., Zou, L., Zhao, H., *et al.* (2012). In vitro differentiation of human umbilical cord mesenchymal stem

cells (hUCMSCs), derived from Wharton's jelly, into choline acetyltransferase (ChAT)-positive cells. *International Journal of Developmenta`l Neuroscience, 30*(6), 471-477. https://doi.org/10.1016/j.ijdevneu.2012.05.006

Stem Cell Therapy for Autism in India (2022) https://www.lyfboat.com/stem-cell-therapy-for-autism-treatment/

Parida, K. U. (2023, February 25). Navi Mumbai hospital's registration revoked for using stem cell therapy for autism 'illegally'. Times of India. Retrieved from https://timesofindia.indiatimes.com/city/navi-mumbai/navi-mumbai-hospitals-registration-revoked-for-using-stem-cell-therapy-for-autism-illegally/articleshow/98220914.cms

Cite this Article

Joshi, P., Surti, A. (2023). Is stem cell treatment an effective treatment for autism? *SCRIBE, 4*(1), 67-77.

CAR T- CELLS: A CANCER IMMUNOTHERAPY

Vidushi Kandpal, Rajbinder Kaur Dehiya
Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Dr. Rajbinder Kaur Dehiya, Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai.
Email: rajbinder.dehiya@sophiacollege.edu.in

Abstract

The immune system prevents cells from becoming cancerous, but cancer cells find ways to evade detection and multiply. Immunotherapy stimulates the immune system to generate a stronger defense against cancer. Cancer immunoediting explains the interplay between immune and cancer cells through equilibrium, elimination, and escape stages. CAR T-cell therapy is an immunotherapy method using synthetic Chimeric Antigen Receptors (CARs) to enhance T-cell effectiveness in locating and eliminating cancer cells. It shows promise in treating hematologic and solid malignancies, including relapsed tumors. CAR T-cell therapy is a promising approach to potential cancer treatment.

Introduction

Cancer is a fatal disease whose incidence is rapidly increasing and thus needs to be slowed down. Cancer progression is closely linked to the interaction of immune cells and tumor forming cells. Immunity can be defined as an individual's ability to remain free of an infection or a disease to a significant extent (Willey *et al.*, 2008). The immune system recognizes a protein structure called antigen, present on the surface of a cell or any foreign substance like bacteria, toxins, drugs, etc., and generates an immune response by replicating specific cells that produce several types of proteins to protect the individual (Henochowicz, 2020)

The branch of immunology kick started with two major discoveries that gave insight into two major immune responses (Henderson, 2022):

1. Discovery of phagocytic cells by Elie Metchnikoff, forming the basis for innate immunity, which is the first line of defense that responds with similar intensity when interacting with any antigen.
2. Identification of antibodies by Emil Behring and Paul Ehrlich, forming the basis for acquired immunity, which has immunological memory and its response is more efficient when the same antigen enters the body.

Both innate and adaptive immunity is responsible for providing defense against cancer (Willey *et al.*, 2008).

Paul Ehrlich (1909) postulated that the immune system can identify and destroy early tumors, thereby keeping a check on cancerous cells. Despite this, cancer cells

manage to escape the immune system surveillance by suppressing major histocompatibility class (MHC) I expression, lacking co-stimulatory signals, secreting immunosuppressive products, and avoiding inflammatory signals (Cancer Australia, 2014).

Over the last decade, immunotherapy is emerging as a challenging strategy altering the approach to cancer treatment. It relies on the enhancement of the ability of the immune system to treat life-threatening diseases. Unlike chemotherapy, which aims on destroying rapidly growing cells, immunotherapy targets specific altered or overexpressed genes or proteins that are found on cancer cells, without damaging the nearby healthy cells (Sonpavde, 2019).

Cancer immunoediting is a dynamic process that allows the immune system to function as an extrinsic tumor suppressor on one hand and prevent further growth of the tumors by inducing an immune response intrinsically, on the other (Tavakoli *et al.*, 2021). It recognizes that every immune mechanism known, plays a part in destroying cancer cells. This can be explained by the cancer immunoediting theory of the three Es stages: Elimination, Equilibrium, Escape (Miliotou & Papadopoulou, 2018). This theory implies that a dynamic period exists where there is a destruction of tumor by the immune cells, but with time cancer cells develop mechanisms to escape this destruction. The term immunoediting is used as it views all the phases of interactions between immune cells and cancer cells (Abbott & Ustoyev, 2019).

In the elimination phase, (Figure 1) there is cooperation between the innate and the adaptive immune systems where they work together to detect and kill the developing tumors, much before they can be detected in clinical procedures. Natural immunity functions to identify cancer cells. The release of cytokines starts once the tumor grows beyond 2-3 mm, which further triggers the activation of NK cells, γδT-cells, macrophages, and dendritic cells. These recognize and kill cancer cells (Borroni & Grizzi, 2021).

Many malignant cells tend to escape the elimination phase and proceed into the second phase, i.e., the Equilibrium phase, which is the longest phase of cancer immunoediting, utilizing adaptive immunity to prevent their outgrowth. This phase is still not well-understood, but several researches suggest that an equilibrium state is achieved by the action of IL-12 and IL-23, where IL-12 promotes elimination and IL-23 promotes persistence of the tumor cells (Abbott & Ustoyev, 2019; Teng *et al.*, 2012). Despite this, cancer cells are able to develop immune evasion strategies with rapid proliferation and improper DNA damage control, which helps them to survive and enter the escape phase.

In the escape phase, cancer cells undergo genetic and epigenetic changes making them inexpressive to the major histocompatibility complex (MHC) and resistant to destruction by the immune system. This allows these cells to proliferate and become clinically detectable (Borroni & Grizzi, 2021).

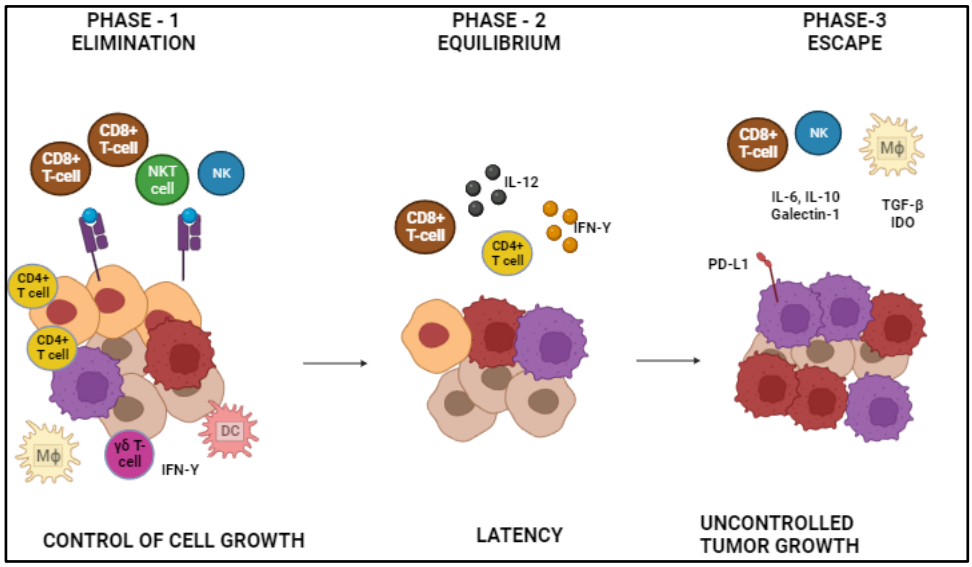


Figure 1. The Cancer Immunoediting Concept (Constantino *et al., 2017).*

Cancer immunotherapy is recognized as an innovative approach towards cancer treatment that utilizes the body's immune system to eliminate cance cells, simultaneously fighting the mechanisms that allow cancer cells to go unnoticed by the immune surveillance (Fiona S, 2023).

Cancer immunotherapy

Cancer treatment using immunotherapy consists of several types of immunotherapies (National Cancer Institute, 2019).

1. Immune Checkpoint Inhibitors

These are protein molecules that function as a switch to help regulate the intensity of the immune response. (American Cancer Society - Immune checkpoint inhibitors and their side effects, 2022). They target inhibitory signaling pathways, namely CTLA-4 (Cytotoxic T-Lymphocyte associated protein 4), PD-1/PD-L1 (programme death receptor-1/ligand-1), reactivating immune cells, mostly T-cells, in order to maximize tumor elimination (Fiona S, 2023).

Certain drugs, like pembrolizumab and atezolizumab, are used to block these checkpoints to have a strong immune response towards cancer cells. (National Cancer institute, 2019). Pembrolizumab, a humanized monoclonal IgG4 kappa antibody, targets PD-1 protein found on T-cell surfaces. It disrupts the immune suppression by preventing PD-1 from binding to its ligands (PD-L1 and pD-L2 on antigen presenting cells), leading to tumor destruction through T-cell activation (Flynn & Gerriets, 2023). Atezolizumab is a humanized monoclonal anti-programmed death-ligand 1 (PD-L1) antibody that blocks PD-L1 from binding to PD-1 and allows the killing of cancer cells by tumor-specific cytotoxic T-cell immunity (Aleem & Shah, 2023).

2. Therapeutic Antibodies

Lab-designed proteins, known as monoclonal antibodies, bind to specific cancer cell targets, marking them for easy detection by the immune system (National Cancer Institute, 2019).

Antibodies have immunomodulatory properties that allow the elimination of cancerous cells by several immune-mediated cell-killing mechanisms, like T-cell regulation, antibody-dependent or complement-dependent cell-mediated toxicity, or by direct action of antibody resulting in apoptosis or blocking a receptor and ultimately destroying tumor cells (Abbott & Ustoyev, 2019).

Pembrolizumab, Atezolizumab, Ipilimumab are examples of checkpoint inhibitor monoclonal antibodies, while Blinatumomab (approved to treat acute lymphoblastic leukemia) targets both CD19 on B-cells and CD-3 on T-cells, activating cytotoxic T-cells to eliminate CD19 expressing malignant lymphoblasts (Eltarhoni *et al.*, 2022).

3. Treatment vaccines

These are vaccines that fight cancer by boosting the response of the immune system toward tumor cells. These vaccines initiate a desired anti-tumor response either directly delivering tumor antigens or activating antigen-presenting cells to process and present tumor antigens effectively to T-cells. Some of such vaccine formulations include peptide-based vaccines, whole tumor cell vaccines, viral vector-based vaccines, and nucleic acid-based vaccines (Fiona S, 2023).

4. Immune system modulators

These helps boost the immune response towards cancer, by either targeting certain parts of the immune system or enhancing the functions of the entire immune system. Cytokines, like interferons (INFs) and interleukins (ILs) are used to boost immune response and WBCs production, respectively, thereby promoting an immune response and helping B cells target cancer cells. Thalidomide, lenalidomide, pomalidomide, and imiquimod are some drugs that release cytokines while inhibiting new blood vessel formulation crucial for tumor growth (National Cancer institute, 2019).

5. T-cell transfer therapy

This therapy, also known as adoptive cell therapy (ACT), enhances the body's ability to fight cancer by stimulating the T cells. ACT exploits the natural ability of immune cells to recognize and kill tumor cells (Fiona S, 2023).

T-cell transfer therapy is of two major types:

a) Tumor-infiltrating lymphocyte (TIL) therapy: Active TILs are selected and cultured ex vivo, then reintroduced into the patient’s body, overcoming the tumor’s ability to evade immune recognition and promoting destruction (National Cancer Institute, 2019).
b) Chimeric antigen receptor (CAR) T-cell therapy: Patient’s T-cells are genetically modified to express a synthetic receptor binding to a specific tumor antigen. These CAR T-cells are expanded and infused back into the patient’s body to target and destroy chemotherapy-resistant tumor cells without the involvement of endogenous T-cell receptors (Feins *et al.*, 2019). Engineered CAR binding to tumor-associated antigen (TAA) activates the killing of tumor cells by various immune responses such as secretion of granzymes and perforins

along with expression of TRAIL and FasL (Jogalekar *et al.*, 2022).

Structure of CAR T-cell

A typical CAR structure comprises an extracellular domain, hinge, transmembrane domain, and an intracellular signaling domain (Figure 2).

CAR T- cells recognize tumor antigens via the ectodomain, which initiates signal transduction through the hinge, transmembrane, and co-stimulation domains in order to activate transcription factors, proliferate, survive, and release cytokines resulting in the cytotoxic response (Naing & Hajjar, 2020)

Ectodomain or Extracellular Domain (ECD) - It functions as a tumor-associated antigen (TAA), similar to a lock and key for target antigen specificity. A target TAA (can be any carbohydrate, protein, or glycolipid), is universally expressed on cancer cells, rarely lost in refractory disease, and is not expressed on non-essential normal tissue. ECD comprises an antigen-binding moiety (scFV) and a spacer. Single-chain fragment variable (scFv) is a type of antigen-binding moiety that can be derived from mouse monoclonal antibodies (mAbs), humanized Abs, or complete human Abs. It is responsible for detecting and binding TAAs on the tumor cells (Miliotou & Papadopoulou, 2018).

Hinge (Spacer) - It connects the scFv to the transmembrane domain and impacts overall function, T-cell expansion, and cytokine production. It is obtained from the constant Fc portion of IgG subclass immunoglobulins. Its length can impact the flexibility of the scFv, but can also make Fc more vulnerable towards off-target FcR receptors (Naing & Hajjar, 2020).

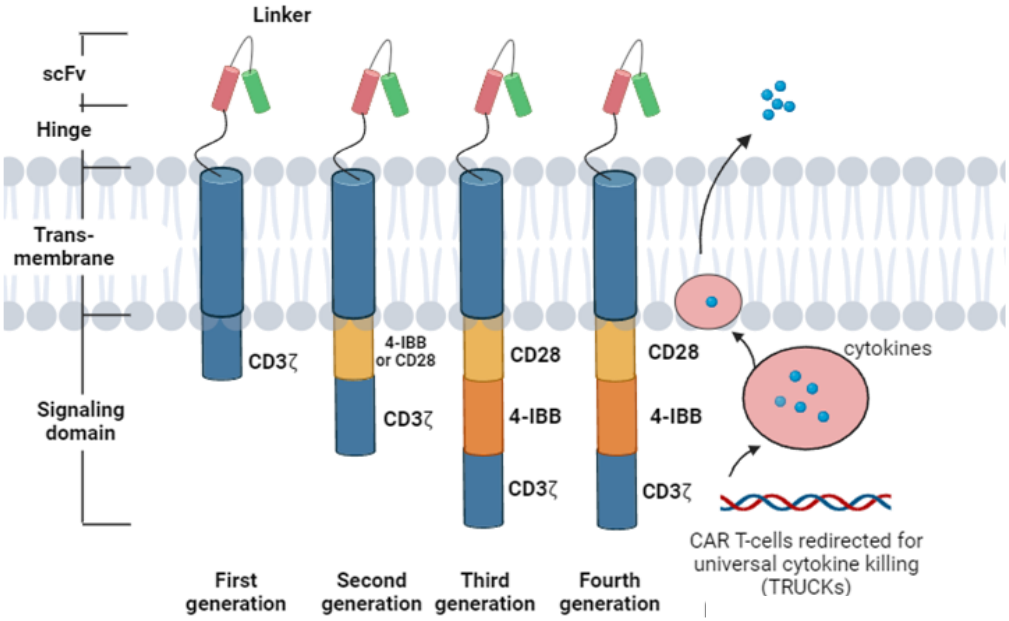


Figure 2. Structure of CAR-T cells (Miliotou *et al.*, 2018)

Transmembrane Domain - It is situated between the hinge and the ECD supporting the cell membrane and serves as a signal gateway to the intracellular compartment. It is derived from CD3-ζ, CD4, CD8, or CD28 molecules (Naing & Hajjar, 2020).

Intracellular Domain - It is connected to the transmembrane domain and contains signaling moieties like CD3ζ which delivers the first signal for the activation of T-cells (Miliotou & Papadopoulou, 2018).

As per the structure of the intracellular domain, CAR-T cells are of four generations (Naing & Hajjar, 2020):

1. The first generation of CARs consists of a ζ chain of complex TCR/CD3 (CD3ζ).
2. The second-generation CARs have a dual signal for T-cell activation. Antigen recognition triggers one and co-stimulatory molecules like CD28 trigger the other. This results in the synthesis of IL-2 and T-cell activation occurs.
3. The third-generation CARs combine co-stimulatory sequence signals which tend to enhance cytokine production which results in enhanced T-cell functions.
4. Fourth-generation CARs or "armored" CARs, include additional stimulatory domains, such as T-cells redirected for universal cytokine-mediated killing (TRUCK), which secretes pro-inflammatory cytokine IL-12, stimulating the innate immune cells that resist tumors and inhibitory elements of the tumor microenvironment (Hong *et. al.,* 2020).

CAR-T cell therapy has shown promise in treating malignancies, including leukemia and lymphomas and thus can be considered the breakthrough therapy of this century (Naing & Hajjar, 2020). The breakthrough case of Emily Whitehood is one of the most popular cases of acute lymphoblastic leukemia (ALL). She became a part of the NCT01626495 trial, along with patients suffering from blood malignancies and solid tumors at the age of six (Hartmann et al, 2017) and is cancer-free to date (Emily Whitehead Foundation, 2022).

Production/ generation of CAR-T cells

The procedure begins with leukapheresis, which is a process of separating the white blood cells from the blood sample using a blood separator, and the T-cells of the patient or the donor are collected. These T-cells are put in a cell-processing center where the selected T-cells are exposed to IL-2 (interleukin-2) or anti-CD3 (anti-cytoplasmic domain 3) antibodies which allow them to grow. Using a retroviral or lentiviral vector, CAR genes are transfected into T cells, and then these cells are allowed to expand. After synthetically creating these CAR T-cells, these are injected into the patient (Feins *et al.*, 2019).

Before infusing the CAR T-cells into the patient, the endogenous T-cells are reduced using chemotherapy (lymphodepletion). Stimulation of cytokines like IL-7 and IL-5 is followed which helps in the growth of CAR T-cells (Makita *et al.*, 2017).

Limitations and their potential strategies

Despite being a helpful tool in treating cancer, certain limitations were observed to be hindering the efficiency of CAR-T cell

therapy. Some of these limitations with their potential resolutions are as follows:

1) Antigen Escape

Antigen escape occurs when cancer cells lose expression of CD19 surface antigen, leading to disease relapse post-CAR-T cell therapy due to an inability of anti-CD19 antibodies present in CARs to bind to CD19 antigen (Majzner & Mackall, 2018). Such relapses usually arise from pre-existing antigen-negative cancer cells or cells with altered/mutated antigen expression.

This occurs because of genetic and epigenetic changes. In pediatric B-ALL patients at the Children's Hospital of Philadelphia (CHOP), there was loss of surface antigens as a result of splicing CD19 into different variants that lack critical parts of the CD19 molecule resulting in relapse after CAR-T cell therapy (Majzner & Mackall, 2018). In another case, there was loss of CD19 antigen due to the loss of CD81, a chaperone protein for CD19, in a patient after blinatumomab therapy (Majzner & Mackall, 2018)

In order to resolve this issue, other targets or antigens should be explored. For example, TCR-like CAR-T cells are supposed to target intracellular oncoproteins thereby activating antitumor responses (Huang *et al.*, 2022). Similarly, CAR designs targeting multiple receptors, such as dual targeted CAR-T cells targeting CD19/CD22 (efficient in cases of ALL) or CD19/BCMA (efficient in cases of multiple myeloma), are found to be effective (Sterner & Sterner, 2021). In addition to that, combining therapies has shown to enhance CAR-T cell activity, allowing non-antigen specific killing of leukemia cells (Huang *et al.*, 2022).

2) On Target off-tumor Effects

Targeting solid tumor antigens poses challenges due to potential off-target toxicity in normal tissues. Lack of defining the specific tumor antigen would increase the potential risk of significant off-target off-tumor toxicity (Martinez and Moon, 2019). A way of overcoming incorrect off-tumor targeting of solid tumor antigens is to target tumor-restricted post-translational modifications such as solid tumor overexpressed truncated O-glycans (Sterner & Sterner, 2021). It is important to select tumor-associated antigens (TAAs) safely because even a lower number of them on normal tissues can cause severe toxicity. A case involving a patient receiving human epidermal growth factor receptor 2 (HER2) targeted CAR-T cells died due to damage of the epithelial cells of lungs (Martinez and Moon, 2019). Thus, it is important to target antigens that are overexpressed on the tumor cells and less expressed in the normal tissues.

3) CAR T-cell trafficking and tumor infiltration

The characteristics of solid tumors such as the immunosuppressive Tumor Microenvironment (TME) and physical tumor barriers like the tumor stroma tend to limit the ability of CAR T-Cells to traffic and infiltrate the solid tumors. This is not seen as such in hematological malignancies. A TME that has low oxygen levels, poor vessel formation and an increased amount of extracellular matrix often causes limited

infiltration of T-cells and a poor sense of recognition for specific tumor antigens by them (Daei Sorkhabi *et al.*, 2023).

One strategy to improve upon these limitations is to make use of local administration and alternate delivery routes which eliminates the need for CAR T-cells to traffic to disease sites as well as limits the on-target off-tumor toxicities. Pre-clinical models of CAR T-cells being injected and targeting HER2 and IL-3Ra2 in breast cancer, brain metastases and glioblastoma, have shown superior therapeutic efficacy. However, this injection method is limited to single tumor lesions and/or oligometastatic disease (Sterner & Sterner, 2021).

4) Immunosuppression

In the tumor microenvironment (TME), immunosuppressive cell types like MDSCs (myeloid-derived suppressor cells), TAMs (tumor-associated macrophages), and Tregs infiltrate solid tumors. These cells produce cytokines, chemokines, and growth factors that support tumor growth. Immune checkpoint pathways like PD-1 and CTLA-4 further reduce anti-tumor immune responses. Some reasons that cause the weak response to CAR T-cell therapy are poor T-cell expansion and short-term T-cell persistence. Scientists guess that this might be due to co-inhibitory pathways. Thus, it has been recommended that combination immunotherapy with CAR T-cells and checkpoint blockade be the next immunotherapy frontier. This is because it provides the two elements necessary for strong immune responses as follows:

a) CAR T-cells that provide infiltration.
b) PD-1/ PD- L1 blockade that ensures sustained T cell persistence and function (Sterner & Sterner, 2021).

For example, in multiple myeloma (MM), CAR-T cell therapy is majorly affected by cancer-associated fibroblasts (CAFs) present in the tumor microenvironment. In order to rectify this, researchers suggest a dual-target strategy which aims on targeting both CAFs and MM cells to increase anti-tumor activity and better functioning of CAR-T cells. This strategy however, causes infiltration of TAMs and MDSCs, leading to immunosuppression and failure of CAR-T cell therapy in cases of Hodgkin's lymphoma. Gemtuzumab ozogamicin or Phosphoinositide 3-kinase (PI3K) blockade is then employed to target TAMs or MDSCs and boost CAR-T cell activity against various cancers (Huang *et al.*, 2022).

5) CAR T-cell-associated toxicities

CAR-T therapy often faces high toxicity rates, leading to life-threatening complications, especially in ALL/LBL patients, where most patients were found to have minor amount of toxicity and 23-46% of them suffered with in-vivo T-cell expansion and severe supraphysiologic cytokine production (Sterner & Sterner, 2021).

Such cytotoxic levels of systemic cytokine release and severe immune cell cross-activation in patients results in Cytokine release syndrome (CRS), Hemophagocytic lymphohistiocytosis (MAS) and immune effector cell-associated neurotoxicity syndrome (ICANS). CRS involves excessive

cytokine production and T-cell expansion. MAS is an inflammatory syndrome characterized by elevated ferritin, hemophagocytosis, renal failure, and more. ICANS causes cerebrospinal fluid cytokine elevation and brain barrier disruption (Sterner & Sterner, 2021).

In order to overcome these shortcomings, CAR-T cells are to be designed to secrete anti-IL-6 scFv and IL-1RA, in order to neutralize IL-6 and IL-1β, since CRS is closely linked to IL-6 and IL-1β, which will thereby help in reducing chances of CRS and ICANS in treated patients. Moreover, granulocyte-macrophage colony-stimulating factor (GMCSF) has been identified as a key CRS-promoting protein released from CAR-T cells and needs to be replaced or neutralized in order to achieve less of CRS-associated cytokine without hindering the anti-tumor ability of CAR-T cells (Huang *et al.*, 2022).

Blood cancer treatment by CAR T-cell therapy

Hematological malignancies are easier to target as compared to solid tumors. The first few applications for hematological malignancies have been for Acute Lymphoblastic Leukaemia (ALL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). The Food and Drug Administration (FDA) has approved five CAR T-cell therapies, four of which make use of CD19 as the target antigen. For treating multiple myeloma, the FDA recently approved an anti-BCMA CAR T-therapy (Idecabtagene viclaucel) (Haslauer *et al.*, 2021).

Acute Lymphoblastic Leukaemia

ALL is caused by malignant precursor B- or T- lymphocytes which affect the production of normal cells in the bone marrow. FDA has approved only one CAR-T cell therapy to date, which was given by Novartis and is named Kymriah and is effective and is effective in treating B-ALL. It targets the surface marker CD19, leading to a well-tolerated rise in B-cell numbers as an off-tumor effect (Haslauer *et al.*, 2021).

Chronic Lymphocytic Leukaemia

In CLL, CD5 or CD19 double-positive B cells accumulate in peripheral blood and lymphoid compartments, together with some dysregulation of the immune system which may include dysfunctional T-cells with impaired synapse formation, and diminished proliferation capacity of T-cells, a damaged phenotype and a diminished ability to execute cytotoxicity. CAR T-cell therapy using CD19 as a target in cases of relapsed or refractory disease has not shown desirable results (Haslauer *et al.*, 2021).

Richter's Syndrome

Richter's syndrome is a malignant form of CLL, like a diffuse large B-cell lymphoma (DLBCL), which has a poor recovery rate. A study was done at Ohio State University, in which nine patients underwent CD19 targeted therapy, axicabtagene-ciloleucel. One died because of infections, and the remaining eight were pre-treated with kinase inhibitors, five of which showed a complete, with a partial response in the remaining three. The results were encouraging and so far, however, one patient died. Still, further

investigations are needed. (Haslauer *et al.*, 2021)

Lymphoma

40% of all lymphomas comprise non-Hodgkin lymphomas (NHL), of which diffuse large B-cell lymphoma (DLBCL) is the most common form. A ZUMA (Zelboraf and Unum Therapeutics in Monoclonal Antibody is a series of clinical trials conducted to evaluate the safety and efficacy of CAR-T cell therapies for treating various types of cancer, particularly lymphomas) study was conducted in which CD19-targeted CAR-T cells, Yescarta, were used to treat patients of DLBCL, in which 58% showed a complete response while 25% showed a partial response. Breyanzi (Lisocabtagene marleucel), a new CAR-T cell therapy for the treatment of refractory large B-cell lymphomas was approved by the FDA in March 2021. Tecartus (Brexucabtagene autoleucel) is another anti-CD19 CAR T-cell therapy that FDA approved for treating mantle cell lymphoma (MCL) (Haslauer *et al.*, 2021).

Multiple Myeloma

Multiple myeloma is cancer where malignant plasma cells accumulate in the bone marrow, affecting normal hematopoietic cell production and osteoblast function. This leads to the production of non-functional immunoglobulins called paraproteins. There is a low amount of expression of CD19 on the surface of the cells due to which the CD19-targeted CART-T therapy is ineffective in MM patients. Clinical trials are ongoing to investigate different targets, such as BCMA on mature B-cells and plasma cells which can be effective for CAR-T therapy in MM. Syndecan-1 or CD138 is a new target in interest that is expressed on MM cells, whose safety and efficiency when explored had about an 80% of response rate (Haslauer *et al.*, 2021).

Acute Myeloid Leukemia

AML is a disease occurring because of genetic or epigenetic changes affecting the myeloid blood cell lineage and disturbing the normal blood cell production in the bone marrow. CAR-T therapy is done with a combination of targets as there is no particular targetable antigen for AML, and healthy hematopoietic stem and progenitor cells (HSPCs) also express many myeloid antigens which can lead to bone marrow destruction (Haslauer *et al.*, 2021).

Solid tumors

It is a challenge to target solid tumors with CD19 CAR-T cells therapy due to a range of factors (Miliotou & Papadopoulou, 2018):

1. The tumor cells are genetically unstable which might prevent antigenic expression on them that T-cells target.
2. There has been limited success as of now with CAR T-cell therapies for solid tumors due to the tumor histopathological characteristics, the inadequate "trafficking" of CAR T-cells to tumor sites, and the difficulty in identifying and targeting specific antigens in tumor tissue, and tumor heterogeneity.
3. Activation of T-cells causes a dense tumor microenvironment that produces hypoxia, low pH, lack of arginine or

tryptophan, inhibitory effects of tumor-derived cytokines, and fast depletion of CAR T-cell function and efficacy. Many cases of rapid death have been linked to the "off-target" cross-reaction of CAR T-cells.

These challenges have been tried to overcome using various strategies, which include:

a) **Introducing a chemokine receptor gene**: These are used to match the chemokines produced by tumor or tumor-associated cells. E.g., CCR2b binds to CCL-2 secreted by neuroblastoma cells (Hong *et. al.*, 2020).

b) **Engineering CAR T-cells** with basement membrane-degrading enzyme: Heparan sulfate proteoglycan (HSPG) is the primary component of ECM present in the stroma, which has to be degraded for T-cells in tumors surrounded by stroma. Engineering CAR-T cells with the enzyme Heparanase (HPSE) has been found useful in degrading HSPG and showed the improved capability of CAR-T cells (Ma *et al.*, 2019).

c) **Developing iCARs**: iCARs are antigen-specific inhibitory CAR-T cells that are capable of recognizing antigens on tumors as well as off-target tissues which can be restricted to tumors only, to prevent damaging the off-target tissues. They are designed to prevent rather than treat the complications arising from inadequate T-cell specificity.

d) **Logic-gated CARs**: Genetically engineered CAR T-cells express multiple specific receptors that act as sensors to recognize specific antigens. This strategy is based on George Boole's algebraic principle of Boolean logic gates. Such genetically engineered CAR T- cells are highly effective in detecting tumors based on combinations of multiple antigens expressed by cancer cells (Savanur *et al.*, 2021).

e) **Combined T-cell therapies** with immunomodulatory agents such as checkpoint inhibitors, cytokines, or small molecules like adenosine, which block pathways critical to tumor growth, may result in enhanced anti-tumor responses.

Conclusion

Immunotherapies, like CAR-T cell therapy, have revolutionized cancer treatment. CAR-T cell therapy demonstrates effectiveness in both hematological and solid cancers. While many patients in trials have seen complete or partial success, accessibility is hindered by its high cost. It offers hope in cancer treatment, but more research and strategic adaptations are needed to make it successful to a broader spectrum of cancer. Exploring new solutions and combining different immunotherapies will likely enhance its functionality, efficiency, and accessibility.

References

Abbott, M., & Ustoyev, Y. (2019). Cancer and the immune system: The history and background of immunotherapy. *Seminars in Oncology Nursing*, *35*(5), 150923. https://doi.org/10.1016/j.soncn.2019.08.002

Aleem, A., & Shah, H. (2023, January 9). Atezolizumab - *StatPearls - NCBI Bookshelf*. https://www.ncbi.nlm.nih.gov/books/NBK567758/

Borroni, E. M., & Grizzi, F. (2021). Cancer immunoediting and beyond in 2021. *International Journal of Molecular Sciences*, *22(24)*, 13275. https://doi.org/10.3390/ijms222413275

Cancer Australia. (2014). The immune system and cancer. EdCaN. Retrieved May 10, 2022, https://www.edcan.org.au/edcan-learning-resources/supporting-resources/biology-of-cancer/defining-cancer/immune-system

Constantino J, Gomes C, Falcão A, Neves BM, Cruz MT. (2017). Dendritic cell-based immunotherapy: a basic review and recent advances. *Immunol Research*, *65*(4), 798-810. https://doi.org/10.1007/s12026-017-8931-1

Daei Sorkhabi, A., Mohamed Khosroshahi, L., Sarkesh, A., Mardi, A., Aghebati-Maleki, A., *et al.* (2023). The current landscape of car T-cell therapy for solid tumors: Mechanisms, research progress, challenges, and Counterstrategies. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1113882

Emily Whitehead Foundation - Activate the Cure® for Childhood Cancer. (2022, May 12). Celebrating 10 Years cancer free. Emily Whitehead Foundation. Retrieved May 15, 2022, from https://emilywhiteheadfoundation.org/news/celebrating-10-years-cancer- free/

Feins, S., Kong, W., Williams, E. F., Milone, M. C., Fraietta, J. A. (2019). An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer. *American Journal of Hematology*, *94*(S1), S3-S9. https://doi.org/10.1002/ajh.25418

Fiona, S. (2023). Targeting the immune system: Mechanistic insights into the immunotherapeutic approaches for cancer treatment. *J Pharmacol Ther Res*., 7(4), 158 10.35841/aajptr-7.4.158

Flynn, J. P., & Gerriets, V. (2023, June 26). Pembrolizumab – *StatPearls – NCBI Bookshelf*. https://www.ncbi.nlm.nih.gov/books/NBK546616/

Haslauer, T., Greil, R., Zaborsky, N., Geisberger, R. (2021). CAR T-Cell Therapy in Hematological Malignancies. *International Journal of Molecular Sciences*, *22*(16), 8996-9007. https://doi: 10.3390/ijms22168996. PMID: 34445701; PMCID: PMC8396650.

Hartmann, J., Schüßler-Lenz, M., Bondanza, A., Buchholz, C. J. (2017). Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. *EMBO Molecular Medicine*, *9*(9), 1183–1197. https://doi.org/10.15252/emmm.201607485

Henderson, E. (2022). Humoral vs cell-mediated immunity. *News-medical.net.* Retrieved May 10, 2022, https://www.news-medical.net/health/Humoral-vs-Cell-mediated-Immunity.aspx

Henochowicz, S. I. (2020). Immune response: Medlineplus medical encyclopedia. *MedlinePlus*. Retrieved May 10, 2022.

https://medlineplus.gov/ency/article/000821.htm

Hong, M., Clubb, J. D., Chen, Y. Y. (2020). Engineering CAR-T cells for next-generation cancer therapy. *Cancer Cell*, 38(4), 473–488. https://doi.org/10.1016/j.ccell.2020.07.005

Huang, J., Huang, X., Huang, J. (2022). Car-T cell therapy for hematological malignancies: Limitations and Optimization Strategies. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.1019115

Huang, X., Chen, S., Li, W., Li, Y., Zhang, Y., *et al*. (2022). Gemtuzumab ozogamicin or Phosphoinositide 3-kinase (PI3K) blockade is employed to target TAMs or MDSCs and boost CAR-T cell activity against various cancers. *Journal of Cancer Research and Clinical Oncology, 148*(3), 789-799. https://doi.org/10.1007/s00432-021-03718-4

Immunotherapy for cancer. National Cancer Institute. (2019, September 24). Retrieved May 10, 2022, from https://www.cancer.gov/about-cancer/treatment/types/immunotherapy

Immune checkpoint inhibitors and their side effects. American Cancer Society. (2022, March 22). Retrieved May 10, 2022, from https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/immune-checkpoint-inhibitors.html

Jogalekar, M. P., Rajendran, R. L., Khan, F., Dmello, C., Gangadaran, P., *et al*. (2022). Car T-cell-based gene therapy for cancers: New Perspectives, challenges, and clinical developments. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.925985

Ma, S., Li, X., Wang, X., Cheng, L., Li, Z.,*et al*. (2019). Current progress in car-T cell therapy for solid tumors. *International Journal of Biological Sciences, 15(12),* 2548–2560. https://doi.org/10.7150/ijbs.34213

Makita, S., Yoshimura, K., & Tobinai, K. (2017). Clinical development of anti-CD19 chimeric antigen receptor T-cell therapy for B-cell non-Hodgkin Lymphoma. *Cancer Science*, 108(6), 1109–1118. https://doi.org/10.1111/cas.13239

Martinez, M., & Moon, E. K. (2019). Car T cells for solid tumors: New strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Frontiers in Immunology,* 10, 128. https://doi.org/10.3389/fimmu.2019.00128

Majzner, R. G., & Mackall, C. L. (2018). Tumor antigen escape from car T-cell therapy. *Cancer Discovery*, *8*(10), 1219–1226. https://doi.org/10.1158/2159-8290.cd-18-0442

Miliotou, A. N., & Papadopoulou, L. C. (2018). Car T-cell therapy: A new *era in cancer immunotherapy*. *Current Pharmaceutical Biotechnology*, *19*(1), 5–18. https://doi.org/10.2174/1389201019666180418095526

Naing, A., & Hajjar, J. (2020). Immunotherapy. *Advances in Experimental Medicine and Biology,* 1244(3), 215–220. https://doi.org/10.1007/978-3-030-41008-7

Savanur MA, Weinstein-Marom H, Gross G. (2021) Implementing Logic Gates for Safer Immunotherapy of Cancer. *Front Immunol.*;12:780399. https://doi.org/10.3389/fimmu.2021.780399 PMID: 34804073; PMCID: PMC8600566.

Sonpavde, G. P. (2019). Immunotherapy: What you need to know. *Harvard Health Blog.* https://www.health.harvard.edu/blog/immunotherapy-what-you-need-to-know-2019012415788

Sterner, R. C., & Sterner, R. M. (2021). Car-T cell therapy: Current limitations and potential strategies. *Blood Cancer Journal*, 11(4). https://doi.org/10.1038/s41408-021-00459-7

Tavakoli, F., Sartakhti, J. S., Manshaei, M. H., Basanta, D. (2021). Cancer immunoediting: A game theoretical approach. *In Silico Biology*, *14(1-2)*, 1–12. https://doi.org/10.3233/isb- 200475

T-cell transfer therapy - immunotherapy. *National Cancer Institute*. (2022). Retrieved May 12, 2022 https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/t-cell-transfer-therapy

Teng, M. W., Vesely, M. D., Duret, H., McLaughlin, N., Towne, J. E., *et al.* (2012). Opposing roles for il-23 and IL-12 in maintaining occult cancer in an Equilibrium State. *Cancer Research, 72*(16), 3987–3996. https://doi.org/10.1158/0008-5472.can-12-1337

Willey, J. M., Woolverton, C. J., Sherwood, L. M., Prescott, L. M., Harley, J. P., *et al.* (2008). Prescott, Harley, and Klein's microbiology (7th ed.). McGraw-Hill.

Cite this article:

Kandpal, V., & Dehiya, R. K. (2023). CAR-T Cells: A Cancer Immunotherapy**.** *SCRIBE, 4*(1), 78-91.

Nobel Prizes in Science (2022)

Nobel Prize in Chemistry 2022: Newer and better molecules: Just a click away

Samika Pandey, Rajani Khandagale
Department of Chemistry, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Sr. Rajani Khandagale, Department of Chemistry, Sophia College for Women (Empowered Autonomous), Mumbai. Email: rajani.khandagale@sophiacollege.edu.in

The Nobel Prize, instituted by Alfred Nobel, has, for years, been the benchmark par excellence, and the highest possible honor for any scientist, reserved for those whose works have the potential to change the direction of the world. In 2022, the Nobel Prize in Chemistry was awarded to Carolyn R. Bertozzi, Barry Sharpless and Morten Meldal, for the development of 'Click chemistry and bioorthogonal chemistry'.

Abstract

Chemists are forever locked in the quest for making better and more useful molecules with improved properties also in finding better ways to synthesize them. Click chemistry as a conceptual tool helps accomplish these exact goals. The Nobel Laureates for Chemistry 2022; Dr. Sharpless and Dr. Meldal with their discovery of the copper catalyzed azide-alkyne cycloaddition reaction (widely regarded as the crowning jewel of click chemistry) have laid the foundations for click chemistry as a whole new area of research. Within the larger scope of click chemistry Dr. Carolyn Bertozzi pioneered the field of bioorthogonal chemistry which made use of reactions that were chemoselective in biological systems. By adapting the CuAAC to biological systems, Dr. Bertozzi unlocked a world of prospects for bioorthogonal chemistry, for which she was jointly awarded the Nobel Prize in Chemistry for 2022.

The Concept

Click chemistry is a conceptual field that deals with molecular rearrangements and syntheses to form molecules with desired properties. The guiding principle as articulated by Dr. Barry Sharpless was the idea that any target set of properties required either in material science, synthetics, drug development or any of the myriad other fields of study can be obtained by the joining of small molecular "building blocks" in a certain way; given that a suitable, specific and reliable method for forming that particular bond is known. Dr. Sharpless laid down a set of criteria for a reaction to qualify as a click reaction. Click chemistry thus deals with simple high yielding reactions with a vast scope. Dr. Sharpless realized that the process of creating an inventory of possible molecular species as candidates for drug development could be streamlined to a great extent by utilizing more robust efficient reactions for preparation. The criteria for a reaction to

qualify as a click reaction are: 1) wide-scoped, modular reactions, 2) simple operating conditions and high yield 3) they should occur without solvent or using only benign solvents, and 4) should be stereoselective and highly selective for one pproduct.

Several reactions, including nucleophilic ring opening of strained heterocyclic rings and Diels-Alder reaction were listed as candidates for click chemistry but the most promising was the reaction which would become synonymous with click chemistry – 1,3-dipolar cycloaddition reaction between azides and alkynes.

The term “bioorthogonal chemistry” was introduced by Dr. Carolyn Bertozzi to be representative of a group of highly selective, high-yielding chemical transformations that proceed without side reactions and do not commonly occur in biological systems. These reactions are intrinsically chemoselective, can easily achieve site-selective labeling, are non-toxic and occur at biocompatible pH and temperatures. Hence, a vast range of biomolecules can be tagged using bioorthogonal chemistry-based reactions allowing for a range of utility (Scinto *et al.*, 2021).

Bioorthogonal reactions proceed such that only one target functional group of the biomolecule undergoes reaction, the rest remain unaffected. Dr. Bertozzi and her team worked to bring about these reactions specifically the azide-alkyne cycloadditions in biological systems making use of strained cyclic alkynes instead, which are capable of undergoing strain-promoted azide-alkyne cycloadditions (SPAAC) eliminating the use of copper.

How it happened

In the 1950's and 60's, several eminent scientists worked on the mechanism for 1,3-dipolar cycloadditions reactions including the likes of Linus Pauling, Kurt Alder and Rolf Huisgen. Huisgen, studied resonating structures of azides containing a 1,3-dipole which allowed them to react with alkenes and alkynes to form 5-membered ring structures. This laid the foundations for Dr. Barry Sharpless to identify this reaction as a candidate for click chemistry.

However, the low rate of reaction for the synthesis at room temperature and the poor regioselectivity under ambient conditions was a major barrier. Heating the system in turn resulted in the azide acting as an explosive at high temperatures therefore making the process unfeasible. The breakthrough came in 2001 when Dr. Morten Meldal and his team were working on methods of introduction of the 1,4-substituted 1,2,3-triazole as pharmacophore in peptides and discovered that use of copper as catalyst for the cycloaddition of azide to terminal alkynes substantially increased the rate of reaction. The reaction had high yields of up to 95% and took place at ambient temperatures. They used a system with both aryl and alkyl azides and found the resultant chemistry to be compatible with solid phase peptide synthesis.

Around the same time, Dr. Sharpless and his colleagues independently discovered that use

of copper salts in conjunction with a reducing agent like ascorbate proceeded with similarly high yields for a variety of starting materials and produced 1,4-disubstituted 1,2,3-triazole exclusively irrespective of pre-existing groups thus showing high specificity.

Thus, the CuAAC reaction became the very embodiment of click chemistry – the efficiency with which this reaction proceeds to completion and just within a few hours at ambient temperature in a variety of solvents including alcohols and water, robustness and ease of operation; all these properties rapidly made it the preferred method of transformation of one structural entity to be conjoined with another irrespective of steric or electronic effects of the substituents present on either of the reactants.

The versatility of the CuAAC reaction made it perfect for various applications including biological ones but the chief stumbling block was the toxicity of copper to living cells. However, Dr. Carolyn Bertozzi and her team used surface engineered cells to express azide-containing glycoproteins. Using biotinylated (the process of attaching biotin to macromolecules) cyclooctyne rings which are highly reactive to cycloadditions with azides, owing to their highly strained ring structures, they successfully found an alternative to the 1,3-dipolar cycloaddition in biological systems which did not need copper to proceed. Dubbed the strain-promoted azide alkyne cycloaddition (SPAAC) these reactions could be performed directly in living cells and marked a breakthrough in conjugation chemistry in living systems. Thus, the SPAAC reaction vindicated Dr. Bertozzi's long standing search for reactions that showed chemoselective ligation, orthogonal coupling and native chemical ligation with respect to biological systems and gave impetus to the field of bioorthogonal chemistry.

Why is their discovery relevant?

Click chemistry is increasingly relevant because it makes for easier, quicker syntheses avoiding any by-products altogether. Click reactions are quick and easy. Copper catalyzed azide-alkyne cycloaddition has also been used for studying cell and molecular structures: fluorochromes for labeling proteins containing azidohomoalanine (Aha) and homopropargylglycine (Hpg) are bound by using CuAAC allowing visualization of molecular components (Beatty *et al.*, 2010). Researchers are studying the use of click chemistry to detect plant biomolecules *in vitro.* CuAAC click chemistry also has immense potential in the study of natural drugs where it can act as an important pharmacophore in incorporation of essential functional groups and help in bringing about necessary modifications in structure for the natural ingredients to function effectively in a medicinal setting (Xiao *et al.*, 2023). Click reactions are also similarly useful in DNA mapping. Material science too stands to benefit tremendously from application of click chemistry to form materials which are tailored to have specific properties for the intended usage.

Researchers are also working with bioorthogonal chemistry for use in anti-cancer therapeutics, tumor detection and

imaging and development of nanotherapeutics by a combination of nanotechnology and bioorthogonal chemistry (Zhang *et al.*, 2023).

In keeping with the need for sustainable green chemistry-based solutions; click chemistry reactions; especially CuAAC can also be conducted to bring about synthesis of 1,2,3–triazole derivatives as a microwave reaction eliminating the need for solvent (George *et al.*, 2022).

Their lives and careers

An alumnus of the Danish Technical University, Dr. Morten Paul Meldal completed his PhD in the synthetic chemistry of carbohydrates. Born in Copenhagen in 1954, Dr. Morten has, in an illustrious career, worked on various facets of chemical biology including combinatorial chemistry, click chemistry, polymer chemistry, organic synthesis, automation in synthesis, artificial receptors and enzymes, nano assays, biomolecular recognition amongst others. With over 300 publications and 21 patents to his name he currently serves as a professor of chemistry at the University of Copenhagen and head of the Center of Evolutionary Chemical Biology

Born in Boston, Massachusetts in 1966, Dr. Carolyn Bertozzi is a chemistry graduate from Harvard. Dr. Bertozzi completed her PhD in 1993 from UC Berkeley on chemical synthesis of oligosaccharide analogues and her postdoctoral research from UC San Francisco in 'the activity of endothelial oligosaccharides in promoting cell adhesion at sites of inflammation.' She was made a MacArthur Fellow in 1999 and has been awarded prizes such as the Lemelson-MIT Prize, the Heinrich Wieland Prize, the ACS Award in Pure Chemistry and the Chemistry of the Future Solvay Prize. She served at the Howard Hughes Medical Institute as an investigator from 2000. Dr. Bertozzi is currently a professor of chemical and systems biology at Stanford University and the Baker Family Director of Stanford ChEM-H- an interdisciplinary institute of Chemistry, Engineering & Medicine for Human Health.

Dr. Karl Barry Sharpless was born in Philadelphia in 1941. An alumnus of Dartmouth College, he completed his PhD from Stanford University in organic chemistry in 1968. His post-doctoral work was in inorganic/organometallic chemistry from Stanford and in enzymology from Harvard. His list of accolades includes the Benjamin Franklin Medal, the Wolf Prize, the Jose Medal of Honor, the Andrew D. Dorsey Memorial Award amongst others. For his previous work on chirally catalyzed oxidation reactions, he has also won the Nobel Prize in Chemistry in 2001.

Conclusion

All in all, the developing twin fields of click chemistry and bioorthogonal chemistry hold tremendous promise with the glimmer of new exciting prospects on the horizon unlocking synthesis methods and molecules of desired utility, tailored properties and which are biocompatible too. The 2022 Nobel Prize honors the immense contribution of the three chemists whose work brought about this seismic discovery - one that might just change our world.

References

Beatty, K. E., Fisk, J. D., Smart, B. P., Lü, Y., Szychowski, J., *et al.* (2010). Live-Cell imaging of cellular proteins by a Strain-Promoted Azide-Alkyne cycloaddition. *Chem Bio Chem*, *11*(15), 2092–2095.

Scinto, S. L., Bilodeau, D. A., Hincapie, R., Lee, W., Nguyen, S. S., *et al.* (2021). Bioorthogonal chemistry. *Nature Reviews-Methods Primers*, *1*(1).

Zhang, H., Wang, J., Han, R., Sun, B., & Luo, C. (2023). Bioorthogonal chemistry-driven anticancer nanotherapeutics. *Trends in Chemistry*, *5*(9), 697–710.

Team, C. S. (2022). *Key insights on Click Chemistry and bioorthogonal chemistry.*

Xiao, Z., Gu, Y., Dong, H., Liu, B., Jin, W., *et al.* (2023). Strategic application of CuAAC click chemistry in the modification of natural products for anticancer activity. *European Journal of Medicinal Chemistry Reports*, *9*, 100113.

Cite this Article:

Pandey, S., & Khangale, R. (2023). Newer and Better Molecules: Just a click away. *SCRIBE, 4*(1), 92-96.

Nobel Prize in Physics 2022: Illuminating the Mysteries of Quantum Entanglement.

Yeshashvi Marolikar, Meeta Saxena
Department of Physics, Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding author: Dr. Meeta Saxena, Department of Physics, Sophia College for Women (Empowered Autonomous), Mumbai. Email: meeta.saxena@sophiacollege.edu.in

Abstract

The Nobel Prize in Physics is an esteemed honor awarded to individuals whose remarkable contributions advance our understanding of the universe. In 2022, the Nobel Prize in Physics was bestowed upon Dr. Alain Aspect, Dr. John F. Clauser, and Dr. Anton Zeilinger for their groundbreaking experiments and theoretical work on quantum entanglement. Their contributions have unraveled the mysteries of entanglement, a phenomenon that defies classical explanations and has profound implications for quantum mechanics and information science. Dr. Aspect's experiments in the 1980s confirmed the non-local nature of entanglement, providing compelling evidence against local hidden variable theories. Dr. Clauser's work involved experimental tests that directly demonstrated the violation of Bell's inequalities, validating the entangled nature of quantum physics. Dr. Zeilinger's experiments with photons advanced the understanding of entanglement over large distances, paving the way for quantum communication and quantum information processing.

Introduction

Mechanics is a field of physics concerned with the motion and interactions of different entities. It comprises two branches: classical mechanics and quantum mechanics. Classical mechanics, also known as Newtonian mechanics, is a mathematical discipline that explores the movement of large-scale objects and the forces influencing them. On the other hand, quantum mechanics is a subfield of physics that delves into the behavior of particles such as atoms, electrons, and photons, operating at the molecular and sub-molecular levels. It was developed in the early 20^{th} century to explain phenomena that classical mechanics could not account for, such as wave-particle duality and the uncertainty principle.

Quantum mechanics introduces a probabilistic framework to describe the behavior of particles, using mathematical tools like wave functions and operators. It has applications in various fields, including quantum physics, quantum chemistry, and quantum computing. Quantum mechanics has revolutionized our understanding of the fundamental nature of matter and the behavior of particles at the atomic and subatomic levels.

A notable distinction between the behavior of quantum systems and classical rigid bodies lies in the phenomenon of entanglement – two (or more) particles existing in the "entangled state" reflect instantaneously whatever happens to either of them, irrespective of their spatial separation.

Einstein himself had called this “spooky action at a distance”. This unique characteristic of quantum mechanics forms the foundation for numerous applications that rely on the entanglement of particles, regardless of their physical distance from each other.

First proposed by Albert Einstein, Boris Podolsky, and Nathan Rosen in the famous EPR paradox, quantum entanglement refers to the mysterious connection that exists between particles, regardless of the distance separating them. This mind-boggling phenomenon has perplexed scientists for decades, offering a glimpse into the deep and intricate nature of the quantum world.

One of the fundamental aspects of quantum entanglement is the violation of Bell's inequality. Proposed by physicist John Bell in the 1960s, this inequality provides a way to test whether a theory adheres to the principles of classical physics or requires a quantum explanation. Numerous experiments have verified the violation of Bell's inequality, conclusively demonstrating that the predictions of quantum mechanics hold true and that entanglement is an inherent feature of the quantum world.

Interestingly, as early as 1949 Chien – Shiung Wu had documented the phenomenon using a cyclotron, photomultiplier tubes and a scintillation counter, though she was not mentioned when the Nobel was awarded.

About the Research

John Clauser, affiliated to Lawrence Berkeley National Laboratory and the University of California, Berkeley, together with Stuart Freedman, a graduate student, were the pioneering researchers who translated Bell's experiment from theory to practical realization. Clauser recognized that the experiment would be more viable if it employed polarized photons, which are particles of light, instead of spinning electrons.

John F. Clauser, along with Stuart Freedman, made significant contributions to the experimental verification of Bell's theorem. Bell's theorem mathematically established the conflict between quantum mechanics and local realism, suggesting that the predictions of quantum mechanics were incompatible with the concept of hidden variables. Clauser developed a rigorous experimental test to measure the correlations between entangled particles, known as the Clauser-Horne-Shimony-Holt (CHSH) inequality.

In a path breaking experiment conducted in the late 1970s, Clauser and his collaborators conducted measurements on entangled photon pairs, confirming the violation of the CHSH inequality and providing strong experimental evidence against local realism. This experiment solidified Bell's theorem and underscored the non-local nature of entanglement, significantly impacting our understanding of the quantum world.

Alain Aspect's work in the 1980s played a pivotal role in experimentally verifying the violation of Bell's inequalities, which are fundamental tests of the validity of local hidden variable theories in quantum mechanics. In a series of pathbreaking experiments, Aspect and his team

demonstrated that entangled particles exhibit correlations that cannot be explained by classical theories. His experiments involved measuring the polarization of entangled photon pairs (generated by focussing a laser on Calcium atoms) at various angles and distances, confirming the non-local nature of quantum entanglement. (Initially, many senior scientists had even called it “silly” and “a waste of funds”- perhaps a lesson for us to pursue sound logic- based experimentation and not give up!)

Aspect's experiments provided strong evidence against local hidden variable theories, bolstering the principles of quantum mechanics. His work laid the foundation for subsequent research, showcasing that quantum entanglement is a genuine phenomenon that cannot be explained by classical physics alone.

Anton Zeilinger's pioneering work in quantum information and entanglement has pushed the boundaries of quantum mechanics and opened up exciting avenues for practical applications. Zeilinger's experiments have showcased the fundamental aspects of quantum communication, cryptography, and teleportation.

One of Zeilinger's notable achievements was the demonstration of quantum teleportation, which involves the instantaneous transfer of the quantum state of a particle to a distant location using entanglement. His experiments have not only confirmed the viability of quantum teleportation but also highlighted the potential for secure quantum communication and quantum computing.

Furthermore, Zeilinger's research has explored the phenomenon of quantum entanglement on a large scale, involving systems with increasing complexity. These investigations have paved the way for understanding the limits and possibilities of quantum entanglement in macroscopic systems, contributing to the emerging field of quantum technologies.

In conclusion, the Nobel Prize awarded to Alain Aspect, John F. Clauser, and Anton Zeilinger represents a significant recognition of their transformative research on quantum entanglement and the violation of Bell's inequalities. Their experiments have shattered classical notions of locality and realism, solidifying the profound non-local and probabilistic nature of the quantum world. The findings of Aspect, Clauser, and Zeilinger have not only deepened our understanding of fundamental physics but also sparked new avenues for quantum information processing, communication, and cryptography. Another application of quantum entanglement is in the diagnosis of breast cancer. Their contributions will continue to shape the development of quantum technologies and inspire future generations of physicists to unravel the mysteries of the quantum realm.

As research on quantum entanglement continues to progress, it is expected to drive transformative technologies and shape our understanding of the fundamental laws of the universe. The exploration of entanglement's potential applications and its integration with other fields holds the promise of remarkable advancements and innovations in the coming years.

References

Erhard, M., Krenn, M., & Zeilinger, A. (2020). Advances in high-dimensional quantum entanglement. *Nature Reviews Physics, 2*(7), 365-381. https://doi.org/10.1038/s42254-020-0193-5

Hill, H. M. (2022). Physics Nobel honors foundational quantum entanglement experiments. *Physics Today, 75*(12), 14-17. https://doi.org/10.1063/PT.3.5133

Nobel Prize in Physics 2022 (2022, October 6). Drishtiias. Retrieved June 30, 2023 from https://www.drishtiias.com/daily-updates/daily-news-analysis/nobel-prize-in-physics-2022

The Nobel Prize in Physics 2022 (2022, October 4). Nobelprize.org. Retrieved June 29, 2023, from https://www.nobelprize.org/prizes/physics/2022/press-release/

Vedral, V. (2014). Quantum entanglement. *Nature Physics, 10*(4), 256-258. https://doi.org/10.1038/nphys2904

Wood, C. (2022, October 4). Pioneering Quantum Physicists Win Nobel Prize in Physics. Quantamagazine. Retrieved June 30, 2023 from https://www.quantamagazine.org/pioneering-quantum-physicists-win-nobel-prize-in-physics-20221004/#0

2022 Nobel Prize in Physics winners announced (2022, October 4). Institute of Physics. Retrieved June 29, 2023 from https://www.iop.org/about/news/nobel-prize-physics-2022-winners-announced#gref

Cite this Article:

Marolikar, Y., Saxena, M. (2023). Nobel Prize in Physics 2022: Illuminating the Mysteries of Quantum Entanglement. *SCRIBE, 4*(1), 97-100.

Nobel Prize in Physiology or Medicine 2022: From Bones to Breakthroughs: Nobel Laureates illuminate Neanderthal Legacy

Muqadas Wani, Saida Bano Sayyed, Geetanjali Ganguli
Department of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai
Corresponding Author: Dr Geetanjali Ganguli, Department of Life Sciences, Sophia College for Women (Empowered Autonomous), Mumbai. Email: geetanjali.ganguli@sophiacollege.edu.in

Abstract

Before the discoveries made by the Nobel winning scientist Svante Pääbo, our knowledge of human evolution revolved around records from fossil, archaeological findings, and limited genetic information from modern humans. In spite of these sources providing valuable insights, there were still limitations in understanding the genetic makeup of extinct hominins (a group consisting of Homo sapiens and extinct members of human lineage) and their relationship to modern humans. Through genomic sequences of extinct hominins, such as Neanderthals and Denisovans, unprecedented insights into ancient human migrations, interbreeding events, genetic variants, and the evolutionary history of our species was understood. These studies do not show practical applications in medical research and healthcare but also provide a substantial understanding in human evolution. This article highlights the significant contributions to the field of human evolution which eventually resulted in the 2022 Nobel Prize for Physiology or Medicine.

Introduction

The 2022 Nobel Prize winner in Physiology or Medicine Prof. Svante Pääbo, discovered genomes of extinct hominins and human evolution. Pääbo's initial breakthrough was sequencing Neanderthal Mitochondrial DNA (mtDNA), retrieved from humeral bone using PCR amplification with primers of 105-bp-segment of the human mtDNA control region (Matthias *et al.,* 1997). mtDNA is often used for tracing maternal lineages and constructing phylogenetic trees. While mtDNA analysis indicates that Neanderthals did not contribute their mtDNA to modern humans, it does not exclude the possibility of them contributing other genes (Wielgus *et al*., 2023) Pääbo's team obtained Neanderthal bone samples from the Vindija site in Croatia, with one sample dated to be 42,000 years old (Wielgus *et al*., 2023 and Richard E. Green *et al.,* 2010). In 2010, Pääbo published the Neanderthal nuclear genome sequence, estimating the divergence between Neanderthal and present-day human nuclear DNA sequences at 825,000 years (Green, R. E., Krause, J., Briggs *et al.,* 2010).

According to Prof. Svante Pääbo's research on ancient DNA sequencing revealed that Neanderthals were genetically distinct and that gene transfer had occurred from these hominins to *Homo sapiens* around 70,000 years ago. Furthermore, Neanderthals were found to be closely related to both Europeans and East Asians, with more genetic similarity

to non-Africans than Africans. This suggested gene flow (introgression) between Neanderthals and the ancestors of non-Africans during their coexistence. The analysis also indicated that between 1% and 4% of Eurasian genomes are derived from Neanderthals.

Pääbo's contribution to the field was also the discovery of a new hominin species-Denisova. It was named so because it was found in the Denisova Cave in the Altai mountains of Russia Hagymási K. (2023). He extracted the mtDNA from the finger bone and sequenced it. The mtDNA sequence from the finger bone were compared to various reference genomes, including present-day humans, Neanderthals, chimpanzees and bonobos.

The researchers found that the Denisova mtDNA was more divergent from present-day humans than Neanderthal mtDNA, suggesting that Denisovans were a distinct hominin species. The most recent common mtDNA ancestor of Denisovans, Neanderthals, and modern humans was estimated to be around one million years ago (Krause *et al.,* 2010; Reich *et al.,* 2010). In the phylogenetic tree predicted in (Fig 2) we have used mitochondrial gene for the following organism: Banonos, Chimpanzee, Human, Neanderthal.

Neanderthal and humans are arising from the same ancestor and are closely related. All the organisms shown common ancestry.

The researchers also sequenced the nuclear genome from the Denisova finger bone, mapping it to human and chimpanzee reference genomes. The Denisova nuclear genome was found to be similar but distinct from Neanderthals, indicating that they were sister groups.

Denisovans diverged from present-day humans roughly 640,000 years ago, while Neanderthals and Denisovans diverged 380,000-470,000 years ago. The divergence between modern humans and Neanderthals/Denisovans occurred 550,000-

Figure 1: Phylogenetic analysis of genomes of extinct hominins and human evolution (Wielgus et al., 2023)

760,000 years ago. They also found evidence of inbreeding in Neanderthals and low genetic diversity, suggesting a small population size.

The findings for interbreeding within Neanderthals, Denisovans, and early modern humans has led to extensive research in understanding the gene flow between them.

These studies can help understand the interactions between modern humans, therefore enhance our understanding in human variation and adaptations, disease susceptibility, etc.

Investigation which resulted from Pääbo's work led to unraveling of functional significance of archaic gene variants found in modern humans. Phenotypes, positive selection and functionality of the genome is correlated with the variants. Examples include genes associated with high-altitude adaptation, microbial recognition, and allergic reactions. The current area of research is based on the studies of functional significance of these unique gene variants (Green, R. E., Krause, J., Briggs *et al.,* 2010).

Pääbo's findings in Neanderthal and Denisovan genome sequencing have established a way for researchers to recover and sequence genomes from other extinct hominin species. This research of a diverse range of extinct hominins, gave a better understanding into the history and dynamics of human evolution.

Numerous studies have revealed that archaic introgression has influenced human immune functions and host defense, enabling genetic adaptation of modern humans to new pathogens. In the context of the COVID-19 pandemic, Svante Pääbo and Hugo Zeberg have identified genetic variants inherited from European Neanderthals that increase the risk of requiring the ventilator for breathing up to three times. These variants are present in the chromosomal region that consists of six genes (Zeberg & Pääbo, 2022). Additionally, it was found that a protein encoding region on chromosome 12 which plays a crucial role during infections with RNA viruses, was inherited from Neanderthals which acts as a protective agent against severe diseases. This research provides an understanding of the impact caused by sequencing of ancient DNA in research (Zeberg & Pääbo, 2020; Wielgus *et al.,* 2022).

In summary Pääbo's work paved a way into our evolutionary past and provided significant insights with respect to understanding the Neanderthal genetics and their relationship to modern humans. His

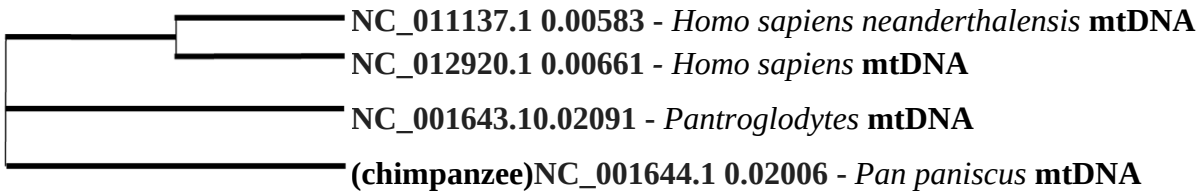


Figure 2: The phylogenetic comparison between hominis species. The analysis was performed using Clustal omega multiple sequences alignment tool.

research opened doors to the field of Paleogenomics (a field based on the construction and analysis of genetic information in extinct species) which resulted in sequencing of archaic hominin and ancient *Homo sapiens* genomes (Dannemann, M., & Kelso, J., 2017). This predominantly enhanced the understanding of human evolution and the relationship between ancient modern humans, Neanderthals, and Denisovans.

References

Dannemann, M., & Kelso, J. (2017). The Contribution of Neanderthals to Phenotypic Variation in Modern Humans. *American Journal of Human Genetics*, *101*(4), 578–589. https://doi.org/10.1016/j.ajhg.2017.09.010

Hagymási, K. (2023). The Nobel prize in physiology and medicine - 2022. *Structural chemistry*, *34*(2), 733–736. https://doi.org/10.1007/s11224-023-02124-0

Krause, J., Fu, Q., Good, J. M., Viola, B., Shunkov, M. V., *et al.* (2010). The complete mitochondrial DNA genome of an unknown hominin from southern Siberia. *Nature*, *464*(7290), 894–897. https://doi.org/10.1038/nature08976

Krings, M., Stone, A., Schmitz, R. W., Krainitzki, H., Stoneking, M., *et al.* (1997). Neanderthal DNA sequences and the origin of modern humans. *Cell*, *90*(1), 19–30. https://doi.org/10.1016/s0092-8674(00)80310-4

Reich, D., Green, R., Kircher, M., Krause, J., Price, A. L., *et al.* (2010). Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* 468, 1053–1060. https://doi.org/10.1038/nature09710

Green, R. E., Krause, J., Briggs, A. W., Maricic, T., Stenzel, U., *et al.* (2010) A Draft Sequence of the Neandertal Genome. *Science*, 328, 710-722. https://doi.org/10.1126/science.1188021

Wielgus, K., Danielewski, M., & Walkowiak, J. (2023). Svante Pääbo, reader of the Neanderthal genome. *Acta physiologica (Oxford, England)*, *237*(1), e13902. https://doi.org/10.1111/apha.13902

Zeberg, H., & Pääbo, S. (2020). The major genetic risk factor for severe COVID-19 is inherited from Neanderthals. *Nature*, *587*(7835), 610–612. https://doi.org/10.1038/s41586-020-2818-3

Cite this Article:

Wani, M., Bano, S., Ganguli, G. (2023). Nobel Prize 2022 Physiology or Medicine *SCRIBE*, *4*(1), 101-104

International Year of Millets 2023: A Contemporary Note

The Mighty Millets: Rediscovering Ancient grains for Modern Health

Ayesha Moosa, Ayesha Sorathiya, Deeann Cerejo, Firdos Khan, Gulnaz Ansari, Maryam Malkani, Ritu Rajpurohit, Rohini Nair, Sarah Gonsalves, Shraddha Yadav, Shriya Sharma, Sneha Narayanan, Tania Shroff, Umaima Ansari, Yeshashvi Marolikar, Zeel Lopes, Tressa Jacob, Sree R Nair
Sophia College for Women (Empowered Autonomous), Mumbai

Corresponding Authors: Tressa Jacob, Sree Nair
Email: tressa.jacob@sophiacollege.edu.in, sree.nair@sophiacollege.edu.in

Abstract

Millets are a diverse species of small seeded grasses cultivated all over the world since 8000 BC. All members of the millet family show great diversity of more than 20 species, each adapted to their specific environment. Additionally, many cereal millet varieties have been found to grow exceptionally well in regions with high temperatures and water scarcity, making them well suited for cultivation in arid regions also. They are attributed with having many nutritional and medicinal properties making them an ideal crop for consumption. To further showcase the importance of millets and to promote awareness and emphasize millet cultivation, the United Nations has announced the year 2023, as the international year of the millet. Owing to this, we address and highlight the importance of these forgotten indispensable grains here.

The Mighty Millets: Rediscovering Ancient grains for Modern Health

Millets are small seeded crops widely known as cereal crops, belonging to the family *Poaceae.* There are more than 6000 varieties of millets across the world. Millets are an important crop in Asia and Africa and are widely grown and consumed. It is particularly favored in developing countries like India, Nigeria and Niger due to its high productivity, short growth period and favorability to dry and hot temperature conditions. Originally millets were consumed as a staple food which later on was replaced by wheat and rice resulting in a declined production of millets. Some of the most commonly grown types of millet include: Finger millet (Ragi) Pearl millet (Bajra) Foxtail millet (Kakum) and Proso millet (Chena). These millets are used in a variety of ways, including as a staple food, for animal feed, and in the production of alcoholic beverages.

History of Millets

Millets have been cultivated as food crops for thousands of years in ancient Egypt, Greece, and Rome, where they were used as a staple food for the lower classes (Karuppasamy, 2015). They were cultivated in Europe, Asia, Africa and North America a few hundreds of years ago. There have been reports that Millets, known to be the "Grain for the poor" in India, have been cultivated in Asia way back in the Neolithic Age. There is archaeological evidence that shows it was grown in China, India and Southeast Asia. It is during the colonial period that the cultivation of millets declined that led to the loss of the knowledge too. This was then replaced by tea, coffee and rubber plantation.

In Europe, millets were first introduced by the Romans, who brought foxtail millet (*Setaria italica*) and broomcorn millet (*Panicum miliaceum)* from Asia. In the 18th and 19th centuries, the cultivation of millets declined in Europe as the production of wheat and other grains increased. In North America, millets were introduced by European settlers in the 17th and 18th centuries. In the 19th and 20th centuries, the cultivation of millets in North America declined as other crops like corn and wheat became more popular. However, millets continue to be grown in some regions of North America, particularly in the Great Plains region, where they are used as a food for both humans and livestock (Kirleis *et al.*, 2022).

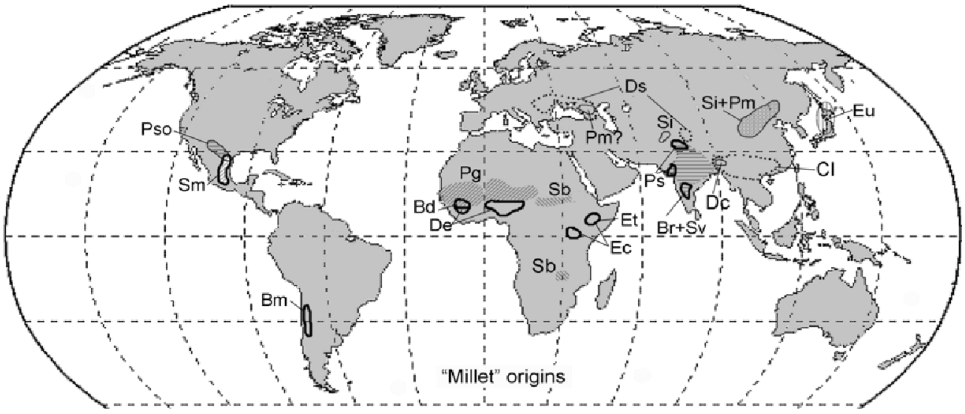


Figure 1. The map of likely centers of origin for "millets."

Millets abbreviated: Pso: *Panicum sonoran*; Sm: *Setaria cf. macrostachya*; Bm: *Bromus mango*; Bd: *Brachiaria deflexa* ; De: *Digitaria exilis*; Pg: *Pennisetum glaucum*; Sb: *Sorghum bicolour*, including Southern African zone where the race kafir may be an independent domesticate; Ec: *Eleusine coracana*; Et: *Eragrostis tef*; Ds: *Digitaria sanguinalis*; Pm: *Panicum miliaceum*, a separate Western origin remains unconfirmed; Si: *Setaria italica*; Ps: *Panicum sumatrense*: Br: *Brachiaria ramosa*; Sv: *Setaria verticillata;* Dc: *Digitaria cruciata*; Cl: *Coix lachrymal-jobi*; Eu: *Echinochloa crus-galli var. utilis.* The striped zone in India indicates the broader Indian millet zone within which several domestications remain to be better localized (*Paspalum scrobiculatum, Echinochloa colonum, Setaria pumila*), in addition to possible multiple domestications of *Brachiaria ramosa* (Weber & Fuller, 2008)**.**

Nutritional Value of Millets

Millets are rich in protein and nutrients and consumed as food and fodder worldwide and are considered as golden crops because of their richness in proteins and nutrients like calcium, iron, and magnesium among others providing all the necessary things in one grain. They can be used to make bread, beer, cereal, and other dishes. They are extraordinarily superior to rice and wheat in their nutritional composition and therefore are a solution for the malnutrition that affects a vast majority of the Indian population providing a viable option to live a healthy life and reduce the incidence of lifestyle diseases as they are gluten free and non-allergenic. Consumption of millets decreases triglycerides and C-reactive protein, thereby preventing cardiovascular disease. All millets are rich in dietary fiber (Shankaramurthy & Somannavar, 2019).

	Nutritional value (per 100g)							
Type Of Grain	Energy	Carbo-hydrates	Protein	Total Fat	Fibers	Minerals	Vitamins	Anti-oxidants
Millets								
● Sorghum	339 kcal	72-75g	11.3g	2-3g	6.7g	yes	yes	yes
● Kodo millet	353 kcal	65-70g	8.03g	3-5g	9.2g	yes	yes	yes
● Finger millet	328 kcal	72-73g	7.3g	1-3g	3.5g	yes	yes	yes
● Foxtail millet	331 kcal	60-70g	2.94g	3-4g	6.7g	yes	yes	yes
● Pearl millet	361 kcal	65-70g	0.31g	4-5g	8.0g	yes	yes	yes
Cereals								
● Rice	130 kcal	28.7g	2.7g	0.3g	0.4g	yes	yes	yes
● Wheat	339 kcal	71.2g	12.6g	1.5g	12.2g	yes	yes	yes

Table 1. Nutritional value of known millets (Shankaramurthy & Somannavar, 2019).

SORGHUM:

Sorghum millet, known for its nutritional richness, provides a very strong profile that contributes to a fit and balanced diet. With an energy content of 339 kcal per 100 grams, Sorghum millet acts as a beneficial source of sustained energy (Shankaramurthy & Somannavar, 2019). Abundant in complex carbohydrates, it provides 72-75g of carbohydrates. It also facilitates exceptional energy release throughout the day. The protein content of total 11.3 grams, is significant which supports muscle maintenance and overall bodily functions (Shankaramurthy & Somannavar, 2019). Moreover, the relatively low-fat content of 2-3g is preferred because it focuses on healthier fat intake. Sorghum contains multiple vitamins such as B1 (Thiamine), B2 (Riboflavin), B3 (Niacin) and B6 (Pyridoxine). This millet is a good source of minerals like iron, potassium, phosphorus, and magnesium. These minerals act as a cofactor for many physiological functions (Shankaramurthy & Somannavar, 2019). Sorghum grain is rich in bioactive phenolic compounds which are known to provide many health benefits including antioxidant, anti-inflammatory and anti-diabetic activities. Sorghum millet's nutritional composition, coupled with its gluten-free nature, makes it an attractive choice for those seeking a nutrient-dense alternative (Shankaramurthy & Somannavar, 2019).

KODO MILLET:

Kodo millet emerges as a powerhouse of nutrients, contributing abundance of health benefits with its high nutrient profile. It has an energy content of 353 kcal per 100 grams, it serves as a strong source of energy. Rich in complex carbohydrates, ranging from 65 to 70 grams, kodo millet supports a balanced release of energy. The protein content in kodo millet is 8.03 grams, which makes it a good protein source for not only adults but also for young children. It has a low-fat content of 3-5 grams providing the consumer with better alternatives to common grains. Kodo millets are rich in vitamin B3, vitamin B6 and folic acid as well as minerals such as calcium, potassium, magnesium and zinc (Bunkar *et al.*, 2021). Kodo millet had the highest total phenolic content, making it richest in antioxidant properties (Shankaramurthy & Somannavar, 2019).

FINGER MILLET:

Finger millet, also known as Ragi, is a nutritious grain that is widely consumed in many parts of the world. It is rich in energy, carbohydrates, proteins, and low in fat. It is a great source of energy due to its high carbohydrate content of 72-73g. As carbohydrates are essential for providing fuel to our bodies it becomes an excellent support for various bodily functions. Additionally, finger millet contains dietary fiber of 3.5g, which aids in digestion and helps maintain a healthy weight. When it comes to protein, it is packed with essential amino acids. Proteins are crucial for building and repairing tissues, as well as supporting the immune system. In terms of fats, finger millet is naturally low in fat consisting of just 1-3g, making it a healthy choice. It contains primarily unsaturated fats, which are beneficial for heart health. Overall, finger millet is a nutritious grain that provides energy, carbohydrates, proteins, and healthy

fats. It is a fantastic addition to a balanced diet! (Shankaramurthy & Somannavar, 2019).

FOXTAIL MILLET:

Foxtail millet has a great quantity of nutrients that contributes to a balanced and healthy diet. Along with the nutrients, a 100g serve provides approximately 331 kcal of energy which makes it an excellent source of sustained fuel. The protein content is relatively higher than most millets which is 12.94g per serving. It helps with repair, enzyme production which catalyzes biochemical reactions in the body. It facilitates digestion, cellular functions along with nutrients and energy. Carbohydrates consisting of 60 to 70 g per 100 g contributes to the release of energy and regulating blood sugar levels. Additionally, foxtail millet is rich in vitamins including niacin and folic acid that helps in cell division. Antioxidants help like phenolic compounds help with oxidative stress and inflammation in the body (Shankaramurthy & Somannavar, 2019).

PEARL MILLET:

Pearl millet has a rich nutritional profile that supports overall health. Energy of approximately 361 kcal per 100g. The energy derived from the pearl millet is utilized by the body to support various metabolic functions such as cellular activities, maintaining body temperature and organ function. Consuming an appropriate amount of energy is important for maintaining a healthy weight and diet. The protein content of 10.31g supports the immune system that helps the body to defend against infections and illnesses. It also provides structural support for the bones and joints. (Shankaramurthy & Somannavar, 2019) The carbohydrates composition of 65-70g helps to regulate blood flow. Pearl millet includes various B-complex vitamins such as B1, B2, B3, B6, B9, B5, and vitamin E. Iron and magnesium enhance its nutritional value. Some antioxidants present in pearl millet are Phenolic compounds, Beta-carotene and lutein which helps neutralize harmful free radicals in the body, strengthen immune system and maintain eye health respectively (Shankaramurthy & Somannavar, 2019).

Distribution and Economic Contribution of Millets

India is among the top 5 exporters of millets in the world. India contributes nearly 20% of the global export value of millet. World export of millet has increased from $380 million in 2019 to $402.7 million in 2020 globally. India exported millets worth $64.28 million in the year 2021-22, against $59.75 million in 2020-21. Share of Millet based value added products is negligible. The top three importers of millets from India in 2020-21 were Nepal ($6.09 million), UAE ($4.84 million), and Saudi Arabia ($3.84 million). The other seven destinations in the top-ten list of India’s millet export are Libya, Tunisia, Morocco, the UK, Yemen, Oman and Algeria (Press Information Bureau, 2023).

Millets are widely cultivated in many parts of the world today, particularly in developing countries of Africa and Asia where they are a staple food. Globally, millets are cultivated in more than 90 countries. Among the millets, Sorghum is the most widely cultivated crop.

About 97% of millets are produced and consumed by Africa and Asia. India is the largest producer of millet with 26.6% of the world and 83% of Asia's millet cropping area (Meena *et al.*, 2021).

Myths and Facts

Millets have been an important part of human diet since the very beginning and yet few people are aware of its benefits and uses. This has in turn led to people mixing up myths and facts on millets.

Some of the myths are –

Bajra and Ragi millets cause thyroid- Each millet is immensely different, only two contain flavonoids in a form that might affect thyroid metabolism and that too only if the consumer is also deficient in iodine and has a primary thyroid disorder.

These two millets have been consumed by local, rural and adivasi/aboriginal communities for generations with no reports or anecdotes mentioning endemic goiter. However, the presence of beneficial anti-cancer, anti-aging and antioxidants in millet, far outweigh the enormously minuscule danger of hypothyroidism (Saleh et al, 2013). Millets are hard to digest: This is a myth because properly cooked millets are not hard to digest. Millets are high in fiber and eating them in wrongly cooked manner can bring digestion problems for anyone. However, this is true about consumption of many food items in general.

Millets are a new-fangled fad: Rather than being a marketing scam, millets are in fact a way to obtain nutritional security and food security. Due to this, India put forth to the United Nations to declare 2023 as the international year of millets. Countries like India have a large percentage of soil only being able to support dryland crops/rain-fed crops such as millets. However, due to the less demand for millets in the global market the governments of the world are trying to do their bit by advocating millets on a large scale so that their populations can have food security, nutritional security and even water security.

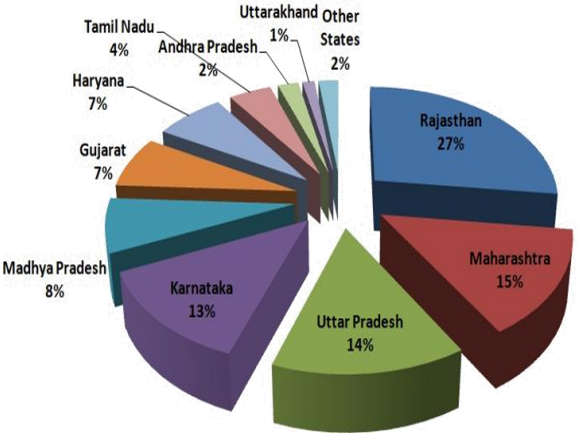


Figure 2. State Wise Production of Millets, 2021-22 (4th Adv, Estimate)

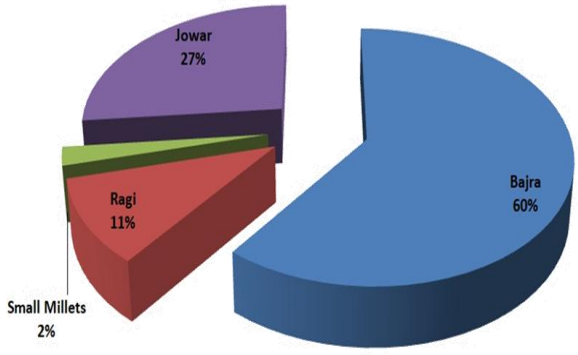


Figure 3. Courtesy - Agriculture and Processed Food Products Export development and Authority (APEDA) - Ministry of Commerce and Industry, Government of India.

Millets are grown using pesticides: This is also not true. It is also generally grown without chemical pesticides and has a long tradition of sustainable farming practices in countries like India, where a recent government push to use pesticides and chemical fertilizers in rural communities has met with deep resistance (McDonough et al, 2000).

Conclusion

Understanding the above we can conclude that millet is not only a good source of proteins, fibers, vitamins and minerals but also has potential health benefits which include protecting cardiovascular health, preventing the onset of diabetes, helping people achieve and maintain a healthy weight, and managing inflammation in the gut. Millets have proteins that are reasonably rich in several essential amino acids and sulfur-containing amino acids, including methionine and cysteine. Nutritionally, most of the millets are superior or at least equivalent to major cereal grains including rice and wheat. Interestingly millets can survive extreme conditions like drought, flooded areas and swampy lands as well. If encouraged to cultivate, it could turn out to be a valuable crop in a country dependent on the vagaries of monsoon for its irrigational purpose. The use of millets in commercial/packaged food will encourage farmers to grow millets and will open new opportunities and revitalize the farmers for cultivation of more millets. The inclusion of millet-based foods on international, national and state- level feeding programs will help to overcome the existing nutrient deficiencies of protein, calcium and iron in developing countries.

Various cuisines and countries have adapted innumerable recipes and preparations for millets over years of practice and traditions. Additionally, the reintroduction of millets into daily cuisine as part of the awareness effort to showcase the diversity of dishes that can be prepared with millets various new and innovative recipes have also been created in recent years.

References

Saleh, A. S. M., Zhang, Q., Chen, J., & Shen, Q. (2013). Millet grains: Nutritional quality, processing, and potential health benefits. *Comprehensive Reviews in Food Science and Food Safety*, *12*(3), 281-295. https://doi.org/10.1111/1541-4337.12012

Bunkar, D. S., Goyal, S. K., Meena, K. K., & Kamalvanshi, V. (2021). Nutritional, functional role of kodo millet and its processing: A review. *International Journal of Current Microbiology and Applied Sciences*, *10*(01), 1972-1985. http://doi.org/10.20546/ijcmas.2021.1001.229

Karuppasamy, P. (2015). Overview on Millets. *Trends in Biosciences*, *8*(13), 3269-3273.

Kirleis, W., Dal Corso, M., & Filipović, D. (2022). *Millet and what Else?: The Wider Context of the Adoption of Millet Cultivation in Europe* (p. 328). Sidestone Press. http://doi.org/10.59641/o7235ra

Kumar, A., Tripathi, M. K., Joshi, D., & Kumar, V. (Eds.). (2021). *Millets and millet technology* (p. 438). Singapore: Springer. http://doi.org/10.1007/978-981-16-0676-2

McDonough, C. M., Rooney, L. W., & Serna-Saldivar, S. O. (2000). The millets. In *Handbook of cereal science and technology, revised and expanded* (pp. 177-201). CRC Press.

Meena, R. P., Joshi, D., Bisht, J. K., & Kant, L. (2021). Global scenario of millets cultivation. *Millets and millet technology*, 33-50. http://doi.org/10.1007/978-981-16-0676-2_2

Press Information Bureau. (2023, March 18). APEDA organizes the Global Millets (Shree Anna) Conference. *Press Information Bureau*. Retrieved from https://pib.gov.in/PressReleasePage.aspx?PRID=1908330

Shankaramurthy, K. N., & Somannavar, M. S. (2019). Moisture, carbohydrate, protein, fat, calcium, and zinc content in finger, foxtail, pearl, and proso millets. *Indian Journal of Health Sciences and Biomedical Research kleu*, *12*(3), 228-232. http://doi.org/10.4103/kleuhsj.kleuhsj_32_19

Weber, S. A., & Fuller, D. Q. (2008). Millets and their role in early agriculture. *Pragdhara*, *18*(69), e90.

Cite the article:

Moosa A., Sorathiya A., Cerejo D., Ansari G., *et al.* (2023). The Mighty Millets: Rediscovering Ancient Grains for Modern Health**.** *SCRIBE, 4*(1), 105-112.

Bhulabhai Desai Road, Mumbai
400026, Maharashtra
www.sophiacollegemumbai.com