A Review on the Teratogenic Effects of Isotretinoin

By

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A project work submitted to the School of Pharmacy in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “A review on the Teratogenic Effects of Isotretinoin” submitted by Tahsina Binte Kashem Anisha (19346014), of Summer, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This is a review work which did not include any human or animal subjects. The author acknowledges the contributions of all relevant sources of information, and any discrepancies or limitations in the data are reported in a transparent and honest manner.

Abstract

This review article presents recent studies on isotretinoin, a potent oral retinoid that is frequently recommended to treat severe acne but has been clearly associated with teratogenic effects when given to expectant mothers. The motive of this review article is to provide an in-depth overview of the teratogenic effects of isotretinoin and the necessity of strict safety measures to protect fetuses. Isotretinoin is known to be teratogenic; prenatal exposure has been linked to a variety of congenital defects, with the central nervous system, cardiovascular system, and face structures being the most commonly affected. In this review comparative studies of pregnant women with teratogenic effect have been analyzed with numerical data from FAERS database about different teratogenic effect found in fetus and other adverse events. The aim of this review is to analyze the FAERS public database to identify the outcomes reported when pregnant women are exposed to isotretinoin. The review article concludes with proposals for more future study directions as well as a discussion of the research's implications for effective policy reforms.

Keywords: Acne, isotretinoin, retinoic acid, teratogenicity and pregnancy.

Dedication

To my friends and family.

Acknowledgement

I extend my heartfelt appreciation to all those who have contributed to the completion of this research. I am deeply grateful to Allah for providing me the opportunity to work with such wonderful people from the School of Pharmacy, BRAC University. I am honored to have been guided by my esteemed supervisor, Farzana Islam, Lecturer at the School of Pharmacy, whose support, guidance, dedication, and expertise have driven me to excel in this field. I would like to extend my gratitude to Professor Dr. Hasina Yasmin, Program Director and Assistant Dean at the School of Pharmacy, for imparting valuable knowledge, and to Professor Dr. Eva Rahman Kabir, Dean at the School of Pharmacy, for her unwavering support and motivation. I also want to acknowledge the efforts of all the faculty members at the School of Pharmacy, BRAC University, and express my gratitude to my friends and seniors for their guidance and my family members for their unwavering support.

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List of Acronyms

Propionibacterium Acnes	P. Acne
Complementary and Alternative Medicine	CAM
Food and Drug Administration	FDA
International Union of Pure and Applied Chemistry	IUPAC
Retinoic Acid Receptor	RAR
Retinoid X Receptor	RXR
Sebocyte	SEB
Cellular Retinoid Binding Protein	CRBP
Retinol Dehydrogenases	RODH
Retinoic Acid Receptor Alpha	RARα
Retinoic Acid Receptor Beta	RARβ
Retinoic Acid Receptor Gamma	RAR γ
Cellular Retinoid Acid Binding Protein	CRABP
TNF-related apoptosis-inducing ligand	TRAIL
Fork-head box O	FoxO
Neutrophil Gelatinase Associated Lipocalin	NGAL
All Trans Retinoic Acid	ATRA
Insulin-like Growth Factor Binding Protein 3	IGFBP-3
European Medicines Agency's	EMA
United States	US
Area Under Cover	AUC
Central Nerves System	CNS
System to Manage Accutane Related Teratogenicity	SMART

Risk Evaluation and Mitigation Strategy	REMs
Adenosine Monophosphate	AMP
AMP-activated protein kinase	AMPK
Tumor Protein 53	TP53
Homeobox	HOX
mechanistic Target of Rapamycin Complex 1	mTORC1
Phosphatase and Tensin Homolog	PTEN
electrochemical Atom Transfer Radical Addition	eATRA
Pregnancy Prevention Program	PPP

Chapter 1

Introduction

1.1 An overview of acne

Acne is a prevalent skin condition that arises from inflammation of the skin glands and hair follicles. Nonetheless, this disorder is more usual in young adults and adolescents, though it can also affect adults at any age and infants (Genebriera & Davis, 2009). Acne take place when the pores of skin get blocked. Sebaceus glands are small oil glands that surround hair follicles which secrete an oily substance known as sebum (Ramli et al., 2012). The natural shedding process of skin cells involves the ascent of keratinocytes to the skin's surface. Acne, in its initial stages, occurs when a combination of hair, sebum, and keratinocytes becomes trapped within the skin pore. This leads to an impediment in the normal shedding of keratinocytes and prevents sebum from penetrating the skin's surface (Abraham, 2018). In this situation, a bacteria known as Propionibacterium acnes, which is typically present on the skin, has the potential to propagate within obstructed hair follicles, triggering inflammation. This inflammatory response, in turn, affects the sebaceous gland, manifesting as acne symptoms such as redness, swelling, warmth, and discomfort. As the wall of the obstructed follicle breaks down, bacteria, skin cells, and sebum escape into the surrounding skin, giving rise to lesions or pimples. The deterioration of the follicle's wall facilitates the leakage of germs, skin cells, and sebum into the adjacent skin, resulting in the formation of lesions or pimples (Abraham, 2018). Acne can have various adverse impacts on teenagers, including physical discomfort, mental stress, feelings of imperfection, and the potential for enduring skin damage. Furthermore, it has the capacity to diminish both the physical and social well-being of individuals, leading to heightened levels of anxiety and embarrassment (Fox et al., 2016).

1.2 Causative factors of acne formation

Acne occurs for a variety of reasons, involving hormonal, genetic, excess sebum production, microbial, climatic, chemical, and psychological factors. Some major reasons are

- Excessive or excess oil production in the pores. (Abraham, 2018)
- Dead skin cell buildup in the pore. (Abraham, 2018)
- Bacterial growth in the pore.(Abraham, 2018)

Other factors which might leads to acne are

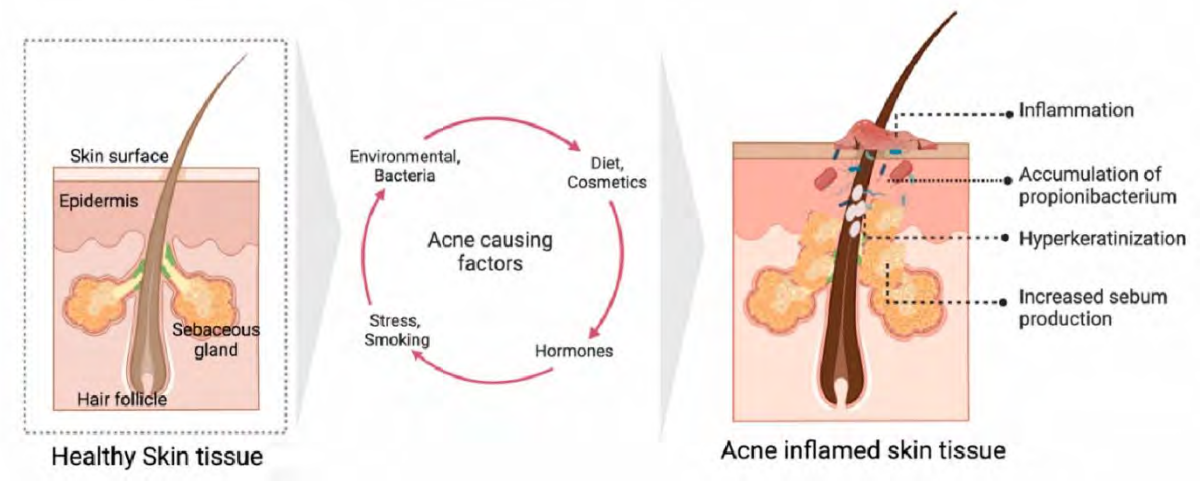

Figure 1: Comparison between healthy skin cell and acne inflamed skin cell due to various acne causing factors (Vasam et al., 2023).

There are several factors that contribute to acne, but genetics is the most significant. Acne affects three out of every four children in case if both parents had acne. One of the reasons of acne is hormonal activity, such as the menstrual cycle and adolescence. When sebaceous glands become hyperactive in comparison to normal activity, they are affected. When the hair follicles get clogged, P. acnes will attack the trapped sebum beneath the skin's surface (Ramli et al., 2012). Excessive sunlight exposure. In addition, wearing underwire brassieres, backpacks,

headbands, shoulder pads, and other occlusive clothing. Endocrine abnormalities include pregnancy and polycystic ovarian syndrome. Moreover, 70% of patients who are female experience this (Imperato-McGinley et al., 1993).

1.3 Acne Types

Acne manifests in various forms, including acne conglobate, acne rosacea, acne fulminans, acne cosmetica, acne excoriee (picker's acne), acne medicamentosa, acne chloracne, with acne mechanica being one of them. However, acne vulgaris is the most prevalent, constituting 99% of all acne cases. It is the most common type and is characterized by two primary lesion types: non-inflammatory (open and closed comedones) and inflammatory (papules, pustules, nodules, and cysts). Comedones, appearing in two distinct forms open comedones, commonly known as blackheads, and closed comedones, referred to as whiteheads are characteristic features of acne vulgaris (Vasam et al., 2023).

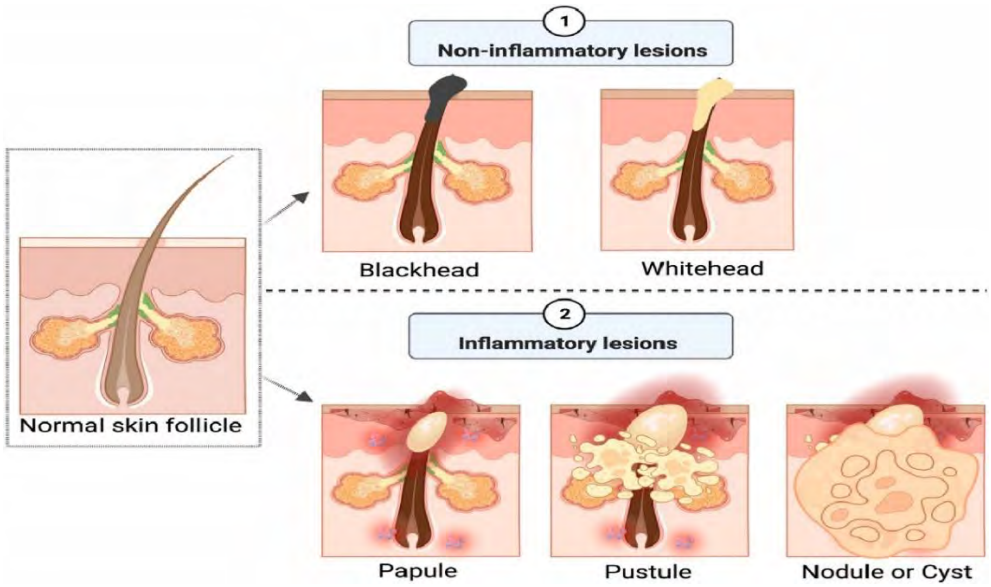

Figure 2: Diagram illustrating the major distinctions between two types of lesions (non-inflammatory and inflammatory) and their pathogens (Vasam et al., 2023).

1.4 Common acne treatment options

The main objectives of treating acne are to manage and treat pre-existing lesions, reduce morbidity, minimize the duration of the condition, and prevent permanent scarring as much as possible. Further, patients should be advised that it might take 3-6 weeks before they observe any improvement (Fox et al., 2016). Some common methods are topical treatment such as Adapalene, isotretinoin, motretinide, retinoyl-β-glucuronide, tazarotene, and tretinoin are examples of retinoids. Antibiotics: erythromycin and clindamycin. Besides, systemic treatments such as Retinoids isotretinoin, Antibiotics: levofloxacin, lymecycline, minocycline, roxithromycin, clindamycin, co-trimoxazole, doxycycline, erythromycin, alongside hormonal: birth control pills. Moreover, some other methods like CAM, physical treatment are associated with the acne (Fox et al., 2016).

1.5 Isotretinoin

An oral derivative of vitamin A called isotretinoin, also known as 13 cis retinoic acid, is used to treat a variety of dermatological conditions, including ichthyosis, hidradenitis suppurativa, rosacea, scarring alopecia, and the prevention of non-melanoma skin cancer. However, it is primarily used to treat sebaceous gland pathology (Draghici et al., 2021).

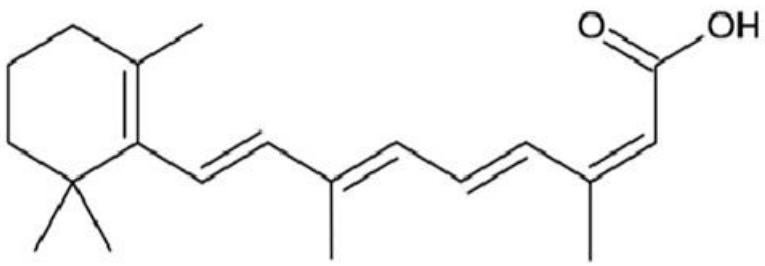

Figure 3: Chemical structure of isotretinoin

The IUPAC name assigned to isotretinoin is (2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl cyclohexen-1-yl) nona-2,4,6,8-tetraenoic acid. As per research by Kapała et al. in 2022, its

molecular formula is C20H28O2. Isotretinoin is marketed under various brand names, including Accutane, Amnesteem, Claravis, Myorisan, Absorica, and Zenatane.

Isotretinoin is FDA-approved in the US and Europe. Its primary use is to treat severe, intractable instances of acne vulgaris. Isotretinoin is a beneficial therapy for treating acne. It functions by reducing the number of sebaceous glands, lowering sebum excretion, regulating cell division, and decrease keratinization. The follicular microenvironment can be changed via isotretinoin, which lowers the quantity of Cuti-bacterium acnes (Paichitrojjana & Paichitrojjana, 2023). It is an effective treatment option for a variety of skin conditions due to its anti-inflammatory and immunomodulatory properties, which include lowering monocyte TLR-2 expression and decreasing the inflammatory cytokine response. (Paichitrojjana & Paichitrojjana, 2023).

1.6 Retinoids

Retinoids are the ‘first line’ and ‘the gold standard’ treatment for acne. The retinoid family encompasses vitamin A, including its natural derivatives like retinol, retinoic acid, retinyl esters, and retinaldehyde. Additionally, this family includes a diverse range of synthetic derivatives that have been developed for various purposes. Retinol, also known as all-trans-retinol, is a 20-carbon molecule characterized by an alcohol end group, a side chain containing four double bonds, and a cyclohexenyl ring. On the other hand, all-trans retinoic acid, commonly referred to as tretinoin, is a carboxylic acid resulting from the oxidation of the alcohol end group of retinol, forming an aldehyde in the process (Mukherjee et al., 2006).

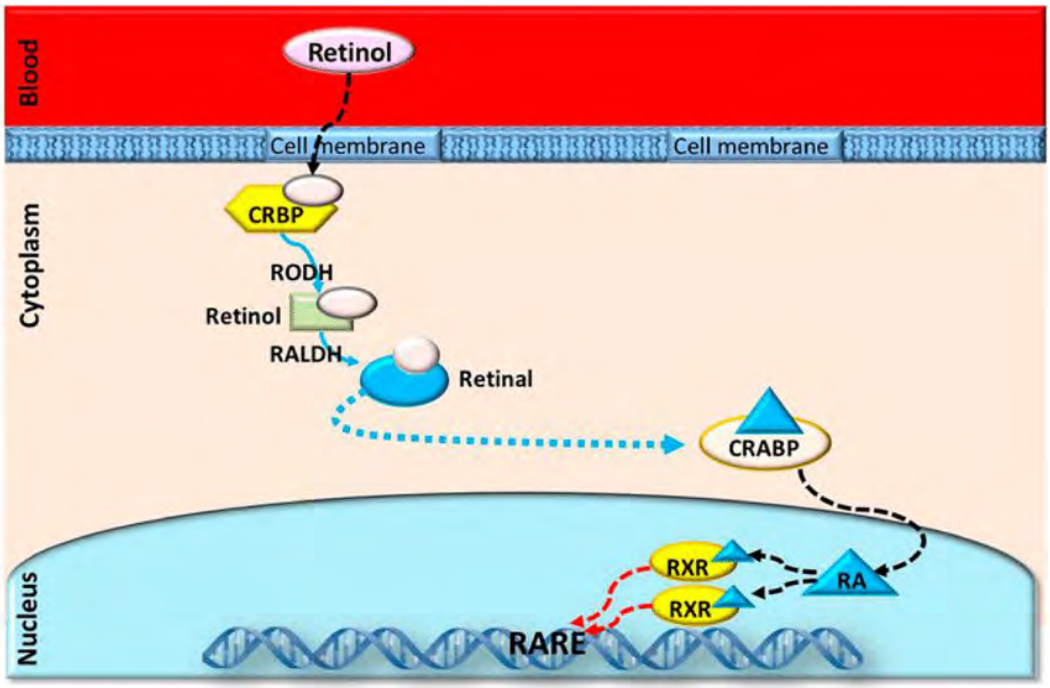

Figure 4: Mechanism pathway of retinoids (Abdelnour et al., 2019).

Retinoids, derived from vitamin A, interact with and bind to retinoic acid receptors (RARs) and retinoid X receptors (RXRs). These receptors, when activated by retinoids, target keratinocytes (Martin et al., 1998). Trifarotene binds to the only RAR-gamma receptor. Retinoids that primarily act on RAR-beta and RAR-gamma receptors involves tazarotene, adapalene, and retinoin. For instance, the RAR-alpha receptor is the target of tretinoin. There are three different types of RAR receptors: α, β, and γ. The binding capabilities of each retinoid drug is different. The anti-acne feature of retinoid stem from its ability to suppress keratinocyte formation and restore normal follicular differentiation, which helps in pore unclogging and restoring normal peeling (Callender et al., 2022). Retinoic acid-receptor complex develop and transferred into the cell nucleus, where they bind to activate the retinoid hormone response element and increase the transcription of multiple regulatory genes (Martin et al., 1998). The most significant effects of this procedure are the normalization of follicular keratinization and the loosening of keratinocyte cohesiveness which reduce the production of micro-comedones (Bershad et al., 2002).

Topical retinoids like tretinoin, tazarotene, adapalene, and trifarotene are recommended for the majority of acne patients as a first line of treatment. These medications effectively treat inflammatory papules and pustules as well as comedones. Individuals primarily suffering from comedonal acne may benefit from topical retinoids as a monotherapy (Martin et al., 1998). Patients suffering with papulopustular acne may benefit from the application of a topical antimicrobial agent such as benzoyl peroxide or a topical antibiotic. Benzoyl peroxide should ideally be used combined with topical antibiotics to lessen the chance of antibiotic-resistant bacteria developing. Topical retinoid should be applied once daily, preferably at night, because of tretinoin's apparent photo ability (Nyirady et al., 2002).

1.6.1 Oral isotretinoin mechanism

Oral isotretinoin, commonly referred to as 13-cis retinoic acid, was first approved by the US Food and Drug Administration (FDA) in 1982 as a treatment for severe acne. Two decades later, isotretinoin is still the most clinically effective anti-acne treatment, helping many patients achieve significant improvement and/or a long-term remission (Abdelnour et al., 2019). Isotretinoin is an orally administered systemic retinoid. At pharmacologic levels, isotretinoin inhibits sebaceous gland function and keratinization, though the exact mechanism of action is unknown. The drug showed a reduction in both sebum production and sebaceous gland size. (Pile & Sadiq, 2022).

The most potent medication for treating severe forms of acne vulgaris is isotretinoin because of its strong sebum-suppressive properties, which are mostly caused by sebocyte death. (Draghici et al., 2021). The reason why isotretinoin is used for the treatment of children's neuroblastoma, promyelocytic leukemia, and basal cell carcinoma is due to its apoptotic effect. Apoptosis can also occur in primary human keratinocytes, B16F10 melanoma cells, adult T-cell leukemia cells, and Dalton lymphoma ascites cells (Draghici et al., 2021). Many studies

have been conducted to investigate the mechanism of action of isotretinoin, with some have been successful in explaining these physio-pathological pathways.

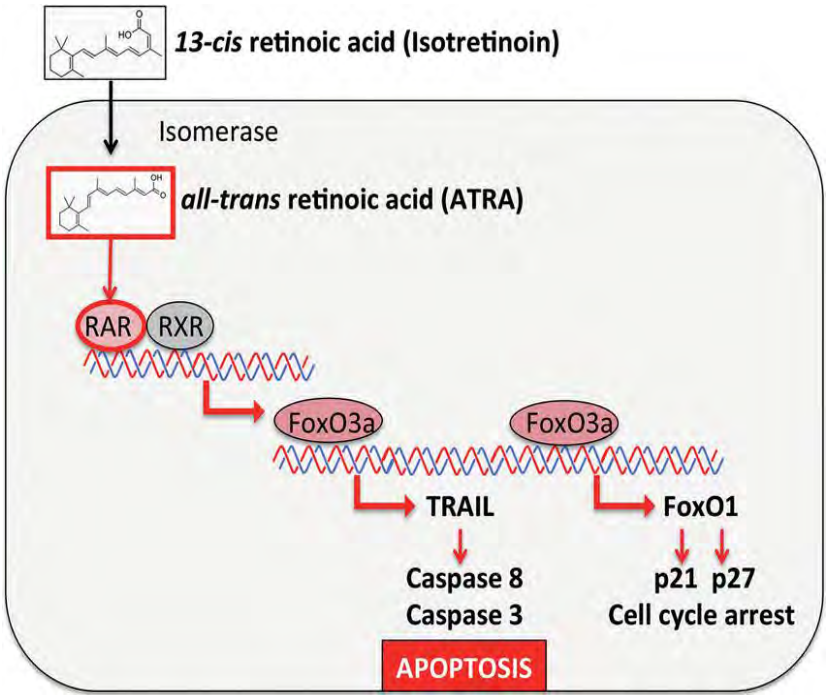

Figure 5: Mechanism action of isotretinoin (Melnik, 2017)

Isotretinoin leads to apoptosis and cell cycle arrest in human SEB1 sebocytes, as shown by Nelson et al. However, isotretinoin also causes apoptosis in the human sebaceous gland, which is further facilitated by the apoptotic proteins tumor necrosis factor related apoptosis inducing ligand (TRAIL) and neutrophil gelatinase associated lipocalin (NGAL). According to Kelhälä et al., acne patients treated with isotretinoin had higher expression levels of NGAL and TRAIL in their skin, which further supported the importance of these molecules. In a number of tumor cell lines, TRAIL has been demonstrated to induce apoptosis; however, more investigation is required (Draghici et al., 2021). Research has shown that TRAIL is relatively nontoxic to normal cells, even though it is constitutively produced in many human organs and has been shown to induce apoptosis in various tumor cell lines (Draghici et al., 2021). TRAIL expression

is initiated in healthy individuals by the nuclear transcription factors fork head box protein O (FoxO), specifically FoxO3a. Isotretinoin isomerizes to all trans retinoic acid (ATRA) in sebocytes. ATRA promotes the expression of FoxO3a, an apoptosis-related transcription factor, and TRAIL (Draghici et al., 2021).

Research indicates that isotretinoin treatment for acne patients causes an increase in TRAIL in their sebaceous glands, which indicates that the treatment may enhance FoxO/TRAIL signaling in these glands and cause sebocyte death (Draghici et al., 2021). In acne patients, the primary antiacne effect of systemic isotretinoin treatment is sebum reduction, which can be achieved via this process. An alternative Fox O-dependent pro-apoptotic protein called IGF binding protein 3 (IGFBP3) is increased by isotretinoin therapy and rises in human sebocytes when isotretinoin is provided (Draghici et al., 2021). The retinoic acid receptor (RAR) and the retinoid X receptor (RXR) are interacting with a nuclear transcription factor known as IGFBP3. Consequently, the interaction between RXR and IGFBP3 contributes to RXR transcriptional activity, which in turn mediates the effects of IGFBP3 on cellular death. Activation of RAR due to FoxO-dependent nuclear IGFBP3 synthesis could lead to overexpression of the apoptotic NGAL. These results collectively indicate that isotretinoin activates proapoptotic protein (FoxO, TRAIL, IGFBP3, and NGAL) expression (Draghici et al., 2021).

1.6.2 Dose and administration of Isotretinoin

According to evidence-based guidelines and current UK recommendations, oral isotretinoin should be provided at a dose of 0.5 to 1.0 mg/kg per day, with a target cumulative dose of 120 to 150 mg/kg. The European Medicines Agency (EMA) and the European Evidence-Based Guidance recommend starting isotretinoin at a dose of 0.3 to 0.5 mg/kg (Daly et al., 2023). Although there is ongoing debate regarding the most effective dosage schedules for treating severe acne and acne that is not responding to conventional therapy, these regimens have been suggested to be helpful in treating these conditions. Lower dosage regimens may be more

economical, more well-tolerated, and more effective, according to recent research. The long-term remission, occurrence of side effects, and comparability of efficacy between lower dosages and higher dosing regimens are unknown, yet. (Daly et al., 2023).

1.6.3 Pharmacokinetics of Isotretinoin

Isotretinoin's pharmacokinetic profile is well-predictable and can be effectively characterized using linear pharmacokinetic theory. Following oral administration, peak concentrations of isotretinoin in the blood are typically observed 2 to 3 hours later. It is important to note that there is a perceived lag time of 0.5 to 2 hours after consuming isotretinoin capsules before the drug enters the systemic circulation. However, after this lag period, the absorption of the drug appears to occur rapidly. In terms of metabolism, 4-Oxo-isotretinoin (Ro 22-6595) has been identified as the primary isotretinoin metabolite in human blood following oral administration of the medication. This metabolite has also been detected in the urine of patients undergoing oral isotretinoin treatment. These findings contribute to our understanding of isotretinoin's pharmacokinetics, shedding light on its absorption, distribution, metabolism, and excretion in the human body (Brazzell & Colburn, 1982). Potential isotretinoin metabolites present in human blood encompass all-trans-retinoic acid (tretinoin) and its 4-oxo-metabolite (Ro 12-4824). However, it is essential to note that these metabolites are typically detected in trace amounts, often near the detection thresholds, after oral isotretinoin therapy. Notably, research utilizing both isotretinoin and tretinoin strongly suggests the elimination of a glucuronide in the urine and bile. Despite this evidence, the precise identity of an isotretinoin conjugate remains unknown. These findings contribute to our understanding of isotretinoin metabolism, emphasizing the presence of specific metabolites and the need for further exploration to identify additional conjugates and their roles in elimination processes (Brazzell & Colburn, 1982).

Nearly all isotretinoin in the bloodstream is carried by plasma albumin. The drug exhibits an extremely high binding affinity, with approximately 99.9% of isotretinoin bound to albumin in human plasma. Importantly, this binding percentage remains constant across the therapeutic concentration range. Even in patients undergoing long-term therapy, the binding observed is consistent with in vitro binding data. This suggests that the presence of isotretinoin metabolites does not significantly displace the drug from its binding sites. To interpret isotretinoin blood concentration data after oral administration, a linear two-compartment pharmacokinetic model with a lag time before the initiation of first-order absorption provides a suitable explanation. (Brazzell & Colburn, 1982). The model-independent values and parameters derived by applying the isotretinoin data to the established model align closely. Following a single dose of isotretinoin, both the pharmacokinetic characteristics of 4-oxo-isotretinoin and isotretinoin suggest that the metabolite is prone to accumulate more than the parent medication after multiple dosages.

When evaluating the area under the concentration-time curve (AUC), the ratio of AUC for 4-oxo-isotretinoin to AUC for isotretinoin typically falls within the range of 3 to 3.5 after a single oral dose of isotretinoin. This indicates a propensity for the metabolite to accumulate to a greater extent over time. Furthermore, when assessing the blood concentration-time curves for the parent drug and its metabolite during a dosing interval at steady-state under chronic treatment, the ratio consistently remains between 3 and 3.5, as per linear pharmacokinetic assumptions. The findings from subsequent multiple-dose trials generally support and validate these theoretical predictions, providing additional confidence in the accuracy of the established pharmacokinetic model for isotretinoin and its metabolite (Brazzell & Colburn, 1982).

A study was conducted recently on acne patients to use the most sensitive high-performance liquid chromatography assay to update the early pharmacokinetic results (Wiegand & Chou, 1998). Over the last four weeks of isotretinoin treatment, the plasma concentrations of

isotretinoin, retinoic acid, and its metabolites were adjusted to 30 mg daily. Oxo-isotretinoin and isotretinoin had higher steady-state concentrations during therapy than retinoic acid and oxo-retinoic acid (Wiegand & Chou, 1998).

1.7 Teratogenic impact of Isotretinoin

Isotretinoin, a potent medication mainly utilized for severe acne treatment, has been available since 1982 under various brand names such as Accutane. Its notable teratogenic effects, capable of causing birth defects when taken during pregnancy, have been a significant concern. The diverse range of congenital anomalies associated with isotretinoin includes craniofacial abnormalities, cardiovascular and neurological deformities, and thymic illnesses. In response to these risks, a pregnancy prevention program was instituted in 1988, mandating monthly pregnancy tests and the use of two forms of contraception during isotretinoin treatment. Additionally, it has been shown that children exposed to isotretinoin during the perinatal period may experience cognitive impairments ranging from 30% to 60%, even when no physical deformities are present (Draghici et al., 2021).

Severe teratogenic effects of isotretinoin incorporate significant abnormalities in the central nervous system, thymic, cardiovascular, and craniofacial systems. The adverse effects of this medication are wide-ranging, affecting the hepatic, neurological, musculoskeletal, cutaneous, ocular, and reproductive systems (Jamkhande et al., 2014). Pregnant women exposed to isotretinoin during the first trimester face a significantly elevated risk of birth defects. While the drug addresses issues like follicle keratinization and sebaceous gland differentiation, it also possesses anti-inflammatory properties. Despite its widespread use, increasing reports highlight negative effects, with teratogenicity being the most significant concern. The prevalent abnormality linked to isotretinoin is the occurrence of conjoined twins in the thoraco-omphalopagus region. Additionally, craniofacial abnormalities such as hypertelorism, dysmorphism, cleft palate, depressed nasal bridge, and ear defects are associated with its use.

Central nervous system abnormalities include cortical and cerebellar defects, hydrocephalus, microcephaly, and facial nerve palsy. Cardiovascular anomalies involve Tetralogy of Fallot, transposition of the great vessels, septal defects, and hypoplasia of the aortic arch. Thymic abnormalities encompass ectopic, hypoplastic, and aplasia conditions. Limb reduction and spina bifida are also observed as outcomes of the medication (Jamkhande et al., 2014).

Isotretinoin, an oral systemic retinoid, is employed to address severe, resistant nodular acne. Its use is cautioned for pregnant or potentially pregnant women due to the potential teratogenic impact on embryos and fetuses. Prior to initiating isotretinoin treatment for severe acne, a prerequisite involves obtaining at least two negative pregnancy test results. Additionally, various adverse effects associated with isotretinoin, such as xerosis and exacerbation of acne, have been documented in research studies (Brzezinski et al., 2022). Research suggests that a significant percentage, ranging from 20% to 30%, of fetuses exposed to isotretinoin during pregnancy may encounter congenital malformations. The term "Accutane embryopathy" is employed to describe a collection of abnormalities affecting various systems, including the craniofacial, cardiovascular, thymic, and central neurological systems. These abnormalities result from exposure to isotretinoin early in pregnancy. Moreover, the use of isotretinoin has been linked to a heightened risk of spontaneous abortion, with rates ranging from approximately 3% to 17%. Furthermore, individuals born without visible birth defects but exposed to isotretinoin in utero may experience cognitive developmental deficits later in life. These findings underscore the importance of avoiding isotretinoin during pregnancy due to its potential adverse effects on fetal development and overall pregnancy outcomes (Brzezinski et al., 2022).

The teratogenic nature of isotretinoin has intermittently posed challenges for its prolonged usage, necessitating heightened patient education efforts and the establishment of an organized, traceable risk-management strategy known as iPLEDGE. To ensure the elimination of most

isotretinoin metabolites within 10 days of the preceding dose, crucial for minimizing risks, given that the most enduring metabolite has an elimination half-life of up to 50 hours. As per current guidelines, discontinuing isotretinoin is advised at least a month before attempting pregnancy. A study on the pharmacokinetics of isotretinoin in a limited cohort of reproductive-age women revealed that the half-life might be more varied than previously believed, impacting the timeframe required for safe conception following drug discontinuation (Brzezinski et al., 2017). The extensive cell death observed in vertebrates during neural development and the crucial role of apoptosis in the formation of the nervous system, contingent upon proper apoptotic signaling throughout embryogenesis, can elucidate these abnormalities. Furthermore, ATRA (all-trans retinoic acid) is accountable for instigating the reprogramming of gene expression in the cranial neural crest, fostering heightened apoptosis in this process (Draghici et al., 2021).

Isotretinoin is a synthetic retinoid known for its ability to inhibit sebaceous gland development, address keratinization defects within the follicle, and exhibit some anti-inflammatory effects. However, as it becomes more widely used, a matter of concern is arising about its side effects, the most serious effect is teratogenicity. Despite the fact that isotretinoin is clearly labeled as contraindicated during pregnancy, birth defects caused by in utero exposure to the drug are still being documented shortly after dispensing, and these include significant craniofacial, cardiovascular, thymic, and central nervous system anomalies (Malvasi et al., 2009). The inherent risk of abnormalities in the general population typically falls within the range of 3 to 5%. However, in women who are exposed to isotretinoin during the initial trimester of pregnancy, this risk has been observed to escalate to more than 30%. Isotretinoin's teratogenicity in humans has been well-documented, and it has a larger risk of abnormalities (35% vs. 20%) than thalidomide. According to current estimates, 50% of pregnancies are unintended. A portion of these pregnancies include women with various types of acne. It is

simple to anticipate the possible catastrophe of isotretinoin usage during pregnancy given the drug's substantial teratogenic potential and the prevalence of its use by women of reproductive age (Garcia-Bournissen et al., 2008).

1.7.1 The correlation between isotretinoin and teratogenicity

Pregnancy and isotretinoin treatment are linked to a higher risk of teratogenicity. It is widely acknowledged that averting fetal exposure to isotretinoin is a fundamental safety concern. The iPLEDGE program represents the latest in a series of FDA-mandated risk management initiatives designed to assist female patients using isotretinoin in preventing pregnancy (Melnik, 2017). Both humans and lab animals are teratogenous affected by isotretinoin. Humans have a distinct pattern of malformations affecting the central nervous system, heart, thymus, and craniofacial regions. Thymic ectopia or aplasia, cerebellar vermis agenesis, microtia, anotia, micrognathia, conotruncal heart defects, aortic arch abnormalities, and various neuronal migration anomalies are some of the most distinctive abnormalities (Melnik, 2017).

Intracellular isomerization of isotretinoin results in the generation of All-Trans Retinoic Acid (ATRA). Subsequently, ATRA plays a pivotal role in inducing the expression of p53 and HOX transcription factors through the retinoic acid receptor (RAR). The activation of the TP53 promoter is facilitated by HOX, leading to an orchestrated response. Moreover, p53 activation stimulates the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and death receptors (DR4/5). This coordinated action of p53, HOX, TRAIL, and death receptors contributes collectively to the initiation of both the extrinsic and intrinsic pathways of apoptosis. These molecular events highlight the intricate signaling mechanisms through which isotretinoin influences apoptosis, ultimately affecting cellular responses in various pathways (Melnik, 2018).

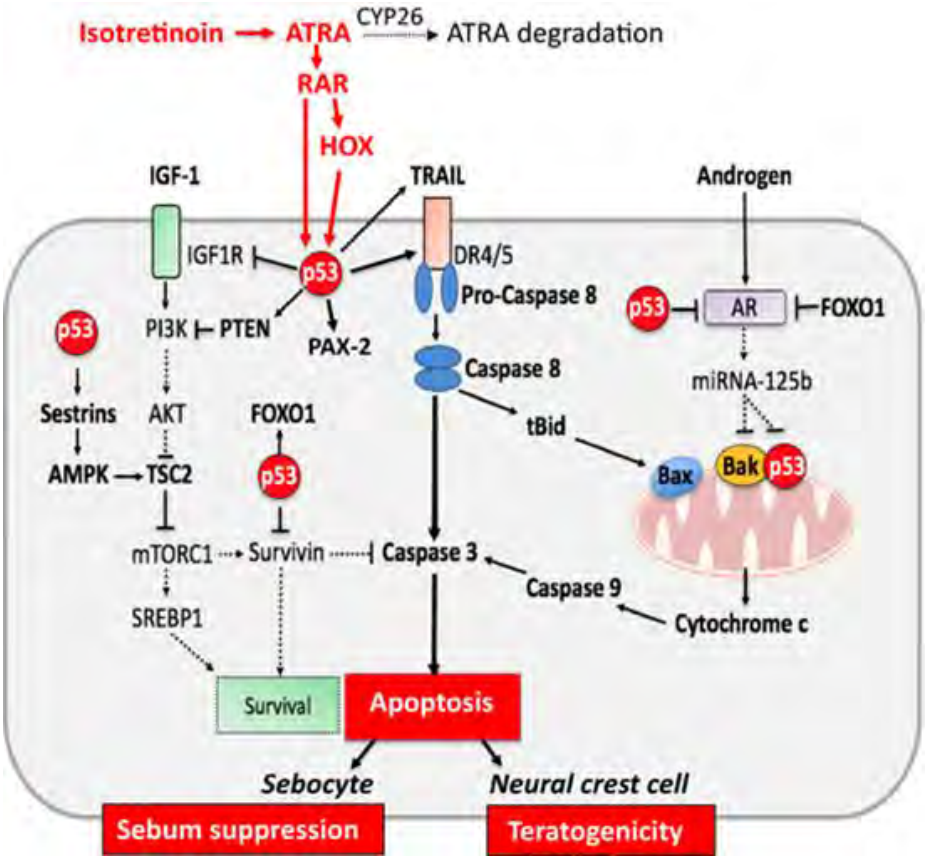

Figure 6: Model of isotretinoin-induced suppression of sebum production and teratogenicity (Melnik, 2018).

By inhibiting the androgen receptor (AR), insulin-like growth factor 1 receptor (IGF1R), and surviving an essential negative regulator of caspase 3, yp53 plays a role in diminishing prosurvival signaling. Additionally, p53 induces the expression of FoxO1, phosphatase and tensin homolog (PTEN), and sestrins, leading to the activation of AMP-activated protein kinase (AMPK), a key negative regulator of the mechanistic target of rapamycin complex 1 (mTORC1). Consequently, the overexpression of p53 induced by ATRA results in a reduction of prosurvival signaling and an increase in pro-apoptotic signaling. The desired sebum-suppressive effect of isotretinoin is attributed to an elevated rate of sebocyte apoptosis. Simultaneously, the teratogenicity of isotretinoin is associated with apoptosis in neural crest cells during embryogenesis. These molecular mechanisms underscore the dual impact of isotretinoin on cellular processes, leading to its therapeutic effects in treating acne but also

highlighting potential risks during pregnancy due to its impact on embryonic development (Melnik, 2018).

The impact of the medication on neural crest cells elucidates the primary characteristics of the syndrome. In vertebrates, massive cell death takes place during neural development. In order to ensure the appropriate differentiation and maturation of cells in the developing nervous system, programmed cell death, or apoptosis, which is a conserved element of nervous system development throughout evolution, requires a meticulously regulated equilibrium of apoptotic signaling during crucial developmental phases. It is noteworthy, that the root cause of foetal alcohol syndrome is also elevated apoptosis of neural crest cells. Increased apoptosis is the outcome of ATRA-induced widespread reprogramming of cranial neural crest gene expression. Isotretinoin administration has been shown in animal studies to increase neural crest cell apoptosis. When isotretinoin is administered during the ganglion formation stage, excessive cell death occurs, which appears to be restricted to trigeminal ganglion neurons of placodal origin and results in abnormalities. Following administration, there is an excess of cell death that appears to be restricted to trigeminal ganglion neurons of placodal origin of isotretinoin during the development of ganglions and causes deformities (Melnik, 2017).

Isotretinoin-induced craniofacial malformations are primarily caused by increased neural crest cell apoptosis. Teratogenic effects of isotretinoin include abnormalities of the aortic arch and heart defects. Because isotretinoin impairs neural crest cell migration, it causes a distinct pattern of cardiac defects. The coordinated regulation of cell proliferation and apoptosis is necessary for the morphogenesis and developmental re-modelling of cardiovascular tissues. The developing coronary vasculature, cardiac valves, conducting system, and embryonic outflow tract have all been shown to be influenced by focal apoptosis in the heart. EATRA's specific targets in the cardiovascular domain include effects on the specification of cardiovascular tissues during early development, including the formation of the endocardial

cushion, left/right decisions, and the early heart's anteroposterior patterning (Melnik, 2017). In medium containing 10–6M isotretinoin, the proliferation in the heart tissue of whole chick embryo cultures was inhibited to 62% of the control level in the myocardium. When combined, these findings emphasize that the primary cause of isotretinoin's teratogenicity is neural crest cell apoptosis (Melnik, 2017).

The outcomes of taking isotretinoin orally in single doses supported our earlier findings. One dose of 100 mg/kg isotretinoin did not demonstrate any embryotoxicity on any of the gestational days examined. 200 mg/kg of isotretinoin given orally once resulted in a moderate embryotoxic response; on day 10.5 and day 11, 19% and 25% of the fetuses exposed had cleft palates but no limb defects, respectively. Isotretinoin, at 400 mg/kg, impacted 33–38% of the exposed fetuses; all of them displayed cleft palates, and most of them also had abnormalities in their limbs (Kochhar & Penner, 1987).

1.8 Measure of safe use of isotretinoin

The iPLEDGE program was established by the US Food and Drug Administration (FDA) in 2006 with the objective of offering more comprehensive guidelines to enhance the prevention of potential pregnancies during isotretinoin treatment. This initiative incorporates monthly pregnancy testing, the documentation of contraceptive methods, and continuous education regarding potential adverse effects to underscore the primary objective. Furthermore, all patients are systematically enrolled in a database. Despite the implementation of these measures, the issue of pregnancies during isotretinoin treatment persisted, and there was no discernible improvement in outcomes compared to the SMART program (Draghici et al., 2021). Enhancing the iPLEDGE program necessitates several measures, such as extending the duration of educational sessions for both women and their partners regarding isotretinoin treatment, potential side effects on infants, and available contraceptive methods, including proper procedures for pregnancy tests. Additionally, obtaining the drug should require a

medical prescription, contributing to increased awareness about the effectiveness of iPLEDGE (Draghici et al., 2021). Regulatory bodies and Hoffmann-La Roche, the drug's producer, made multiple attempts in North America to create initiatives that protected expectant mothers from isotretinoin exposure. In Canada, any physician has the authority to prescribe isotretinoin; however, a pregnancy prevention program (PPP) stipulates that female patients must sign an informed consent form and commit to employing at least two forms of birth control throughout the course of isotretinoin treatment. This includes the use of an oral contraceptive pill (Garcia-Bournissen et al., 2008).

In Europe, the European FDA, working in unitedly with the European Directive governing the prescription of systemic isotretinoin, established a PPP applicable to all female patients undergoing isotretinoin treatment. Additionally, patients are advised to undergo multiple pregnancy tests, including one before the initiation of therapy, one during the course of treatment, and another five weeks after completing the therapy (Draghici et al., 2021).

1.9 Aim

The aim of this project is to conduct a comprehensive review concentrating on the teratogenic impact of isotretinoin and its safety profile.

1.10 Objectives

- From the available literature different types of teratogenic effects are found.
- Using publicly available pharmacovigilance database FAERS, this project attempted to investigate the teratogenic outcomes of fetuses that has been exposed to isotretinoin.

Chapter 2

Methodology

The review task involved selecting relevant literature, analyzing scientific articles, and summarizing this paper. The facts and statistics in this review were compiled from numerous scientific articles on the subject. To gather all of the journals relevant to this topic, an online search was conducted. Following an analysis of the information gathered from the selected recently published articles, a plan was developed to convey the gleaned material in accordance with the project objectives.

The main aim of this review is to acquire more information about the possible improvement of the safety profile of isotretinoin and its teratogenic effect by analyzing the possible mechanism of action that causes teratogenicity in pregnancy and leads to birth defects. The scientific articles are chosen based on the information they contain about isotretinoin and their mechanism of action and teratogenicity adverse effects. The scientific articles are being selected according to the information about isotretinoin, mechanism of action, teratogenic effect and its causes, isotretinoin safe use to interpreted the possible policies changes and proper practice of pharmacovigilance measures.

Research performed a thorough search for studies on PubMed, Google Scholar, Elsevier, Natures, Spriners for studies investigating and ScienceDirect. Search parameters include a 1982–2023, time frame and a solely in English language constraint on the articles. All studies that met the inclusion criteria, including observational studies, books and documents, systemic review was chosen for further investigation.

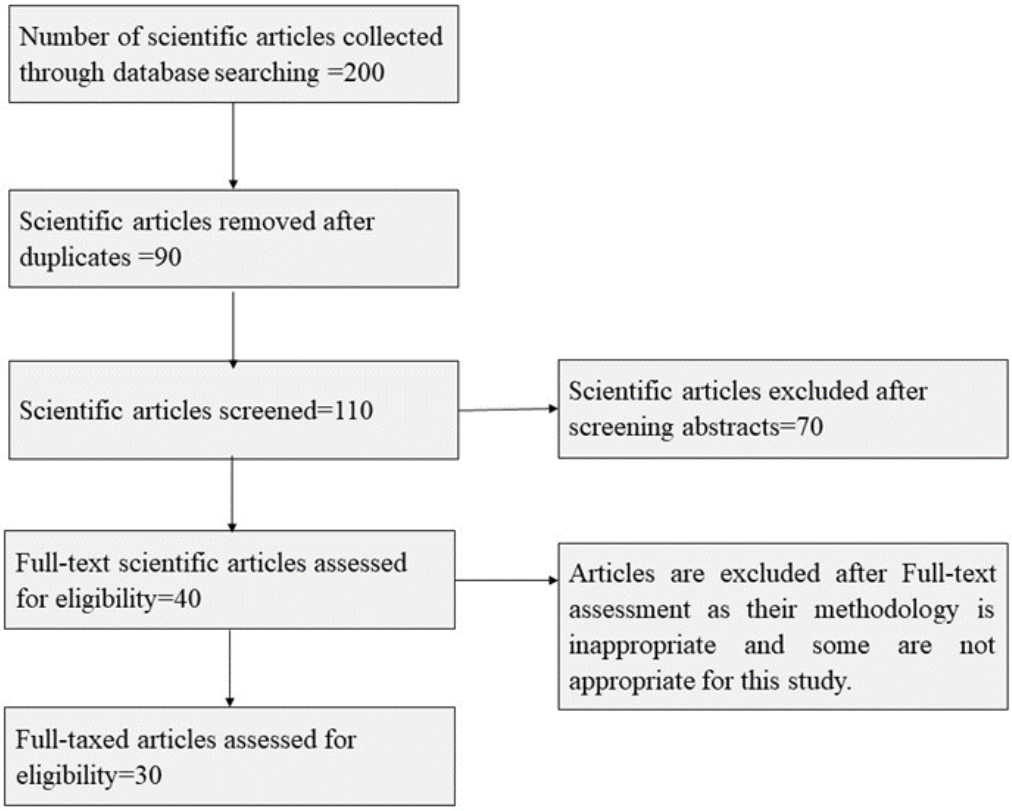

Figure 7: Flowchart for selection of scientific articles for this review

Chapter 3

Result

3.1 Comparison between studies of pregnant women exposed to isotretinoin

Table 1: Comparative investigation on the congenital anomalies observed in pregnant women who were exposed to the drug isotretinoin.

Study name	Count of pregnant women exposed to isotretinoin	Birth defects in the infants	Reference
Pregnancy and neonatal outcomes after periconceptional exposure to isotretinoin in Koreans	51 pregnant women, 42 lived	7 infants had neonatal malformations	(Cha et al., 2022)
Epidemiology of isotretinoin exposure during pregnancy	222 pregnant women, 151 lived	71 fetuses had congenital malformations	(Dai et al., 1992)
A Survey of Pregnant Women Using Isotretinoin	34 pregnant women, 12 lived	12 infants had isotretinoin embryopathy	(Robertson et al., 2005)
Isotretinoin exposure during pregnancy: a population-based study in The Netherlands	57 pregnant women	53 infants had neonatal outcome	(Zomerdijk et al., 2014)

In the given above table, information is organized into four sections, each associated with distinct study names. The table includes details such as the number of pregnant women exposed to isotretinoin, the numerical data of birth defects in infants, and corresponding references.

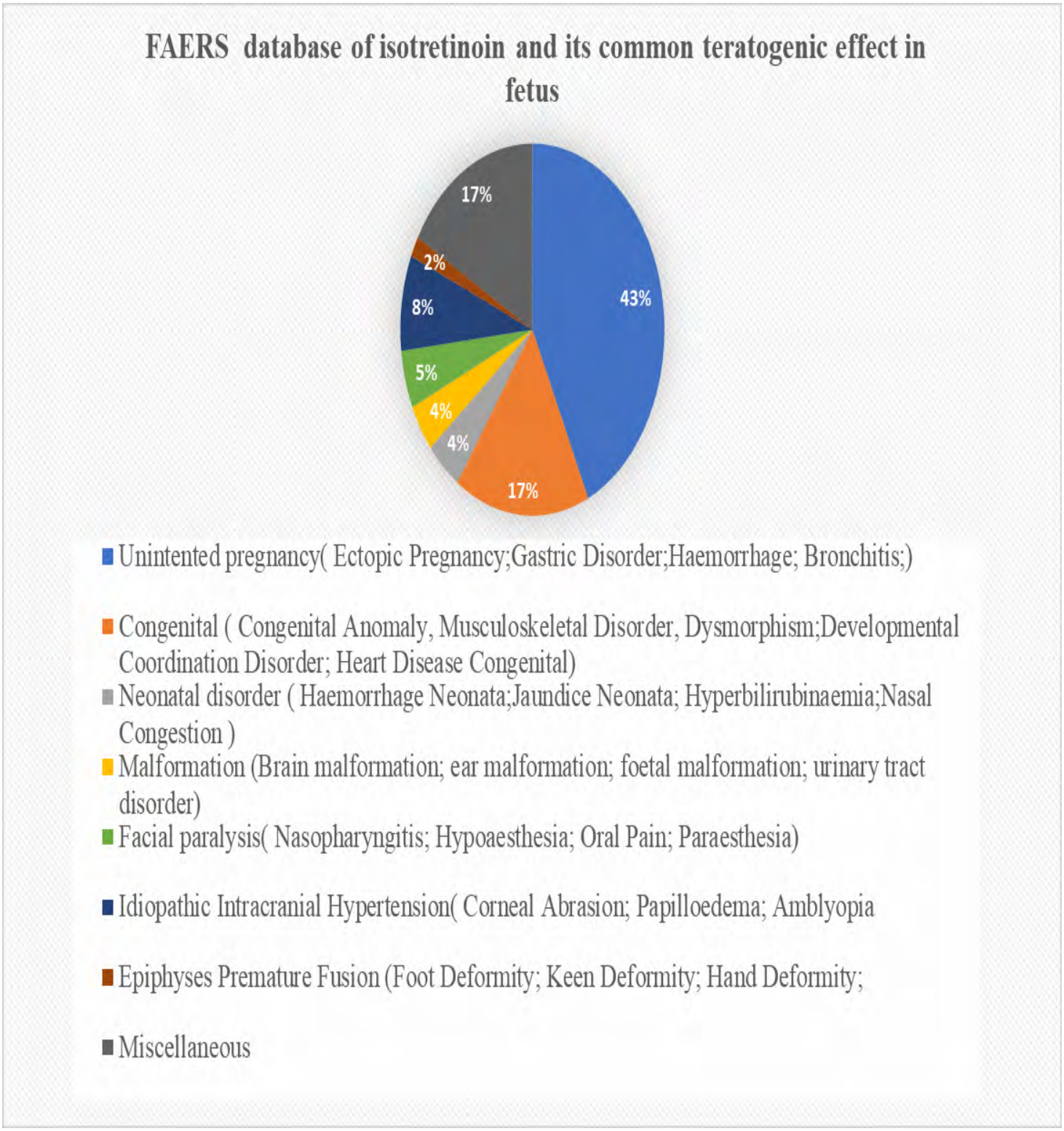

Figure 7: A visual representation of teratogenic conditions found in the FAERS report.

The provided pie chart, sourced from the FAERS database, illustrates adverse reactions in fetuses due to isotretinoin use during pregnancy, with a total of 70,933 patient reports in the

database. The chart is segmented into eight sections, each denoted by a distinct color for clarity. The predominant blue section represents unintended pregnancies, while the orange section indicates congenital disorders. The grey segment corresponds to neonatal disorders, and the yellow portion signifies malformations. Additionally, the green section is attributed to facial paralysis, the navy-blue part to idiopathic intracranial hypertension, and the brown portion to premature fusion of epiphyses. The final black section encompasses miscellaneous adverse reactions, along with their respective percentages.

3.2 Adverse events

Table 2: Along with teratogenic effect the other adverse side effects of isotretinoin (McLane, 2001)

Type of AE's	Example	Incidence
Gastrointestinal disorders	• cheilitis	91.3%
Dry skin	• localized exfoliation • rash erythematous • dermatitis	90%
Epistaxis (nose bleeds)	• respiratory, • thoracic, • mediastinal disorders, • nasal dryness	30%-40%
Eye irritation	• dry eyes	25%
Others,	• Fatigue, hair loss, bone joint pain, Muscular pain	25.2%, 8.9%, 21.1%, 17.7%

The purpose of this table is to provide more information to professionals with additional insights into the anticipated adverse event profile of patients undergoing isotretinoin treatment. It delineates the frequency and severity of common adverse events noted in clinical trials. Predominant side effects of isotretinoin encompass mucocutaneous symptoms affecting the lips, eyes, mouth, and other epidermal surfaces. Furthermore, significant serious adverse events are outlined in the cautionary segments of the medication package insert and label. These encompass psychiatric disorders, pseudotumor cerebri, diminished night vision, corneal opacities, inflammatory bowel disease, hyperostosis, hepatotoxicity, and hypertriglyceridemia (McLane, 2001). Understanding the profile of potential adverse events and effectively communicating this information to the patient increases the likelihood of adherence to prescribed medication and encourages the patient to explore additional therapies that may mitigate or alleviate side effects (Kapała et al., 2022)

Chapter 4

Discussion

Isotretinoin is frequently prescribed to treat severe acne; however, it has a serious side effect known as teratogenicity that affects pregnant women. The facts in the table and the information that follows on exposure to isotretinoin during pregnancy and its side effects give significant insight into the possible risks linked to this drug use.

The studies shown in the table illustrate various levels of adverse effect that isotretinoin exposure caused to pregnant women. The first study was carried out by Cha et al. (2022) and involved 51 pregnant women, 42 of whom survived. Among them, seven infants had birth abnormalities. In the second study, which was carried out by Dai et al. (1992), 151 surviving fetuses, 71 fetuses with congenital abnormalities, and 222 pregnant women were involved. According to Robertson et al. (2005), 12 babies delivered to 34 pregnant mothers who were exposed to isotretinoin experienced isotretinoin embryopathy. In the fourth trial, 53 of 57 pregnant women who were exposed to isotretinoin had malformations in their newborns, according to Zomerdijk et al. (2014).

The pie chart derived from the FAERS database delineates adverse reaction reports associated with fetuses exposed to isotretinoin during pregnancy. The visual representation provides an overview of the diverse range of adverse consequences and their potential impact on fetal health. Notably, 43% of the chart's initial segment accounts for unintended pregnancies, leading to outcomes such as abortion, ectopic pregnancy, bleeding, and bronchitis. In the subsequent 17%, congenital cases result in anomalies, musculoskeletal disorders, dysmorphism, and congenital heart disease. Another 4% documents malformations, encompassing urinary tract disorders, prenatal malformations, brain malformations, and ear malformations. Neonatal conditions, constituting 4%, include acquired hemorrhage, neonatal jaundice, hyperbilirubinemia, and nasal congestion. Facial paralysis, comprising 5%, entails issues like

nasopharyngitis, hypoesthesia, oral pain, and paresthesia. Idiopathic intracranial hypertension, at 8%, involves conditions like corneal abrasion, papilledema, and amblyopia. Additionally, 2% pertains to premature fusion of epiphyses, manifesting as foot deformities, knee deformities, hand deformities, and decreased bone density. The remaining 17% represents miscellaneous reactions documented in the database

In addition, McLane's (2001) study into other isotretinoin adverse effects explains the wider spectrum of challenges associated with this drug. The symptoms include eye pain, dry skin, nosebleeds (epistaxis), stomach problems, and a variety of other problems, with frequency data provided for each. Beyond its teratogenic effects, this evidence broadens our understanding of isotretinoin's overall impact on patients.

In short, the information provided mostly validates the generally held belief that isotretinoin causes serious risks to fetal development during pregnancy. Healthcare professionals and patients must take great care to follow strict safety protocols and contraceptive practices in order to prevent unintended pregnancies while on isotretinoin. The possible adverse effects emphasize how important it is to provide thorough patient education and take regulatory action to reduce the occurrence of birth defects linked to isotretinoin and other problems.

Chapter 5

Conclusion

Isotretinoin is known to be extremely teratogenic, which means that if taken during pregnancy, it might result in serious birth abnormalities. As a result, strong protocols and procedures have been implemented to avoid its usage in pregnant women and to reduce the risk of unwanted pregnancies during isotretinoin therapy. These measures are intended to improve pharmacovigilance and to reduce harmful consequences. Efforts should be directed towards minimizing pregnancies in women undergoing isotretinoin treatment. In consideration of this goal, it is imperative to assess the contextual factors specific to the country where the drug is prescribed and the characteristics of the individual who will be using it (Garcia-Bournissen et al., 2008). Pregnancy Prevention Programs or Risk Evaluation and Mitigation Strategies (REMS) for isotretinoin are presently in place across diverse countries. These initiatives aim to assist women undergoing isotretinoin treatment in preventing pregnancies by ensuring the use of effective contraception. Employing contraception during isotretinoin usage can prevent teratogenicity, and severe cases are rare and often reflective of individual reactions to the medication. Enhancing patient understanding of the adverse event profile and providing explanations increase the probability that patients will adhere to prescribed dosages and seek additional treatments to alleviate or eliminate undesired effects (Kapała et al., 2022).

Pharmacovigilance is the active monitoring of the safety of isotretinoin by regulatory bodies, healthcare practitioners, and pharmaceutical corporations to detect any potential harmful effects. This entails gathering, analyzing, and interpreting data pertaining to the drug's safety profile. Dermatologists and other prescribing physicians should be aware of this potentially fatal side occurrence, and prescription indications should be followed (don't use if when less toxic medications are available), and patients should be properly examined and monitored for signs of depression on a regular and frequent basis during therapy. This critical caution should

be included in pharmacists' counseling of isotretinoin users and their families (Theodoridis & Kraemer, n.d.).

The iPLEDGE program stands as a comprehensive risk management initiative for isotretinoin in the United States. Registration is required for prescribers, pharmacies, and patients alike. Female patients with the potential for childbearing are obligated to use contraception, and both male and female patients must adhere to regular monitoring and reporting protocols. Enhancing the iPLEDGE program could involve implementing several measures, such as extending the duration of educational sessions for both women and their partners regarding isotretinoin treatment, potential side effects on infants, available contraceptive methods, and proper pregnancy test procedures. Additionally, obtaining the drug would necessitate a medical prescription, contributing to increased awareness about the effectiveness of iPLEDGE (Draghici et al., 2021).

In conclusion, these rules and actions indicate a commitment to balance the therapeutic benefits of isotretinoin for severe acne with the need to protect pregnant women from serious adverse effects. These programs may be updated and revised on a regular basis based on ongoing pharmacovigilance efforts and new scientific information. During isotretinoin therapy, patients are encouraged to rigorously follow these guidelines and to disclose any concerns or changes in their health status to their healthcare professionals. The off-label use of isotretinoin presents a viable therapeutic option for dermatological conditions beyond acne. However, there is a need for additional prospective, randomized human trials to ascertain optimal guidelines for prescribing isotretinoin off-label, ensuring both maximum efficacy and safety(Chu et al., 2021). The future direction can be imperative to conduct further research and consider policy implications to assess the safety profile of oral isotretinoin.

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