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ROLE OF METFORMIN IN INSULIN RESISTANCE IN PCOS

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CANDIDATE'S DECLARATION

I hereby certify that the work which is presented in the research work entitled “**Role of metformin in insulin resistance in PCOS**” in fulfilment of the requirement for the award of Degree of Masters in Science in Biotechnology and submitted to the Department of biotechnology, Delhi technological university, Delhi is an authentic record of my own work, carried during a period from 7-jan-2021 to 28-may-2021, under the supervision of Dr. Yasha Hasija.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other university. This work has been communicated in SCI indexed journal with the following details:

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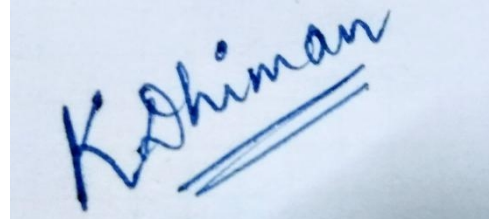
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SUPERVISOR CERTIFICATE

I ,hereby, certify that the the Project Dissertation titled “**ROLE OF METFORMIN IN INSULIN RESISTANCE IN PCOS**” which is submitted by **Kshamta Dhiman, Roll no. 2K19/MSCBIO/22**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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Date : May,31,2021


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Last but not the least, I thank the almighty for his blessings.

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Kshamta Dhiman

ABSTRACT

Poly cystic ovarian syndrome (PCOS) is still being said to be one of the strangest hormonal & metabolic disorder common among among females of the child bearing age group. The various clinical manifestations of PCOS appears not only morphologically but also physiologically. Although psychological effects are also being seen apart from these, but they have been deliberately avoided in this review and the topic has been centralised keeping in demand of the topic where hormonal and metabolic issues have been addressed. Chronic consequences of PCOS also include T2DM and insulin resistance. Metformin, one of the widely used hypoglycemic drug, is also used nowadays in treating the metabolic issues found out in PCOS patients. This drug was also used not in addressing these manifestations but also in inducing ovulation along with combination of some other drugs which were also further helpful in treating the issue of anovulation, infertility and make greater chances of conception. This dissertation is basically a review of all the literature studies I have done in my whole journey. Also there was a variability of data in all these literature studies and therefore only the qualitative data that facilitated in designing my dissertation. Later in this review, it was confirmed that how the metformin usage was useful in regulating all the dysfunctioned consequences of PCOS like control of insulin sensitivity. Biochemical effects included reduced unbounded testosterone levels in blood and an augmented SHBG levels. However, data supporting the weight loss in PCOS still requires a lot of research. Further assessment for the long term effectiveness, who are the beneficiaries, and its optimal duration of usage.

Keywords: Acne, Diabetes mellitus, hirsutism, insulin resistance, metformin, ovulation, PCOS.

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List of abbreviations

S.no.	Abbreviation	Full form
1.	PCOS	Poly Cystic ovarian syndrome
2.	T2DM	Type 2 diabetes mellitus
3.	FSH	Follicular Stimulating Hormone
4.	LH	Luetinizing Hormone
5.	SHBG	Sex Hormone Binding Globulin
6.	IGFBP	Insulin like Growth Factor Binding Protein
7.	FFA	Free Fatty Acids
8.	OCT-1	Organic Cation Transporter-1
9.	AMP	Adenosine Mono-phosphate
10.	AMPK	Adenosine Mono-Phosphate kinase
11.	CBP	CREB- binding protein
12.	CRTC2	CREB- regulated transcription coactivator 2
13.	NADH	Nicotine adenine dinucleotide hydride
14.	LDH	Lactate Dehydrogenase
15.	GPD	Guanosine 5-diphosphate
16.	ATP	Adenosine tri-Phosphate
17.	ADP	Adenosine mono-phosphate
18.	OCP	Oral Contraceptive Pills
19.	PI3K	Phosphoinositide 3-kinase
20.	LHr	Luetinizing Hormone receptor
21.	HSD 3	Hydroxysteroid Dehydrogenase 3
22.	CYP 17	Cytochrome P 17
23.	BMI	Body Mass Index
24.	OHSS	Ovarian Hyper Stimulation Syndrome
25.	PP	Post Prandial
26.	IVF	In-Vitro fertilization
27.	NIDDM	Non insulin Dependent diabetes mellitus

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CHAPTER 1: INTRODUCTION

1.1 General

PCOS is a multifactorial indisposition. Polycystic ovarian syndrome (PCOS) is the most recurrent endocrine pathology in the reproductive age female around the world. PCOS complexity is characterized by elevated androgen levels, irregular menstrual periods, and/or unilateral or bilateral ovaries. This heterogeneous ailment affects at least 7% of grown up females. Evidences imply that 5% to 10% of females from 18 to 44 years of age are affected by PCOS, making it almost a stereotypical hormonal imbalances among females of reproductive age. PCOS affected women are much predisposed towards the probability of emergence of endometrial cancer, Congenital heart disease, and T2DM. The progenitive & metabolic characteristics of PCOS are many a times revocable with lifestyle refinements such as weight reduction and other physical activities. The root cause of PCOS is concealed, but there has been a consequential association with both the insulin resistivity and fat stubbornness. The link with insulin function is anticipated as insulin ameliorates the functioning in synchronizing functions of ovaries, and in turn they also respond to excess insulin by promoting androgens production, an ultimate foundation of anovulation.

Follicular suppuration interruption is an attribute that indicates an ovarian dysplasia. It is absolutely an endocrinopathy with a huge aptitude predisposition factor inside it which initiates profusely many diseases which is also a prominent cause of infertility among women.

The drug named Metformin is the only differentiated and reviewed insulin triggered drug in PCOS. Endocrine Societal Norms clearly advocate the metformin treatment in PCOS patients with T2DM or IGT with abrupt-ed daily routine. It decreases progression from increased glucose tolerance to NIDDM. It is a second-line therapy for irregular menstrual periods in patients with a prophylactic axis for OCPs..

This therapy is also used for adolescents from time and again as monotherapy, which helps in the restoration of normal menstrual cycles, and increased insulin sensitivity, and even though it is still not considered first and foremost drug to treat clinical hyperandrogenism, as it can also upgrade androgen excess feature.

In this paper we are going to review the role of metformin in insulin resistant patients and along with this we will see if metformin is actually a highly effective drug for subsidizing the PCOS patients symptoms.

1.2: POLYCYSTIC OVARIAN SYNDROME:

1.2.1 HISTORY OF PCOS:

PCOS which is being abbreviated for poly cystic ovarian syndrome is both hormonal disorder combined with metabolic disorder. Significantly , in 1935, Stein and Leventhal showed that 7 women patients were diagnosed with common clinical features of hirsutism, disturbance in their periodic cycles of menstruation and heaviness found in the ovaries due to numerous small follicles. Due to the presence of numerous such small follicles, the ovaries are not able to release one mature egg per ovulation and hence further creating complications in conceiving or more peculiarly the fertility issues.

There are various names for the PCOS disorder such as hyperandrogenic, Chronic anovulation, ovarian dysmetabolic syndrome, functional ovary androgenism. All of these names are self explanatory and even helps in providing a proper description of all the clinical features of PCOS.

Table 1.1 : Definition of various terms used for PCOS

<u>S.no.</u>	<u>Terms</u>	<u>Definition</u>
1.	Hyperandrogenic/functional ovary hyperandrogenism	Excessive circulation or production of male hormones such as testosterone inside a female body.
2.	Chronic anovulation	Inability of ovaries to release one mature egg regularly every month is anovulation, characterised along with ammenorhea. Chronic anovulation is one of the cause of infertility.
3.	Ovarian dysmetabolic syndrome	An assembly of all the metabolic disorders resulting primarily from insulin insensitivity, leading to T2DM and other cardiovascular disorders.

1.2.2 Etiology of PCOS:

- Genetic and environmental factors both can be honoured as culprit that can be held responsible for the etiology of PCOS in women.
- Add-ons are ruinous lifestyle, diet or any infections to the body.
- Further resistance in insulin and also the elevated quantity of same effectuates the disturbance in functions of ovary as well as again predisposing again towards higher level of free testosterone in the body.
- The severity of PCOS can also be shouldered upon the associated levels of sufficiently high amount of both androgen and insulin in blood.
- Other hormonal level of FSH, LH and prolactin are also get disrupted in PCOS.
- Mutations in some genes and nucleotides change the transcriptional activities and hence lead to PCOS.

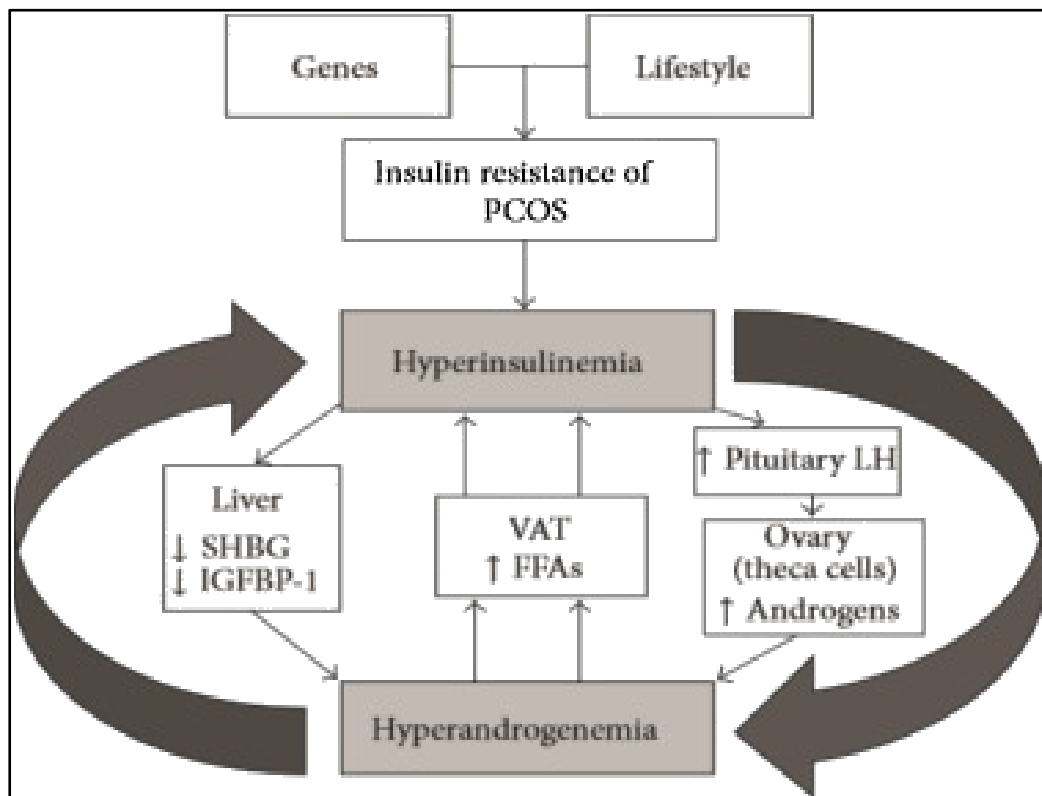


Fig. 1.1: picture showing diagrammatic view of how insulin dysfunctioning disturbance disrupts the functioning of ovarian theca cells.

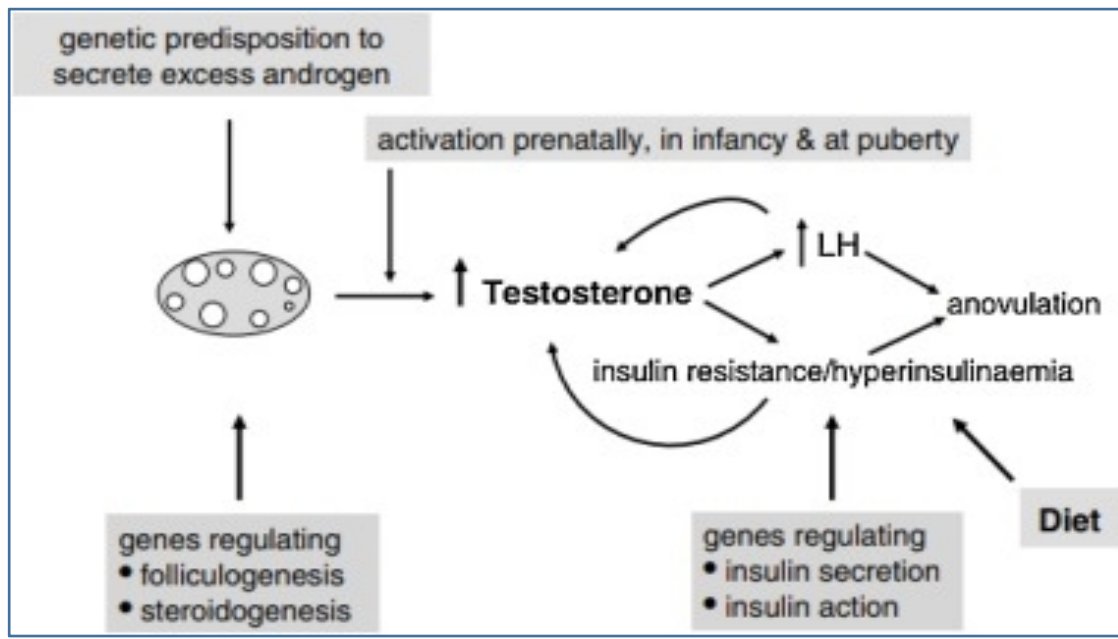


FIG. 1.2: Picture showing diagrammatic representation of how the defect in the pituitary branch embark on to elevated level of LH and testosterone. It further leads to an increase in insulin level. Together all this promote anovulation.

1.2.3 CLINICAL MANIFESTATION OF PCOS:

1.2.3.1: Manifestations of hyperandrogenism :

Table 1.2: phenotypic characters of hyperandrogenism

<i>Hirsutism</i>	excessive growth of hair on chest, abdomen, face and back primarily due to immoderate production of male hormone mainly testosterone.
<i>Acne vulgaris</i>	An extended dermatological condition where there is excessive production of sebaceous glands (oil) along dead skin

	cells clog the hair follicles often due to high levels of male hormone causing severe acne on face, back and chest.
<i>Seborrhea</i>	Production of scaly lesions and red rashes causing stubborn dandruff.
<i>Alopecia</i>	Loss of hair follicles.

1.2.3.2 : MANIFESTATIONS OF OVARIAN DYFUNCTION:

Table 1.3 : table defining various dysfunctionings in the ovary

OLIGOMENORRHEA	Infrequent menstrual cycles or periods occurring at a length of more than 45 days
AMENORRHEA	Absence of more than 3 cycles in a row.
ULTRASOUND POLYCYSTIC OVARIES	Assembly of more than 12 egg follicles In ovaries

1.2.3.3 : OTHER MANIFESTATIONS

Besides the above manifestations, pcos patients have

- BMI > 30ng/m².
- Waist by hip ratio is also measured :

Table 1.4 : waist by hip ratio of women to find out the type of obesity

Waist : hip	Type of obesity
> 0.85	Android obesity
< 0.75	Gynaecoid obesity

- **HYPERINSULINEMIA:** presence of more than normal insulin level in blood.
- **ACANTHOSIS NIGRICANS:** a condition of hyperpigmentation of skin near folds such as armpits, groin, neck, naval and forehead .

1.2.4 CONSEQUENCES of PCOS:

Several long term consequences are faced by patients suffering from PCOS :

1.2.4.1: Hyperinsulinaemia and insulin resistance:

- It has also been seen that women suffering from both hyperinsulinaemia and insulin resistance have a greater probability of facing and having PCOS.
- Also insulin insensitivity is also not a clinical attribute of all the women having anovulation or ovulation. But some studies have shown that some sturdy interrelation between insulin resistance and menstrual irregularity in women.
- Insulin resistance is also being associated with higher degree of production of testosterone.

1.2.4.2: Increased risk of NIDDM:

- Pancreatic beta cell's dysfunctioning was tested by IVGTT, both lean and obese women showed positivity towards it in addition to insulin resistance.
- This metabolic defect indicated the probability of **developing T2DM subsequently by PCOS patients.**
- Dysfunctioning in both secretion and action of insulin and intolerance towards glucose has been detected in adolescents having PCOS.

1.2.4.3: HYPERLIPIDAEMIA:

- Deviation from the normal range of lipids result in hyperlipidaemia with more correlated with cardiovascular disorders.
- Dyslipidaemia is more generally associated with PCOS.

- Low density lipoprotein(LDL),High density lipoprotein (HDL), Triglycerides level were exceptionally high in women having PCOS.

1.2.4.4: . Endometrial cancer:

- Women suffering from chronic anovulation and unresricted exposure of estrogen to endometrium have higher chances of developing endometrial cancer.
- Obese women rather than normal weight women, including hypertension or high blood pressure and hyperglycaemia were found to be some significant causes of endometrial cancer.
- a retrospective study also showed that pcos women having anovulatory cycles can have the risk of developing endometrial cancer at later stages in their lives.
- Thus PCOS serve as a factor of comorbidity for endometrial cancer.
- Women with both the types of diabetes mellitus also have higher probabilities on developing endometrial cancer.

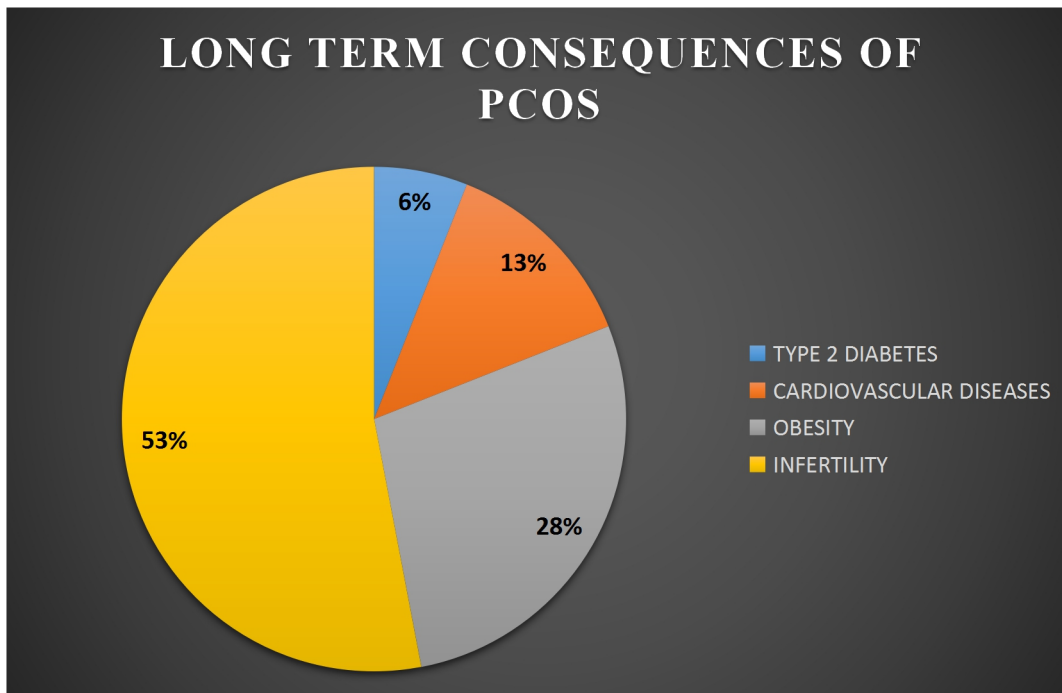


Fig. 1.3 : Pie diagram showing the long term consequence of PCOS percentage

1.2.5 DIAGNOSTIC CRITERIA FOR PCOS:

Table 1.5 : “ Based on the new criteria from the National Institute of Child Health and Human Development (NICHD)/National Institutes of Health (NIH)/American Society for Reproductive Medicine (ASRM)”-[Ndefo et. el., 2013]

NICHD/NIH Criteria (1990)	ESHRE/ASRM Rotterdam Criteria (2003)	Androgen Excess Society (AES) Criteria (2006)
<ul style="list-style-type: none"> ● Hyperandrogenisation ● OligoOvulation/Anovulation ● Elimination Of Other Related Disorders 	<ul style="list-style-type: none"> ● Hyperandrogenisation ● Oligoovulation ● Unilateral or bilateral ovaries 	<ul style="list-style-type: none"> ● Hyperandrogenisation ● Oligoovulation/anovulation ● Unilateral or bilateral ovaries

1.3 : Metformin

1.3.1: History of drug:

Metformin was initially obtained via from galegine, a naturally obtained byproduct of the plant *Galega officinalis*, utilization in herbal medicine. One such more drug was phenformin, similar to metformin derived from the same plant, was later discarded due to occurrence of lactic acidosis. Galegine was an isoprenyl derivative, which was used as lowering agent for blood sugar in early 1920s. Metformin are biguanides because they contain two coupled molecules of guanidine with some additions.

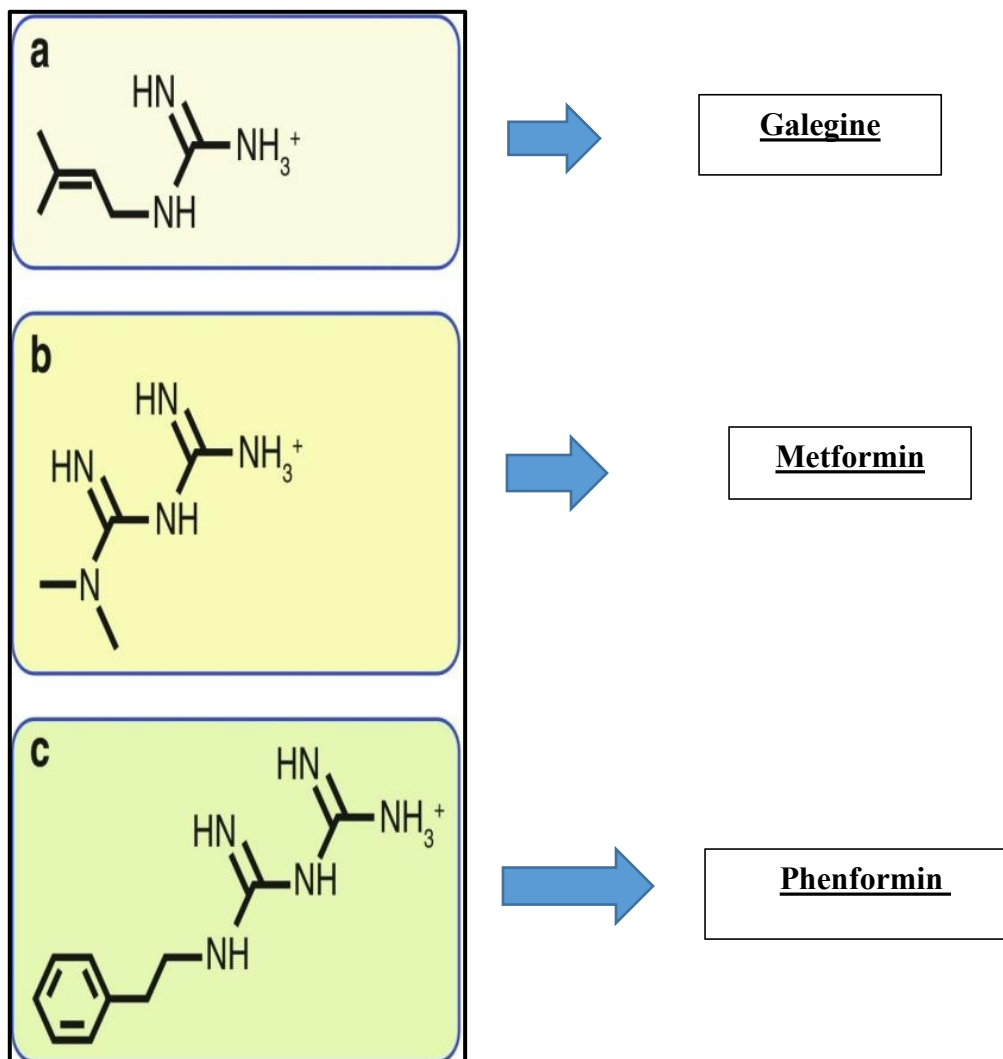


Fig. 1.4 : structures of various derivatives of metformin

1.3.2 : MECHANISM OF ACTION OF METFORMIN:

Metformin has been an important drug used due to its antihyperglycemic property. An oral drug which is being recommended worldwide to all the newly diagnosed T2D patients.

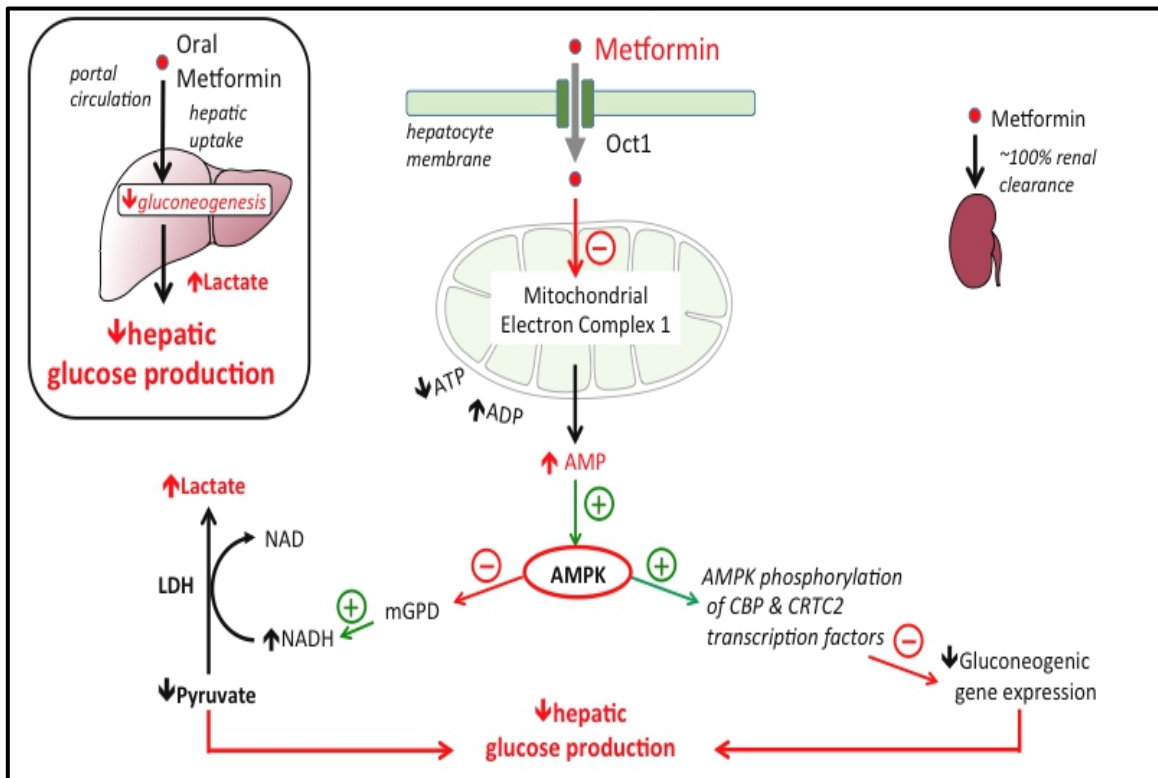


Fig. 1.2: figure showing the action mechanism of metformin whose overall effect was on how to reduce the production of hepatic glucose.

- During the oral administration of metformin, plasma membrane transporters carry the metformin to be absorbed in hepatocytes through portal vein with the help of OCT-1 as well.
- Metformin subsequently hampers respiratory chain complex-1 occurring in mitochondrial matrix. Hence giving rise to an overall escalation in the level of AMP rather than ATP.
- AMP further activate AMPK, which helps in the lowering of glucose in two ways:
 - i. AMPK phosphorylates transcription factors such as CBP and CRTC2, that helps in further decline of gluconeogenic gene expression and hence lowering the glucose production.
 - ii. By inhibiting the enzyme mitochondrial glycerol-3-phosphate dehydrogenase, prompting to an elevated level of production of NADH which would further stimulate the increased production of pyruvate to lactate and hence decrease gluconeogenesis.

CHAPTER 2: LITERATURE REVIEW

2.1 : Why only metformin is chosen for study under this project ?

Based on the research evidence or data that PCOS is sequel of endocrinopathy and metabolic mess. PCOS patients are always at borderline to develop T2DM. Various manifestations of PCOS include pancreatic beta cell malfunctioning, T2D1, insulin resistance which further have comorbidities of infertility, faulty folliculogenesis due to higher level of androgens and LH in bloodstream.

Metformin is being used as a first line of drug due to its clinical feature of decreased gluconeogenesis and also as a blood glucose lowering agent maintaining both basal and postprandial glucose levels in blood and thus helpful in treating the manifestation of metabolism disorder in PCOS. Metformin also has role in enhancement of glucose sensitivity in the liver through the uptake of peripheral glucose and thereby utilising it.

Many observational studies have also shown significant improvement in the reduction of circulating androgen level in PCOS patients and further much more helpful in the reduction of overall body weight, thereby also helpful in reducing the total weight/hip ratio.

Consistent usage of metformin along with OCPs has been proved to be outstandingly evident in treating the females affected through PCOS.

Also effect of metformin has also been seen in inhibiting both androstenedione & testosterone output by thecal cells present in the ovary.

Metformin has been found significant in reducing the circulating level of androgens production through ovary and adrenal gland, reduction in LH, increased usage of circulating SHBG by liver.

2.2 : Database used for the dissertation

- National Centre for Biotechnology Information (NCBI)
- PubMed Central
- Scopus index journal

2.3 : Biostatistics analysis

Various research studies have the meta analysis approach to give affirmation to their results. **Chi-square Values and p-value** corresponding to it are then considered for the significance of the results. The p-values will lead to the confirmation of results whether the results found out after conducting the experiment are **significant or insignificant.**

Chi square tests is a non parametric test which is based on any variables and hypothesis. It is always a best method for the data analysis of numerically based data. They are most frequently used when the data is in frequency such as number of responses in two or more than two categories. P values can be found out by stating alternate and null hypothesis and then the generation of chi-square curve along with p-values.

A small value of chi-square means that correlation is significantly high . There are some basic norms to decide whether to support or reject the null hypothesis:

- If $p > .10 \rightarrow$ “not significant”
- If $p \leq .10 \rightarrow$ “marginally significant”
- If $p \leq .05 \rightarrow$ “significant”
- If $p \leq .01 \rightarrow$ “highly significant.”

Test of association is one such application of chi-square test where the correlation between two discrete parameters is seen. Many events which were considered for the results were the :

- *Endocrine abruption in PCOS women with metformin therapy.*
- *Acne treatment with Metformin in PCOS patients.*
- *Effect on steroidogenesis with metformin therapy.*
- *Effect of metformin on hyperandrogenism and hyperinsulinemia*

2.4 : Methodology:

The topic was selected on the basis of the current issue which women of my age , especially in the reproductive age are commonly and sometimes severely affected because of the inability to conceive in the child bearing age. Other than this, PCOS also cause a turmoil on emotional well being of PCOS patients. Stress,depression, anxiety, physical consciousness- all these issues cause a havoc on the mental well being of PCOS patients. After all this, there was no other option but to conduct my research on this topic. The research papers were selected that were not older than year 2000. The papers were found out according to certain keywords such as diabetes mellitus, PCOS, insulin resistance/ sensitivity, reproductive anomalies, metformin and the subsequent correlation between all these forms. Further, the abstracts were read to finally conclude whether the papers would be assisting in the dissertation writing or not. The papers that I have taken have all the research conducted including meta-analysis and randomised clinical trials which were there in PubMed and scopus by doing the properly literature search for the assessment and critical understanding of the impact of metformin in subjugating the various manifestations of PCOS. Bibliographies and references were taken from the literature review which were shortlisted for the thesis writing for additional studies. the results were put in the concise form which was easier for other people to comprehend.

2.5 : Relationship between PCOS and metformin:

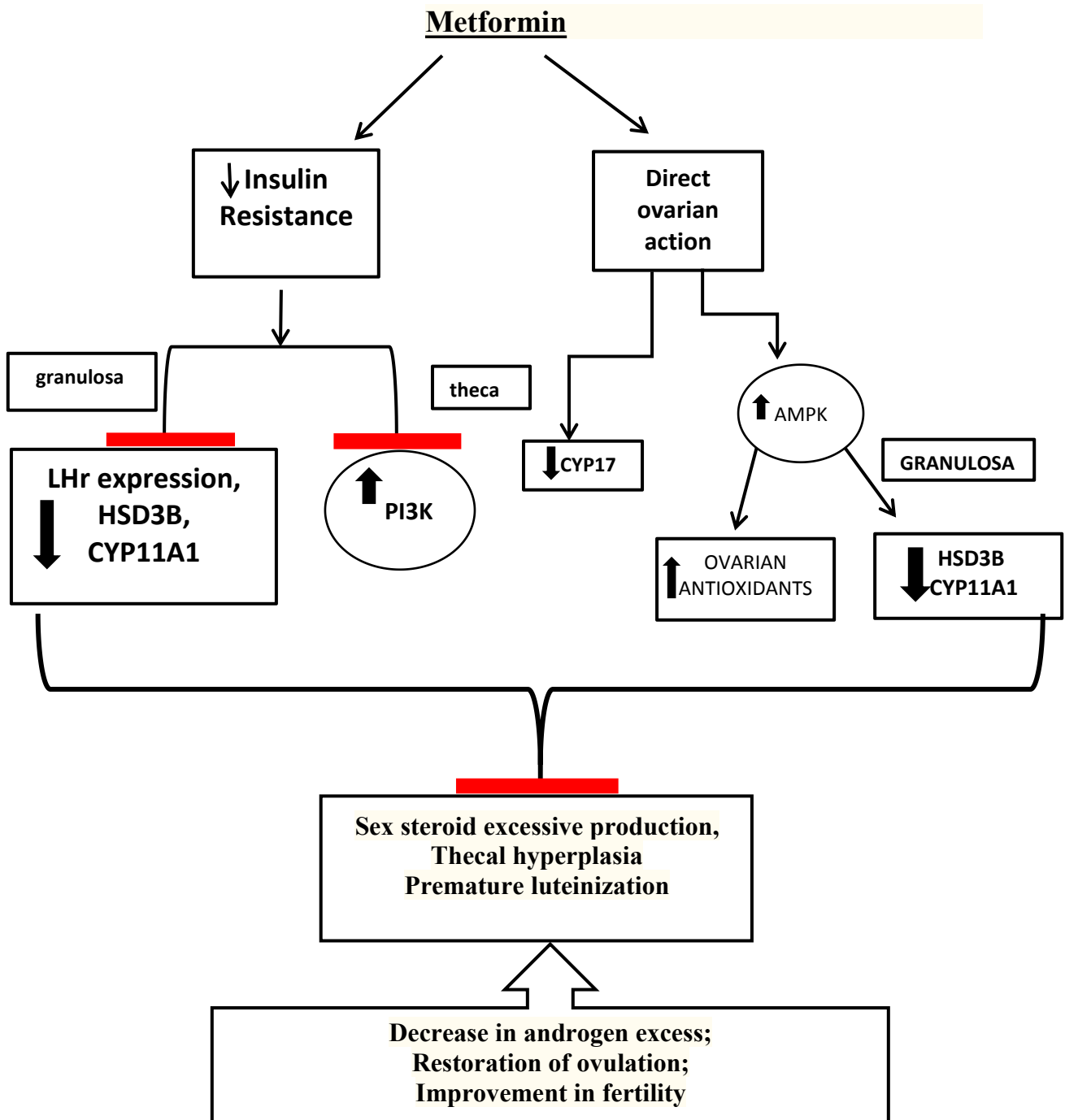


Fig. 2.1 : EFFECT OF USING METFORMIN IN PCOS PATIENTS.

There are two ways in which metformin can act upon the ovary. Metformin works by inhibiting the activity of CYP17 gene either directly or indirectly by decreasing the insulin resistance through suppression of PI3K activity.

Decrease in the levels of blood insulin leads to further suppression of LH receptor expression along with HSD3-beta, CYP11A1 activity in granulosa cells. Additionally, AMPK activation induces further suppression of HSD3-Beta, CYP11A1 activity in granulosa cells along with enhancement of antioxidant defenses in ovarian level. All these mechanisms together contribute towards inhibition of excess androgen production, premature luteinization, thecal hyperplasia. And therefore improves fertility in PCOS women.

Thus, PCOS is a heterogeneous and mysterious disorder which puts about 5-15% of women at prospect of developing infertility, diabetes, obesity as well as cardiovascular diseases.

Metformin also proves to be a boon for proper functioning of ovarian hormones by working on various genes of steroidogenesis and also on LH receptor inhibition.

Metformin has been used in combination with other drugs in order to combat the problems of infertility, improved menstruation, enhancement in insulin sensitivity, and hyperinsulinemia and hyperandrogenemia therefore helpful in attenuating the other symptoms of hirsutism and anovulation.

Table 2.1 : Expected outcomes of metformin usage on PCOS patients

S.no.	<u>PCOS SYMPTOMS</u>	IS METFORMIN HELPFUL IN CONTROLLING THE SYMPTOMS HERE?
1.	<i>Hirsutism</i>	yes
2.	<i>Improved menstruation</i>	yes
3.	<i>Enhancement in insulin sensitivity</i>	yes
4.	<i>Anovulation</i>	yes
5.	<i>Lower BMI</i>	yes
6.	<i>Acne control</i>	yes
7.	<i>Obesity</i>	yes

CHAPTER 3 : Outcomes and Interpretations

3.1 RESULTS:

Many researches have been conducted widely to check the efficacy of an easily available, cost effective product for treating the various consequences of a heterogeneous disorder known as PCOS. Despite of having rigorous studies, the efficacy of this drug and its efficacy in treating the disease symptoms is still questioned. Although theoretically, it has been established that metformin is an insulin sensitive drug that either stimulates or inhibits various genes and factors which had got malfunctioned and leads to the rise in various manifestations of PCOS. Some researchers accept it as the best and an incompetent drug to treat symptoms such as insulin sensitivity, hirsutism, lowering of BMI, obesity, control of acne, improved menstruation.

Kolodziejczyk B.et.al. ,2000 conducted a study for 12 weeks of metformin therapy to check the clinical significance of metformin remedial treatment in PCOS patients. The results shown that the therapy helped in achieving the significant reduction in the free testosterone index. Also the outcomes were crucial for the patients suffering from extreme “hyperandrogenemia” and depends upon the doses of metformin given to the patients.

However there was no such consequential improvement seen in the regularity of menstrual cycle.

Not all women responded well to metformin with a sound improvement in insulin levels. Metformin in combination with OCPs showed improve results followed by lifestyle modifications.

“Although there was remarkable improvement in the clinical outcomes pf PCOS patients which included BMI, High BP, waist to hip ratio. But endocrine and metabolic disorders did not show any important outcomes.”[Rohini patel,et.el.]

“Different combinations of metformin along with many other drugs was also done for the improvement in PCOS symptoms. Also considerable results were there during the clinical studies of this experiment. Metformin combined with OCP and metformin

implemented alone. The results did not show any considerable improvement in BMI index in both cases but when metformin and OCP combination was given there was improvement in the levels of high density Lipoprotein. Also a significant improvement in SHBG levels in both the groups but it was more pronounced in the group where only metformin was given.

In one such other experiment, two groups were formed where one group was given only metformin and other group was given both metformin and OCP. The results showed reduction in the insulin level in both groups. Also there was reduction in BMI index in both the groups but more pronounced results were there in the group of metformin and OCPs”- [Y.yang,2015]

“PCOS women are always at the edge of developing the cardiovascular diseases. Several studies have also experimentally reported that metformin has been highly favourable in improving dyslipidaemia by directly participating in fatty acid metabolism. Also there was a remarkable reduction in the LDL profile of such patients. However the levels of LDL differed greatly and reached the worst when metformin was discontinued”

Metformin has also been said to improve steroidogenesis by inhibiting both androstenedione and testosterone level in the blood circulation produced by theca cells. Metformin when amalgamated with clomiphene citrate improved significantly the rate of ovulation and chances of gravidity other than the times when metformin was administered alone. Thus, solving one more issue which is chronic ANOVULATION in PCOS patients with improved rates in fertility”-[LASHEN H.,2010]

“Reportedly, metformin therapy showed a significant improvement in the menstrual regularity 25% of PCOS affected women. And also there was improvement in insulin sensitivity and basal & PP blood sugar”-[K Unlühizarci et.el.,1999]

Table 2.2 : Resultant repercussions of metformin on PCOS women and how the drug does it all significantly for effective improvement in the clinical features of such cases.

S.no.	Biological Target	Biological Action of Metformin
1.	PCO	<ul style="list-style-type: none"> a) enhanced ovarian physiology b) Diminishing of middling sized antral follicles c) Diminished expression of VEGF
2.	Hyperandrogenism	<ul style="list-style-type: none"> a) diminished expression of androgen-mediated incitement of early stages of follicular growth b) dimunition of granulosa LH receptor expression c) dimunition of number of LH responsive follicles d) Suppressive effect on granulosa-theca cells
3.	Insulin Resistance	<ul style="list-style-type: none"> a) Reduction in response to granulosa cells to gonadotropins. b) inflexion of degeneration of old and small follicles.

3.2 DISCUSSION:

PCOS affects roughly 10-15% women in their child bearing years. It is often being associated with extensively with glucose intolerance, hyperandrogenism, ovulatory dysfunction, insulin resistance and hyperinsulinemia. Metformin therapy has been proved to be significantly efficient in ameliorating insulin sensitivity.

Metformin's long term usage can also serve as an effective therapy for the treatment of acne and other factors such as ovarian hyperandrogenemia.

Many clinical studies have reportedly shown that metformin or OCPs usage among PCOS patients has been efficacious and also cause least side effects. Metformin treatment also showed a significant reduction in the freely available T in the blood circulation. Also there was reduction in the SHBG levels synthesized by liver.

Metformin usage in today has already received a lots of recognition in the past few years due to the obvious reasons of fighting the symptoms of PCOS. But the response has not been an overwhelming one due to many reasons. It may be due to the differences in the morphological, genetical and metabolic parameters of the sample size taken.

Metformin proved to be superior only when combined with some lifestyle modifications which includes various changes in habits such as avoiding processed foods and unwanted carbohydrates and also by following fitness regime. All these things will ultimately help in the weight loss and thus reducing the BMI index. The benefit of all these were seen particularly in the IVF treatment due to the reduction of incidence of OHSS in PCOS patients.

Many patients who have already been on OCP for PCOS treatment have issues of heavy menstrual bleeding, high blood pressure, severe headache when OCPs were discontinued.

However, the long term usage also caused many side effects too which prominently includes lactic acidosis. Also there are chances for the development of hypoglycemia to all the patients who don't have any severe insulin resistivity.

In addition to above manifestations, adverse cardiovascular and these effects were also associated with the usage of only OCPs in the continual treatment of PCOS patients.

However, the usage of metformin along with OCPs was not considered too damagable to PCOS patients.

Here is the summary of what we have read and concluded in the discussion section to get to know about the metformin's usage pros and cons:

PROS:

- ✓ Lowers glucose and insulin levels.
- ✓ Increase peripheral glucose uptake
- ✓ Can improve fertility by regulating the menstrual cycles
- ✓ May improve cholesterol levels
- ✓ Decrease in levels of triglycerides
- ✓ Helps in the improvement of hirsutism symptoms like acne and excess hair growth by increasing SHBG.
- ✓ May help in the prevention of gestational diabetes
- ✓ Could possibly prevent endometrial and ovarian cancer
- ✓ Highly cost effective
- ✓ Oral route

CONS:

- ✓ Nausea
- ✓ Gas bloating
- ✓ Abdominal discomfort
- ✓ Diarrhoea
- ✓ Increases the chances of vitamin B12 deficiency
- ✓ Lactic acidosis
- ✓ Lead to increased weight in children born to moms who took metformin in pregnancy.
- ✓ Can worsen renal function
- ✓ Hear failure
- ✓ Cirrhosis

3.3 CONCLUSIONS :

After going through all the pros and cons of metformin in handling the issues pertaining to PCOS, I can safely conclude that although we have cons of this drug treatment but ultimately the fact can not be denied that it has been proved to be efficacious in treating some of the issues of PCOS. The recent studies we all have shown that metformin has been remarkably effective in improving menstrual irregularities and infertility in PCOS women by lowering the androgen levels in the blood circulation. Metformin, to a larger extent, modulates the ovarian dysfunctioning by working on LH secretion. The results were more pronounced in non obese women who were both hyperinsulinemic and hyperandrogenic. Also any change in the steroidal activity is deeply harmonized with the insulin sensitivity

However, the intensity of the results among the patients depends upon how much or less the symptoms were. It also depends upon the physiological characteristic of patients as well. Further, there has not been a long term study to look out for the effect of metformin on PCOS females as there is always a limitation of sample size at many places during the collection of data. At the end, I would like to add of what I feel that Metformin usage should only be done after proper recommendation from the trained medical practitioner.

References:

1. Lashen H. (2010). Role of metformin in the management of poly cystic ovary syndrome. *TherAdv Endocrinol Metab.* 2010 Jun; 1(3): 117–128.doi: 10.1177/2042018810380215
2. Hayek S., Bitar L., Hamdar L., Mirza F., Daoud G.(2016).Poly Cystic Ovarian Syndrome: An Updated Overview. *Front Physiol.* 2016; 7: 124.Published online 2016 Apr 5. doi: 10.3389/fphys.2016.00124
3. *No authors listed.* History of discovery of polycystic ovary syndrome. *Adv Clin Exp Med* May-Jun 2017;26(3):555-558.doi: 10.17219/acem/61987
4. Oakley A. Hyperandrogenism. February 2014
5. Orzack E.A, George S. Ovarian dysmetabolic syndrome. *Endocr Pract.*Sep-Oct 2000;6(5):410. doi: 10.4158/EP.6.5.410
6. Bilal M., Rehman A., Haseeb A.Relationship of Polycystic Ovarian Syndrome with Cardiovascular Risk Factors. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*Volume 12, Issue 3, May 2018, Pages 375-380
7. Ndefo A.H.,Eaton A., Green R.M. Polycystic Ovary Syndrome-A Review of Treatment Options With a Focus on Pharmacological Approaches. *P T* 2013 Jun;38(6):336-55.PMID: 23946629 PMCID: PMC3737989
8. Ajmal N., Khan Z., Shaikh R. Polycystic ovary syndrome (PCOS) and genetic predisposition: A review article.*European Journal of Obstetrics & Gynecology and Reproductive Biology: X* Volume 3, July 2019, 100060
<https://doi.org/10.1016/j.eurox.2019.100060>

9. Rena G., Hardie D.G., Pearson R.E., The mechanisms of action of metformin. *Diabetologia*. 2017; 60(9): 1577–1585. Published online 2017 Aug 3. doi: 10.1007/s00125-017-4342-z
10. Dumitrescu R., Mehedintu C., Metformin-Clinical Pharmacology in PCOs. *J Med Life*. 2015 Apr-Jun; 8(2): 187–192. PMID: PMC4392089
11. Foretz M. et al. Metformin: from mechanisms of action to therapies. *Cell Metab*, 2014 Dec 2; 20(6): 953-66. doi: 10.1016/j.cmet.2014.09.018
12. Yun yan, Kover K., Moore W. New Insight into Metformin Mechanism of Action and Clinical Application. DOI: 10.5772/intechopen.91148
13. Patel R., Shah G. Effect of metformin on clinical, metabolic and endocrine outcomes in women with polycystic ovary syndrome: a meta-analysis of randomized controlled trials. 2017 Sep; 33(9): 1545-1557. doi: 10.1080/03007995.2017.1279597. Epub 2017 Feb 3.
14. Genazzani A., Lanzoni C., Ricchieri F., Baraldi E., Casarosa E., Jasonni V. Metformin administration is more effective when non-obese patients with polycystic ovary syndrome show both hyperandrogenism and hyperinsulinemia. *Gynecol Endocrinol* 2007 Mar; 23(3): 146-52. doi: 10.1080/09513590701214398
15. Loverro G., Lorusso F., Pergola G., Nicolardi V., Mei L., Selvaggi L. Clinical and endocrinological effects of 6 months of metformin treatment in young hyperinsulinemic patients affected by polycystic ovary syndrome *Gynecol Endocrinol* 2002 Jun; 16(3): 217-24.

16. Tang T., Lord J., Norman R., Yasmin E., Balen A. .Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility.Cochrane Database System Rev2012 May 16;(5):CD003053. doi: 10.1002/14651858.CD003053.pub5.
17. Awartani K. , Cheung A. Metformin and polycystic ovary syndrome: a literature review. J Obstet Gynaecol Can 2002 May;24(5):393-401. doi: 10.1016/s1701-2163(16)30402-9.
18. T Kazerooni 1, M Dehghan-Kooshkghazi. Effects of metformin therapy on hyperandrogenism in women with polycystic ovarian syndrome. Gynecol Endocrinol 2003 Feb;17(1):51-6.
19. Kriplani A., Aggarwal N. Effects of metformin on clinical and biochemical parameters in polycystic ovary syndrome. 2004 May;49(5):361-7
20. Unlühizarci K., Keleştimur F., Bayram F., Sahin Y., Tutuş A.. The effects of metformin on insulin resistance and ovarian steroidogenesis in women with polycystic ovary syndrome.Clin Endocrinol (Oxf)1999 Aug;51(2):231-6. doi: 10.1046/j.1365-2265.1999.00786.x.
21. Chang Y., Choi EJ. Efficacy and safety of metformin or oral contraceptives, or both in polycystic ovary syndrome Published 1 September 2015 Volume 2015:11 Pages 1345—1353 DOI <https://doi.org/10.2147/TCRM.S89737>
22. Sharma S., Mathur D.,Paliwal V.,Efficacy of Metformin in the Treatment of Acne in Women with Polycystic Ovarian Syndrome: A Newer Approach to Acne Therapy.May 1, 2019 J Clin Aesthet Dermatol. 2019;12(5):34–38
23. Palomba S.et.el. Metformin reduces risk of ovarian hyperstimulation syndrome in patients with polycystic ovary syndrome during gonadotropin-stimulated in vitro

fertilization cycles: a randomized, controlled trial. *Fertil Steril* 2011 Dec;96(6):1384-1390.e4. doi: 10.1016/j.fertnstert.2011.09.020. Epub 2011 Oct 7.

24. Genazzani A., Strucchi C., Luisi M., Casarosa E., Lanzoni C., Baraldi E., Ricchieri F., Mehmeti H., Genazzani A.. Metformin administration modulates neurosteroids secretion in non-obese amenorrhoeic patients with polycystic ovary syndrome. *Gynecol Endocrinol.* 2006 Jan;22(1):36-43. doi: 10.1080/09513590500476164

25. Vrbíková J., Hill M., Stárka L., Cibula D., Snajderová M., Sulcová J., Vondra K., Bendlová B.. Effect of long-term treatment with metformin on steroid levels and parameters of insulin resistance in women with polycystic ovary syndrome. *Cas Lek Cesk.* 2001 Nov 8;140(22):688-94.

26. Zelija Velija-Ašimi. Evaluation of endocrine changes in women with the polycystic ovary syndrome during metformin treatment. *Bosn J Basic Med Sci.* 2013 Aug;13(3):180-5. doi: 10.17305/bjbms.2013.2359

Appendices:

- <https://www.statisticshowto.com/probability-and-statistics/chi-square/>
- <https://www.statisticshowto.com/probability-and-statistics/statistics-definitions/p-value/>
- Mahajan's Methods in Biostatistics for Medical Students and Research Workers