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Computational Screening of Phytochemicals against Munc13-1, a Promising target to treat Alcoholism

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ABSTRACT

In silico analysis and characterization has revolutionized target and drug discovery significantly. Alcohol abuse is a big threat to society, economy and wellbeing of people. It has increased the overall disease and injury burden, globally. Recently, a study revealed a brain protein, Munc13-1 C1 domain to play a significant role in development of alcohol tolerance, by binding to alcohol molecules, eventually leading to Alcohol Use Disorder. The aim of this study was to discover a phytochemical that would attach to our target protein, Munc13-1 C1 domain so that it cannot bind with the alcohol molecules. Munc13-1 3D structure obtained from PDB was docked against a library of compounds by MOE software. Ten phytochemicals based on their binding affinity with the target protein were shortlisted i.e. Tannic Acid, Anemone blue anthocyanin 1, Oolonghomobisflavan B, Diosmin, Oolonghomobisflavan A, Neodiosmin, Blepharocalyxin B, 8-Hydroxyhesperetin, Eupatorin and Monotesone A. However, only 8-Hydroxyhesperetin, Eupatorin and Monotesone A followed Lipinski rules. They were non-toxic and non-carcinogenic according to SwissADME. Moreover, have a good drug-like model score as analysed by Molsoft. Further, in-vivo and invitro examinations are required to inspect their role in reducing alcohol tolerance.

Keywords: Insilico, Alcoholism, Munc13-1, Phytochemical, Docking, Lipinski rule, ADMET

INTRODUCTION

Alcohol is an organic substance formed when a hydrogen atom is substituted by a hydroxyl group in a hydrocarbon. Ethanol is the type of alcohol used in alcoholic beverages. It is a product of fermentation of different sugars by yeast. Alcohol is classified as a sedative hypnotic drug that means it acts as a central nervous system depressant (Kuhn et al., 2008).

Alcohol Use Disorder (AUD) or alcoholism is a long-term alcohol addiction. Or drinking problem that becomes severe as defined by The National Institute on Alcohol Abuse and Alcoholism (NIAAA). Alcoholism is a complex problem to treat as it effects a lot of body parts on so many levels. The best approach is to build resistance to tolerance against alcohol so that the patient has less craving and need for alcohol and can eventually quit. Munc13-1 is a phorbol ester-dependent enhancer of spontaneous and evoked neurotransmitters. It is a significant target of presynaptic phorbol ester and diacylglycerol (Betz et al., 1998). According to a new research, Munc13-1 that binds to alcohol molecules inside the brain, can be targeted to cure alcoholism. The protein plays a crucial role in the development of tolerance against alcohol.

Continuous alcohol consumption can develop alcohol tolerance in the consumer. Inhibition of alcohol binding to MUNC 13-1 can aid reduction in tolerance. Reduced tolerance can also reduce addiction. The

binding occurs in a brain synapse, where a cascade of signals is passed through neurons. Alcohol renders long-lasting alterations in neural activities, changing both presynaptic and postsynaptic activity. Research has been conducted on a simple yet similar, Drosophila model. Dunc13 of Drosophila is analogous to MUNC 13-1 of Human. Physiological and behavioural resistance against ethanol sedation is noted in Drosophila on reduction in Dunc13. Researchers believe MUNC13-1 in humans to be a promising target for developing drug against alcohol tolerance by inhibiting its binding with the alcohol molecules (Xu et al., 2018). This paper concentrates on finding a phytochemical that would interact with Munc13-1, the target protein, in such a way as to inhibit its binding with ethanol. Phytochemicals are chemicals isolated from plants with zero nutritive value. They can be polysaccharides, flavonoids, lignin, saponin, carbohydrates, stilbenoids or steroids in nature. They are organic and their disease preventive and protective properties like anti-bacterial, anti-oxidants, enzyme stimulators etc. make then useful in food and pharmaceutical industries.

MATERIAL AND METHODS

Selection of target Protein:

After scrutinizing literature review, Munc13-1 C1 domain was selected as the target protein for development of a drug that reduces alcohol tolerance. The 3D structure of 'Munc13-1 C1 domain' was acquired from PDB database with PDB ID '1Y8F'.

Molecular Docking

For the purpose of docking, 2D conformation of 1010 bioactive phytochemicals, belonging to seven different classes of phytochemicals i.e. alkaloids, aromatic, carbohydrates, flavonoids, lignans, tannins and polycyclic aromatic ligands, were retrieved from different databases including; PubChem (Bolton et al., 2008), MPD3 (Mumtaz et al., 2017) and Zinc database (Irwin & Shoichet., 2005) in sdf file format. This step was followed by the preparation of ligands by adding partial charges via Protonate3D module and energy minimization by selecting MMFF94x force-field. Afterwards each of the selected ligands was added individually to the MOE ligand database for docking purpose. The protein structure preparation included protonation and energy minimization via Protonate3D algorithm and AMBER99 force-field (Labute., 2007). The protein was docked with bioactive phytochemicals retrieved from PubChem database, by employing MOE (Molecular Operating Environment) software. MOE can be used to visualize, model, stimulate and for methodology development in drug discovery (Luo et al., 2011). The idea of conducting docking analysis was to pick compounds with minimum docking score, RMSD value and interacting residues involved. Once the docking was completed, phytochemicals with best conformations were identified on the basis of Root Mean Square Deviation (RMSD) value and S-score. RMSD represents the mean distance amongst the backbone atoms of superimposed proteins and S-score is a mathematical value that demonstrates the binding affinity of ligands with their receptors with all potential binding geometries. LigX tool of MOE was adopted to examine the 2D and 3D plots of receptor ligand interactions that enabled the clear view of receptor ligand interaction of the best docked complexes.

Prediction of Drug-like Properties

Top 10 phytochemicals with the best docking scores were analysed for Lipinski rules. Lipinski rules assess efficiency of a compound and its probability to act as drug in human body, keeping in view its physiochemical and pharmacokinetic properties. These rules are as follows; a

compound having more than 5 HBD (Hydrogen Bond Donors), 10 HBA (Hydrogen Bond Acceptors), molecular weight higher than 500 and Log P higher than 5, have inefficient absorption. Molsoft software (Bordner & Abagyan., 2004) also analyses the drug likeness of a compound by using Molsoft chemical fingerprints. SwissADME software (Daina et al., 2017) is employed to assess whether a compound follows Lipinski rules by evaluating its drug like characteristics i.e. Absorption, Distribution, Metabolism, Excretion, and Toxicity. An online tool ADMETsar execute chemical ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) profiling by integrating 50 high quality QSAR (Quantitative Structure-Activity Relationship) models (Cheng et al., 2012).

RESULTS

Docking Analysis

Phytochemicals from PubChem were docked against the structure of C1 domain of Munc13-1. Top ten bioactive compounds with higher number of interacting residues and lowest binding energies are short listed in Table 1. These compounds are Tannic Acid, Anemone blue anthocyanin 1, Oolonghomobisflavan B, Diosmin, Oolonghomobisflavan A, Neodiosmin, Blepharocalyxin B, 8-Hydroxyhesperetin, Eupatorin and Monotesone A.

Table 1: MOE results of docking analysis of top ten bioactive phytochemicals which were docked against target protein Munc13-1 C1 domain

Pubchem ID	Chemical name	Docking score	Rmsd value	Interacting residues
16129778	Tannic Acid	-17.0020	3.8788	His 567, Cys 616
11979368	Anemone blue anthocyanin 1	-13.1817	4.0438	His 567, Cys 616
14520995	Oolonghomobisflavan B	-11.9863	2.9363	His 567, Cys 616
5281613	Diosmin	-11.3052	1.5186	Gln 566, Asp 610, Asn 613
14520989	Oolonghomobisflavan A	-11.2008	2.6180	His 567, Cys 616
44258230	Neodiosmin	-10.5948	2.7936	Gln 566, Asp 610, Leu 612, Asn 613
10677118	Blepharocalyxin B	-10.3997	1.5375	His 567, Cys 616
42608121	8-Hydroxyhesperetin	-10.3707	1.3222	Gln 566,His 567
97214	Eupatorin	-10.2363	4.8275	Phe 569
10498463	Monotesone A	-10.1622	1.9708	Phe 569

Drug-Like Properties

The purpose of this research is to find a bioactive compound that targets C1 domain of Munc13-1 to reduce alcohol tolerance. Listed bioactive phytochemicals were analysed by molsoft and Molinspiration to assess it for Lipinski rule. Out of 10 only 3 compounds fulfilled Lipinski rule, those are Eupatorin, 8-Hydroxyhesperetin and Monotesone A. Phe 569 of target protein interacts with sidechain acceptor of Eupatorin. Polar Gln 566 and basic His 567 of target protein interacts with sidechain acceptor and backbone donor, respectively, in 8-Hydroxyhesperetin. In Monotesone A, Phe 569 interacts with the compound, interactions of these three compounds with target protein is shown in Figure 1-3. Table 2 shows the data of phytochemicals with respect to Lipinski rule.

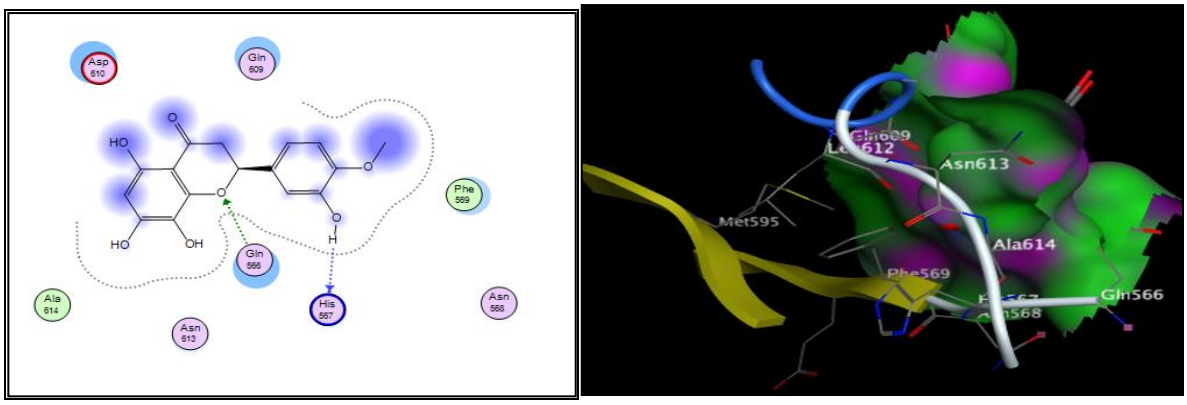

Figure 1: Interaction of ligand 8-Hydroxyhesperetin with Target protein Munc13-1 C1 domain PDB ID: 1Y8F; (A) 2D interactions in which His 567 and Cys 566 are making hydrogen bonds with the ligand while other amino acid residues, present in active sites are also shown in compound's vicinity (B) 3D image of ligand-protein interaction.

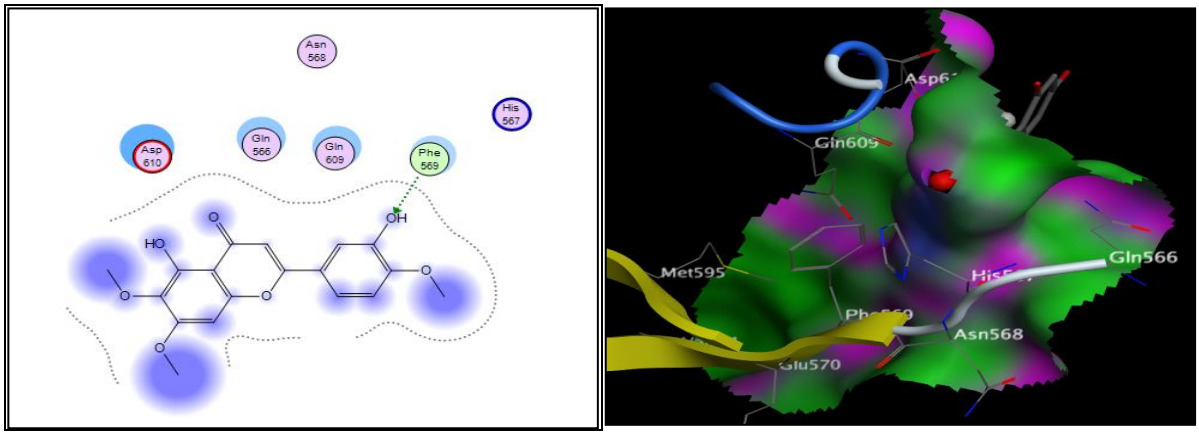

Figure 2: Interaction of ligand Eupatorin '97214'with Target protein Munc13-1 C1 domain PDB ID: 1Y8F; (A) 2D interactions in which Phe 569 is making hydrogen bond with the ligand while other amino acid residues, present in active sites are also shown in compound's vicinity (B) 3D image of ligand-protein interaction.

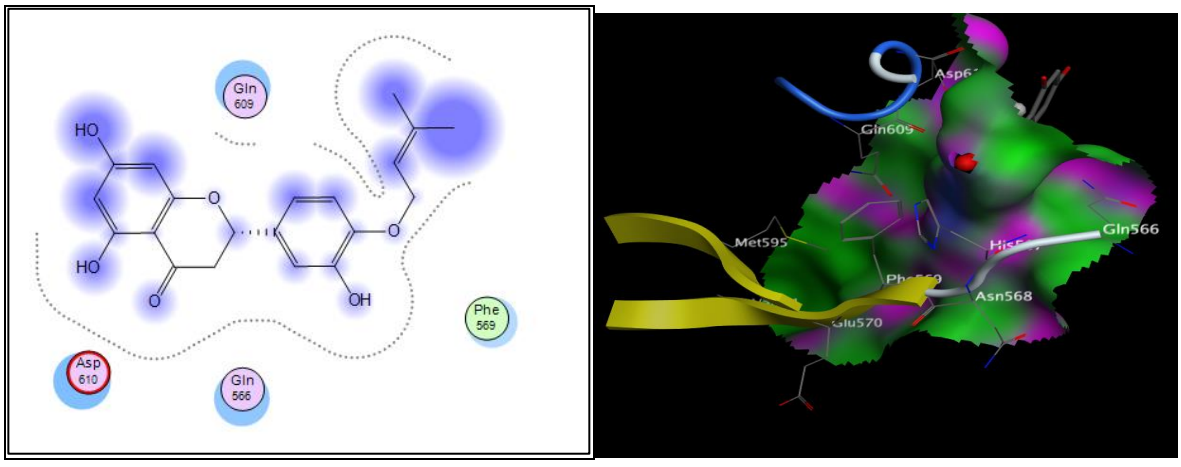

Figure 3: Interaction of ligand Monotesone A '10498463' with target protein Munc13-1 C1 domain PDB ID: 1Y8F; (A) 2D interactions in which amino acid residues, present in active sites are shown in compound's vicinity (B) 3D image of ligand-protein interaction.

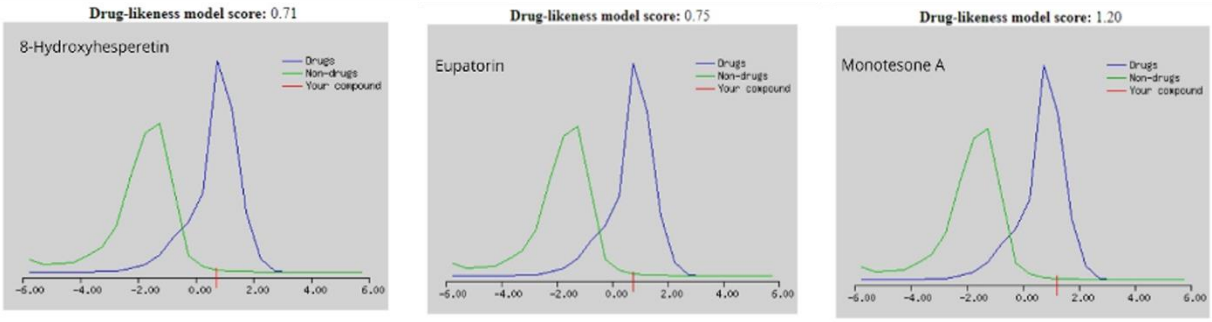

Figure 4: Drug-likeness model and score of ligands 8-Hydroxyhesperetin, Eupatorin and Monotesone via Molsoft

Table 2: Results of compounds examined for Lipinski rule.

Compound	Violations for Lipinski rule
Tannic Acid	No; 3 violations: MW>500, NorO>10, NHorOH>5
Anemone blue anthocyanin 1	No; 3 violations: MW>500, NorO>10, NHorOH>5
Oolonghomobisflavan B	No; 3 violations: MW>500, NorO>10, NHorOH>5
Diosmin	No; 3 violations: MW>500, NorO>10, NHorOH>5
Oolonghomobisflavan A	No; 3 violations: MW>500, NorO>10, NHorOH>5
Neodiosmin	No; 3 violations: MW>500, NorO>10, NHorOH>5
Blepharocalyxin B	No; 3 violations: MW>500, NorO>10, NHorOH>5
Eupatorin	Yes; 0 violation
8-Hydroxyhesperetin	Yes; 0 violation
Monotesone A	Yes; 0 violation

Molsoft software was used to check the drug- likeness model score of the three compounds which was quite satisfactory 0.71 for 8-Hydroxyhesperetin, 0.75 for Eupatorin and 1.20 for Monotesone A (Figure 4). ADMETsar analysed ADMET properties and showed all three compounds to be negative for Blood-Brain Barrier, highly absorbent for Human Intestinal Tract, non-toxic and non-carcinogenic.

DISCUSSION

Drug designing has been radically revolutionized due to in silico analysis and bioinformatics. It has decreased both cost and time going in the drug discovery. Great number of publications on drugs and their targets discovered by bioinformatics tools and softwares are being done. Advances in Chemoinformatics have widened the in silico compound libraries (Simon et al., 2017).

Owing to modern computational methods these compounds can be assessed for their properties and capability to act as drug in human body. These libraries are inclusive of both natural and synthetic compounds, to enable availability of wide range of drugs for customizing. The purpose of this study was to suggest a phytochemical that can be employed as a drug, targeting a brain protein Munc13-1, in order to reduce alcohol tolerance. Alcohol is one of the top 5 most addictive substances today and its misuse is the first leading risk factor in deaths of people aging from 15 to 49 (Bobo., 1989).

3D structure of the target protein, Munc13-1 was taken from PDB database with the ID 1Y8F. This structure was then docked against a library of compounds using MOE

software that has used in many previous studies for docking analysis (Fossa & Cichero., 2015; Perveen et al., 2011; Rahim et al., 2015). Top ten compounds with lowest docking scores were listed in ascending order of their scores that is function of binding affinity and number of residues of protein interacting with the compound. The lower the score the higher the efficiency is. Tannic acid had the lowest score of -17.0020, followed by Anemone blue anthocyanin 1 with -13.1817, then Oolonghomobisflavan B with -11.9863, Diosmin with -11.3052, Oolonghomobisflavan A with -11.2008, Neodiosmin with -10.5948, Blepharocalyxin B with -10.3997, 8-Hydroxyhesperetin with -10.3707, Eupatorin with -10.2363, and finally Monotesone A with -10.1622. The same docking procedure was adopted to analyze the binding capability of chronic acid two sites that can decrease the YopH bacterial virulence activity (Kuban-Jankowska et al., 2016) and to report the phytochemicals action against HCV NS3 protease (Ashfaq et al., 2016).

These top ten compounds were checked for their drug-likeness by judging their ability to meet standards of Lipinski rules, according to which a compound shows inefficient absorption if it has more than 10 H-bond acceptors (HBA), 5 H-bond donors (HBD), Log P (CLogP) more than 5 and Molecular Weight (M.W) is more than 500 (Lipinski et al., 2001). Lipinski rule was evaluated by molsoft and Molinspiration where Molinspiration also determines TPSA (Topological Polar Surface Area) and Molecular Volume, where former one forecasts the transportation ability of the drug within the body and latter one represents features of crossing blood-brain barrier and absorption in intestinal tract (Cheminformatics., 2011). Both Molinspiration and molsoft have already been used for identification of drug-like properties in many previous studies (Lalitha & Sivakamasundari., 2010; Raj et al., 2015; Raj et al., 2014).

Although all the compounds showed good interaction with the target, only 3 compounds (8-Hydroxyhesperetin, Eupatorin, Monotesone A) satisfied all the Lipinski rules without any violations. Although all these compounds had the docking score within the same range, yet owing to good interactions with target protein, all of them can be considered as potential anti-alcoholism drugs. 8-Hydroxyhesperetin (PubChem ID: 42608121) is the eighth best compound on the list having a docking score of -10.3707 and drug like model score of 0.71. It interacts with the target Munc13-1 C1 domain by its Gln 566 and His 567 residues and fulfils all Lipinski rules. It is a flavonoid. Similarly, Eupatorin (PubChem ID: 97214) is the ninth best compound on the list having a docking score of -10.2363 and drug like model score of 0.75, interacts with Phe 569 residue of the target protein and also follows Lipinski rules. It is a metabolite in Brassica napus and has multiple molecular roles i.e. apoptosis inducer, calcium channel blocker, P450 inhibitor, vasodilator, anti-inflammatory and anti-peoplastic agent. And lastly, the ninth best interacting compound Monotesone A (PubChem ID: 10498463) with docking score of -10.1622 and drug like model score of 1.20. It interacts with Phe 569 protein residue and follows Lipinski rules. It is a flavonoid isolated from Monotes engleri. It is an antifungal agent and also a metabolite.

ADMET properties of these three shortlisted compounds were analysed by ADMETsar software. Results depicted that, all of the studied compounds were highly positive for Gastro-Intestinal Absorption and Caco-2 Permeability. Besides, all of them were also reported to be non-toxic and non-carcinogenic in nature. ADMETsar software has been used previously to check

ADMET properties of potential drug compounds (Nisha et al., 2016).

CONCLUSION

In this study, phytochemical library was scrutinized to find a drug to reduce alcohol tolerance by targeting Munc13-1 C1 domain. 8-Hydroxyhesperetin (PubChem ID: 42608121), Eupatorin (PubChem ID: 97214) and Monotesone A (PubChem ID: 10498463) were found out to be the best candidates against the Munc13-1 C1 domain with satisfactory drug-like properties. However, further in-vitro and in-vivo examinations will reveal the exact efficacy of these phytochemicals in biological bodies.

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Analyzing and Identifying the Molecular Targets and Regulators Controlling Cardiac Hypertrophy Progression

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ABSTRACT

Cardiac hypertrophy is the major pathway by which neurohormonal and mechanical stimuli act upon cardiomyocytes which gives the response to these stimuli. It leads to heart failure and ventricular dilation which is the main root of mortality in the western world. Many molecular targets are controlling cardiac hypertrophy development which may influence the growth factors signaling, cytokine release and gene expression. Through clinical trials on different models, recent research shows that cardiac hypertrophy might be inhibited or reversed. These findings have developed a vast drive to recognize specific and novel regulators of cardiac hypertrophy. Many molecular targets and signaling modulators have been studied in this review that induce the hypertrophic response which may involve MAPK pathway, oxidative stress, calcineurin, Cardiac angiogenesis, serum protein concentration, microRNA, and periodontitis. For the treatment of cardiac hypertrophy, the scientific knowledge of these signaling pathways and factors may be translated into potential nutritional and molecular therapies for the betterment of this diseases. The current and previous knowledge of molecular markers can be compiled in this review for the treatment of the molecular pathogenesis of cardiac hypertrophy.

Keywords: Cardiac hypertrophy, miRNA, Angiogenesis, Oxidative stress

INTRODUCTION

Diseases of cardiac remain the prime root of death in the world, with congestive heart failure that illustrates the major cause and rapidly growing subclass over the past decade (Ali, El-Dahshan, & Yahia, 2017). Cardiac hypertrophy is the result of an increase in biomechanical stress on the cell in which the heart undergoes abnormal enlargement, or thickening of the cardiac muscles, as a result increases cardiomyocyte size and changes in other heart muscle components, like the extracellular matrix (DeFrancesco, 2021). Different molecular mechanisms are reported to be involved in the development of cardiac hypertrophy. Many external stimuli and molecular mechanisms categorized cardiac hypertrophy into two types, physiological hypertrophy, mainly in the Athlete's heart, characterized by enhanced contractile function and pathological hypertrophy occurred by

hypertension (Mehdiyev, Mustafaev, & Mamedov, 2021). Structural heart diseases or myocardial Infarction is linked with the re-expression of fetal cardiac genes such as genes that code for β-myosin chain and natriuretic peptides (Dukkipati et al., 2017). Many genes other than fetal genes have been identified by the expression analysis on a large-scale which were involved in the upregulation of hypertrophied heart as well as involved in signaling pathways and energy metabolism by the expression of gene encoding protein (Akasia & Komura, 2003).

Hypertrophic stimulation is responsible for the different gene expression. Cardiac transcription factors directly regulate many cardiac genes and play a leading vital role in the upregulation of hypertrophied myocardium (Churko et al., 2018). Gupta identifies oxidative stress as one of the major factors involved in cardiac hypertrophy development (Gupta, Das, & Sen, 2007). Clinical studies on neonatal cultured cardiomyocytes and transgenic mice show that overexpression of GATA-4 is sufficient for inducing hypertrophy and cardiac angiogenesis (Malek Mohammadi et al., 2017). MicroRNAs have been connected in myocardial disease processes (Kura et al., 2019). Experiments on cultured cardio myocytes identified that the upregulation of many miRNAs in heart failure induced molecular changes that are like seen in cardiac hypertrophy (Kura et al., 2019). Some miRNAs are reported to be downregulated and some are upregulated thus, indicating their role in hypertrophic response (Szczerba et al., 2020).

Other factors like inhibition of FPPS diminished angiotensin II which initiates cardiac hypertrophy and fibrosis by decreasing Rhoda activity (Dai et al., 2017). The concentrations of vitamin D, intact parathyroid hormone, and Fetuin-A in serum were seen to be closely linked with cardiac hypertrophy (Nizameddin et al., 2019). Although a great relationship between periodontitis and cardiovascular disease has been studied, through which it is announced that myocardial hypertrophy might be afflicted by periodontitis (Sato et al., 2017; Seminice et al., 2012). Further In this review we will discuss details that how different molecular mechanisms and factors regulate cardiac hypertrophy and about therapeutic targets in the future that many novel therapeutic drugs and mechanisms are predicted by characterized signaling circuits for heart diseases treatment.

MOLECULAR PATHWAYS AND TARGETS THAT CONTROLLING CARDIAC HYPERTROPHY

Different factors, pathways and regulators have been shown to involve in the regulation of cardiac hypertrophy and its progression including oxidative stress, the impact of miRNAs, Angiogenesis and different pathogenic diseases associate with cardiac hypertrophy (Figure 1).

Oxidative Stress

Oxidative stress hurts cardiac structure and function is unclear but various studies revealed that redox sensitive signaling pathways have a causative role in the development of cardiac hypertrophy (Faria & Persaud, 2017). The increasing level of oxidative stress have a harmful damaging effect on DNA and membrane as well as the enzymes associated with cellular homeostasis (Haque, Nam, Eom, Kim, & Rhee, 2020). In a biological system, the double role of reactive oxygen species is either beneficial or harmful to living entities. Unregulated excessive ROS is cytotoxic, causing damage to cellular macromolecules and are involved in several pathological conditions (Cheng et al., 2021). Defective mitochondrial electron transport chain, dysfunctional nitric oxide

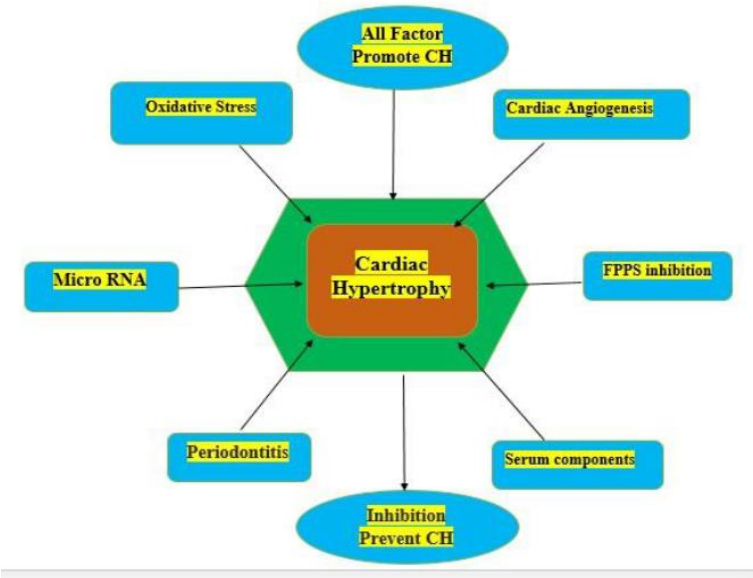

Figure 1: Overview of all Molecular Targets and their Controlling Mechanisms

Oxidative Stress

Oxidative stress hurts cardiac structure and function is unclear but various studies revealed that redox-sensitive signaling pathways have a causative role in the development of cardiac hypertrophy (Faria & Persaud, 2017). The increasing level of oxidative stress have a harmful damaging effect on DNA and membrane as well as the enzymes associated with cellular homeostasis (Haque, Nam, Eom, Kim, & Rhee, 2020). In a biological system, the double role of reactive oxygen species is either beneficial or harmful to living entities. Unregulated excessive ROS is cytotoxic, causing damage to cellular macromolecules and are involved in several pathological conditions (Cheng et al., 2021). Defective mitochondrial electron transport chain, dysfunctional nitric oxide synthase, xanthine oxidase and NADPH are the major sources to produce ROS which is responsible for the induction of cardiac hypertrophy (Zhang, Murugesan, Huang, & Cai, 2020). In cardiovascular cells ROS is continuously produced by NADPH oxidase. Angiotensin II, TNF-α, cyclic stretch and endothelin-1 are the different stimuli that enhanced the activity of ROS (Pena, Brito, El Alam, & Siques, 2020). Insulin-induced ROS generation in an NADPH oxidase-dependent manner, additionally stimulates PI3K and PKC signaling (Biswas, Mukherjee, Tarsal, Singh, & Mukhopadhyay, 2013). Mitochondrial dysfunction and xanthine oxidase are also found to be responsible for CH development.

Many important signaling pathways are modulated by ROS in stretched-induced cardiomyocyte hypertrophy. Serine/ threonine kinases receptor, tyrosine kinases receptor, cardiotrophin-1 receptors are the receptor on cardiac myocytes that is initiated by MAPK signaling cascade (Yongtao Zhang et al., 2020). ROS are important for stretch-induced activation of p38MAPK (J. Liu et al., 2021). Many transcription factors and multiple intracellular targets are phosphorylated by activated p38, JNKs and ERKs have been reported to involve in the remolding of cardiac gene expression.

CARDIAC ANGIOGENESIS

Coronary angiogenesis is impaired in the chronic phase which enhanced in the acute phase. Contractile dysfunction and impaired cardiac growth are caused by the inhibition of angiogenesis in the early phase (S. Liu et al., 2020). Enhanced coronary angiogenesis is associated with physiological cardiac growth having contractile function but impaired coronary angiogenesis is associated with pathological cardiac hypertrophy by reducing contractile function (T. Liu et al., 2020). Precursor cells of angiogenic, angioblasts from the sinus venosus and the proepicardial organ can separate into endothelial cells and assemble in a crude narrow organization in an interaction called coronary vasculogenic. Angiogenesis as well as physiological neovascularization expands the myocardial vascular plexus after birth, in which endothelial progenitor cells are involved (Luton & Carmelite, 2004; Riley, 2012). During the physiological growth phase, the inhibition of coronary angiogenesis is responsible for contractile dysfunction, impaired cardiac growth, and pathological hypertrophy. As the result of pathological hypertrophy capillary density was reduced and in the case of physiological cardiac hypertrophy a significant increase in the number of myocardial capillaries was observed (Oldfield, Duhamel, & Dhalla, 2020). Fibroblast growth factors, angiogenic growth factors, transforming growth factors VEGF sangiopoirtin-1 and -2 and platelet-derived growth factors regulated the myocardial angiogenesis. This study suggested that cardiomyocytes themselves produce angiogenic factors to maintain capillary density, oxygen supply, and function. To enhance myocardial angiogenesis have been investigated due to several strategies including growth factors delivery of angiogenic genes. The angiogenic factors include VEGF-A, VEGFB, stromal cell-derived factor-1, midline, VEGF-B, fibroblast growth factor-5, hepatocyte growth factor, and fibroblast growth factor 2. Immature angiogenesis and increase in vascular permeability occurred due to long-term stimulation with VEGF-A Similarly stimulation with angiopoietin-1 and VEGF-A (Eguchi & Wakabayashi, 2020) are involved in improved cardiac perfusion and porcine models of MI-A combined effect of hepatocyte growth factor and fibroblast growth factor-2 stimulated angiogenesis and prevented the progression of heart failure.

MICRO RNA (MIRNA)

A class of non-coding and single-stranded RNA that is made up of roughly 22 nucleotides in length is commonly known as microRNA (Anusree, Navis, & Prasobh, 2020). About 500 miRNAs are cloned and sequenced in humans, and about 1000 miRNA genes are estimated in the human genome (Anusree et al., 2020). Various pathological and biological processes are directly regulated by miRNA. Increasing evidence shows the involvement of miRNAs in cardiomyopathies (Fulgencio-Covián et al., 2020). miRNA upregulated the CH development by the overexpression of some miRNA whereas some downregulated the cardiomyocyte hypertrophy by the overexpression of miRNA. Mir-199b and miR-133, belonging to the same transcriptional units are reported to be downregulated in inducing cardiac hypertrophy asdemonstrated in mouse and human models (Jiang et al., 2019). The cardiac hypertrophy can be prevented by the overexpression of miR-99b and Mir-133 in vitro whereas in the cell the inhibition of miR-133 by infusion of antimine antisense oligonucleotide cause marked the development of CH. These are due to different regulatory targets that are regulating hypertrophy like Rhoda and cdc-42. However, the overexpression of miRNA-133b reduced the expression of the hypertrophy gene in the cell whereas the downregulation of miRNA-133b induces

the expression of the hypertrophy gene (Y. Liu, Liang, Zhang, & Fu, 2017).

Overexpression of miRNA-199b in human and mouse leads to heart failure by targeting to NFAT or Calcineurin pathway. miRNA-199b direct targets to dual-specificity like tyrosine phosphorylation nuclear NFAT kinase that affect calcineurin-responsive gene expression by increasing Dyrk1a gene expression However, miRNA-199b can be inhibited by a specific antagomir that reduced nuclear NFAT activity by maintaining Dyrk1a expression in the cell which may leads to the reversion of cardiac hypertrophy in the heart failure mouse model (Duygu et al., 2017). These studies show that miRNAs play an essential role in disease formation such as, potential targets of novel therapies (Duygu et al., 2017).

FPPS INHIBITION

Farnesyl pyrophosphate synthase (FPPS) has an assumed part in the pathway of mevalonate. Farnesyl pyrophosphate synthase is a fundamental catalyst for the formation of geranyl pyrophosphate and farnesyl pyrophosphate (Waller, Park, & Tsantrizos, 2019). However, FPP is likewise responsible for the formylation of little GTPases such as Ras; known to be a signal transducer. Heart repairing in the cardio myocytes is solidly connected with the hyperactivity of Ras (Ramos-Kuri et al., 2015). Prior examinations have noticed that hindrance of FPPS lessened angiotensin II that start heart hypertrophy and fibrosis by diminishing movement of Rhoda (Yang et al., 2013). In any case, FPPS overexpression incited cardiovascular hypertrophy and impairment by expanding the expression of RhoA (Yang et al., 2013). Overproduction of Ras support the increment of RhoA in pressing pressure impact heart hypertrophy (Chen et al., 2013) The concealment of farnesyl transferase may likewise increment heart restoring in promptly hypertension rodents by diminishing the action of RAS.

SERUM COMPOSITION CONCENTRATION

The concentration levels of Fetuin A, Vitamin D, and parathyroid hormone were firmly connected with cardiovascular hypertrophy (Zechner & Towler, 2018). With the way toward maturing the occurrence of sarcopenia happen because of involuntary decrease in free of fat muscle mass and heart hypertrophy increments (Chang et al., 2017) . Albeit heart hypertrophy happens with the way toward aging because of cell loaded up with fibrotic (Chang et al., 2017). Nonetheless, the balance between hormone parathyroid and Lit.D. Expanded PTH convinces cardiovascular hyper contractility ultimately causes receptive heart hypertrophy. Fetuin-A is an inhibitor of calcification and it is associated with resistance of insulin (Bourebaba & Marycz, 2019). Fet-A is additionally contributed to the improvement of diastolic cardiovascular arrest (Bourebaba & Marycz, 2019).

PERIODONTITIS AND MYOCARDIAL HYPERTROPHY

Although a great correlation between periodontitis and cardiovascular disease has been studied, through which it is announced that myocardial hypertrophy might be afflicted by periodontitis (Suzuki et al., 2017). However, the clinical information has some sort of study constraint they firmly propose direct cooperation between left ventricular hypertrophy & harshness of periodontitis. The comprehensive mechanisms between periodontitis and myocardial hypertrophy have not been well understood (Suzuki et al., 2017). However, the periodontal bacterial infection is firmly relevant to myocardial hypertrophy. The periodontal pathogen, Aggregatibacter actinomycetemcomitans has been shown to

increase cardiac hypertrophy in murine transverse aortic constriction model, with matrix metalloproteinase-2 activation, however another pathogen Porphyromonas gingivalis (P.g.) did not enhance these pathological changes. In the treatment of slow heartbeat like isoproterenol-induced myocardial hypertrophy model and prohormones gingival is induced myocardial hypertrophy with the help of Toll-like receptor-2 signaling. As our study reported that the periodontitis has a major role in the modulation of chronic inflammation, so it also might have a play role in the medication of myocardial hypertrophy. Table 1: Comparison of molecular targets and controlling mechanism of Cardiac Hypertrophy

Table 1. Comparison of molecular targets and controlling machanism of Cardiac Hypertrophy.

Molecular Targets		Expression	Cardiac Hypertrophy	Controlling Mechanism
Oxidative stress		Increasing	Promote CH	Imbibition
Cardiac Angiogenesis		High Production	Promote CH	Inhibition
Micro RNA	mRNA-199b	Upregulated	Promote CH	Inhibition
	mRNA-133b	Downregulated	Promote CH	Overexpression
FPPS inhibition		Overexpression	Promote CH	Inhibition
Serum components	Vitamin D	High Concentration	Promote CH	Balance Concentration
	Parathyroid hormone	High Concentration	Promote CH	
	Fetuin A	High Concentration	Promote CH	
Periodontitis Bacteria		High Production	Promote CH	Inhibition

CONCLUSION

The cardiac hypertrophy process is highly complicated that involve many molecular targets, signaling mechanism, transcription factors, genes, effectors, and many enzymes that have a scientifically role in the pathogenicity of this process. Latest research has identified some molecular regulators that have significant role in the betterment of these diseases but still some additional regulatory mechanism and targets needs to be identified. For the treatment of cardiac hypertrophy, future research needs to be utilized this scientific knowledge into potential nutritional and molecular therapies for the betterment of this diseases. We can suppress many gene expression by using the CRISPR/ CAS system. By using all the molecular technologies like RNAi, TALAN, or gene silencing for the inhibition of many gene functions. If we successfully control the regulation of genes, we can almost completely control cardiac hypertrophy and can save people from this disease. The implementation of this scientific knowledge for clinical purposes is major challenges for scientists to exciting about this disease.

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A Dire Need To Break The Back Of Hepatitis C Virus In KP-Pakistan: A Meta-Analysis

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ABSTRACT

Viral Hepatitis is the seventh leading cause of death worldwide and prevails in many developing countries specifically in the Middle East and North African regions. The current study focuses on a comparatively low-income province of Pakistan, Khyber Pakhtunkhwa where medical facilities are scarce and the use of blood and its products, free from viral hepatitis and other pathogens, is poorly ensured. The preliminary phase of the study involved a systematic literature review on the epidemiology of Hepatitis C Virus in KP during the period 2000-2018 through PubMed, Science Direct, and Google Scholar. After statistical coding, an initial meta-analysis was conducted to come up with an integrated estimate of the prevalence of Hepatitis C Virus in Khyber Pakhtunkhwa. The prevalence rate of 4% by fixed effect model and of 6% by random effect model is extremely alarming, especially in a low-income region, and the Pakistani government should adopt effective and rapid strategies to eliminate Hepatitis C Virus infection by the end of 2030, as more times announced by the World Health Organization.

Keywords: Hepatitis C virus, Meta-Analysis, Khyber Pakhtunkhwa-Pakistan

BACKGROUND

Viral hepatitis is the main cause of liver cirrhosis, fibrosis, and cancer and is estimated to cause around 700 000 deaths each year. [1,2]. Hepatitis C Virus (HCV) belongs to the Flaviviridae family, which covers a huge group of viruses that are enveloped and have a single-strand RNA genome of positive polarity [3]. Direct-acting antivirals (DAAs) is an extremely effective HCV treatment that can clear HCV infection and lessen HCV disease burden and further transmission [4]. World Health Organization (WHO) has set global targets to eliminate HCV infection by 2030. [5]. HCV is the principal cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). Chronic HCV infection is reckoned to infect 130 to 150 million globally (with an estimate of 55%-85% cases progressing to chronic liver disease, 15%-30% cases progressing to cirrhosis and 1%-5% are expected to die due to decompensated cirrhosis and HCC [6-9]. Globally, 80% of the HCV burden is concentrated in low and middle-income countries (LMICs) [10]. The prevalence varies by region and can be concentrated in a certain type of population e.g., in people who inject drugs (PWID) in which the prevalence can reach up to 67.1% [11]. The prevalence of HCV differs worldwide, but the highest incidence rates are reported in the Middle East and North African regions (MENA countries) and also in some

European countries belonging to the Mediterranean area [12-13]. HCV is widespread in many MENA countries where Egypt shows the highest rate of HCV (estimated at >10%) [14]. HCV infection is massively endemic in Pakistan, with an estimated prevalence of 4.54-8,2%, second only to Egypt. Out of the six major HCV genotypes, the genotype 3a is predominant (69.1%), followed by genotypes 1 (7.1%), 2 (4.2%), and 4 (2.2%) [15]. In Pakistan, HCV transmission is mainly driven by multiple risk factors, such as health care practices, community-based activities (barbering, ear/nose piercing), and injecting drug use [15- 16]. Thus, it is important to investigate the epidemiology of HCV to plan appropriate strategies for detection, treatment, and prognosis. This study aims to determine the incidence of HCV in KP-Pakistan, through an analysis of already published data from 2000–2020 using R- statistical software. Understanding HCV epidemiology in Pakistan is crucial not only in terms of prevention and treatment but mainly for the global purpose of HCV eradication.

MAIN TEXT

This study was conducted following e PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [17].

DATA SOURCE AND SEARCH STRATEGY

The searches were conducted using Google Scholar, PubMed, Science Direct, WHO database, and publications of Health Department KP to identify all the articles reporting the epidemiology of HCV in KP. Information was collected from these databases from 2000 to January 14^{th,} 2018. An extensive search criterion was used with no language constraints. The keywords used were: [Hepatitis C in Pakistan, Hepatitis C in Khyber Pakhtunkhwa, Hepatitis C in Northwest Frontier Province (previous name of KP), Hepatitis C in NWFP, Prevalence of HCV in KP, and Epidemiology of HCV in KP-Pakistan].

STUDY INCLUSION CRITERIA

The study period (2000-2018) was focused on the epidemiology of HCV over the last two decades or from the beginning of the 21st century. All records identified (n=80) were imported into Mendeley, a reference manager, where duplicate publications were identified and excluded (n=13). The studies had to meet the criteria for selection: clearly stated objective, clearly described study design, type of population (e.g., General, PWIDs, Blood donors), adequate sample size, and diagnosis method. Further descriptive information such as reported HCV prevalence, the region of KP, author details, and publication year were collected. There were no age restrictions. After performing primary and secondary selection, 41 studies were included in the study as shown in Figure 1.

DATA ANALYSIS

A total of 41 eligible studies with a cumulative sample size of 316,061 were included and analyzed. Further, the studies were ranked according to their scores into high, medium, or low, relating to HCV incidence. Out of 41, 27 studies represented the general population, and 14 were with high-risk populations. After statistical coding, an initial meta-analysis was conducted to come up with an integrated estimate of the prevalence of HCV in KP. These estimates were found for the fixed effect and the random effect model. To assess the sample heterogeneity Q statistics were calculated. Although Cochran's Q is commonly reported, I^2 has been recommended as a supplement to Q when assessing heterogeneity because, unlike Q, I^2 allows researchers to quantify

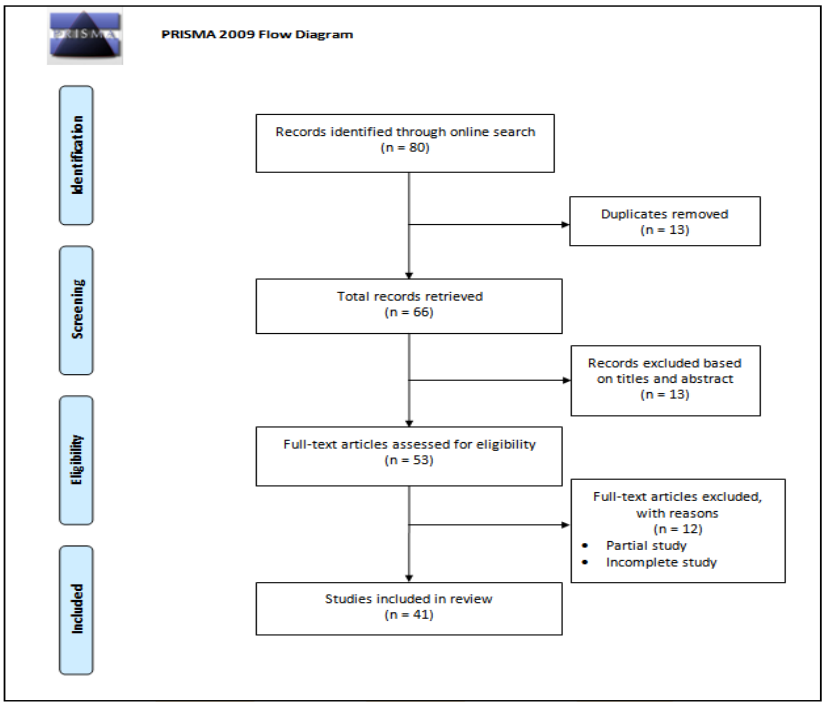

Figure 1: Flow chart of article selection as adapted by PRISMA

heterogeneity and to compare the degree of heterogeneity within different analyses [18,19]. Thus, an I^2 value of 25% indicates that one-quarter of the variation between studies reflects systematic or heterogeneous variation, rather than random sampling error.

The forest plot, useful tool of meta-analysis, was constructed to give a better picture of the prevalence from individual studies and the divergence from the overall combined estimate, 95% confidence interval, and weights were assigned to each study by the fixed effect and random effect model. Further, to highlight the amount of publication bias, funnel plots and regression tests were incorporated. The above process was repeated to compare the gender-wise prevalence of HCV. Out of 41, 26 studies that reported HCV in male and female populations were statistically analyzed, and an odds ratio was used to compare the prevalence of HCV in male and female populations. Further details were found using the Forest plot, funnel plot, and regression test. Although meta-analysis is a useful tool for integrating research findings, one of its major advantages is that it allows an examination of the extent to which findings are due to either random sampling error or systematic variations (heterogeneity) between studies. We conducted a meta-analysis by R (version 3.3.3), a language and software environment for statistical computing [20,21].

STUDY CHARACTERISTICS

Extracted data is graded by study populations' risk of HCV infection in two groups as shown (Table 1 and 2), General Populations and High-Risk Populations.

1. In group 1, eleven studies present general group (Table 1), seven studies present the population of blood donors, five studies report the prevalence of HCV in pregnant women, two studies report the epidemiology of HCV in Health Care Workers, one study reports the epidemiology of HCV in Civil Servants and one study reports the

epidemiology of HCV in Internally Displaced People (IDPs).

2. In group 2, fourteen studies present different types of the population at high risk including patients admitted in hospitals, Cataract Patients, Clinically suspected cases of HCV, Dental Patients, Gynecological patients, Hemophilia patients, Liver disease patients, Orthopedic patients, High-risk groups, and Thalassemia Patients.

The serological methods reported in studies of both the groups were CMIA, ELISA, ICT, IMX, and PCR.

POOLED MEAN HCV PREVALENCE ESTIMATES IN KP

The combined weighted estimate of HCV prevalence in KP, obtained from 41 previously conducted studies is 4% using the fixed-effect model and 6% from the random effect model (Figure 2). Further, the division of these studies in general and high-risk population gives respectively 3% and 8% prevalence of HCV by fixed effect model and 4% and 11% by random effect model, while between-group comparison is highly significant (p-value < 0.0001). The significant *Q* statistics indicate the presence of heterogeneity among the estimate from individual studies while the associated I^2 statistics show that most of the variation between samples is systematic in both groups. These points indicate the existence of substantial moderators (Risk factors) and their relationship with HCV prevalence.

It is observed in the Forest Plot (Figure 2) that out of 27 estimates of the general population mostly larger weight studies possess low or average prevalence and are closing to the central line (combined estimate of fixed effect and random effect model). A study of blood donors carries a maximum sample size of 127,828 and consequently, possesses the largest weight in the fixed effect method (32.9%). The high-risk population studies consist of 25,944 individuals and carry a maximum weight of 8.7%. In the random effect method, the weights are only fractionally varying among the available studies. I^2 statistics and tau^2 statistics indicate highly significant heterogeneity among HCV estimates found in the individual studies. The prevalence of HCV ranges from 1% to 22% in group 1 while in group 2 it varies from 3% to 53%.

HETEROGENEITY ASSESSMENT

The plot (Figure 3) suggested by Baujat pinpoints two studies that are prominent in contributing maximum to the heterogeneity and are the most influential among the studies too [63]. The study by Noor et al (2005) is highly influential while the study by Nazir et al (2016) show maximum contribution in the heterogeneity, the rest of the studies are lying at the left bottom corner having nominal share on both scales. From the direct observation of the funnel plot (Figure 4 a, b, c) the heterogeneity is quite evident. Not only, but most of the plotted dots are also lying outside the funnel, some of the dots are too far on the right, which causes a significant amount of publication bias (p- value=0.01358). In Group 1 and Group 2 upward bias of magnitude 5 and 8 respectively exists, however, the standard error of the bias is substantial which consequently makes the publication bias statistically.

HETEROGENEITY ASSESSMENT

The plot (Figure 3) suggested by Baujat pinpoints two studies that are prominent in contributing maximum to the heterogeneity and are the most influential among the studies too. The study by Noor et al (2005) is highly influential while the study by Nazir et al (2016)

Table 1: Descriptive characteristics of studies reporting HCV prevalence in the general population of KP

General Population	Sample size	Male	Female	HCV (%)	Male (+)	Female (+)	Region	Methods	Reference
General	16,400			4.57%	409	342	Buner	ELISA	[22]
	8,439			0.50			KPK	ELISA	[23]
	4,680	2,870	1,810	13.8	252	393	Swat	ELISA	[24]
	1,978	809	1,169	7.90	74	84	Peshawar	PCR	[25]
	1,431	308	1093	1.47	12	9	Malakand	ICT/ ELISA/q-PCR	[26]
	1,419	757	662	8.52	87	34	Mardan	ICT	[27]
	982	543	439	12.93	84	43	Peshawar	CMIA	[28]
	648	254	394	10.3	30	37	Mansehra	ICT	[29]
	400	300	100	7	12	2	Mansehra	ICT	[30]
	340			17.34%			Peshawar	ELISA	[31]
	180			5	7	2	Peshawar	ELISA/ICT	[32]
Blood Donors	127,828	127,780	48	2.46	3145	0	Peshawar	ELISA	[33]
	41,613	41,613		2.23	938		Swat	EIA	[34]
	32,042	32,042	0	1.97	632	0	Peshawar	ELISA	[35]
	7,148			3.13			Peshawar	ICT/ ELISA/ RT-PCR	[36]
	5,318			2.95			Mardan	ICT	[37]
	4,000	3,95	47	2.20	88	0	KPK	IMX or	[38]
		3						Axsym	
	356	356		22.2	79		Quetta	ICT	[39]
Pregnant Women	10,288	0	10,288	1.42	0	146	Peshawar	ICT	[40]
	5,607		5,607	2.60		146	Swat	ICT	[41]
	2,050		2,050	5		103	Peshawar	ICT	[42]
	500		500	8.60%		43	Hazara	ELISA	[43]
	360		360	2.22		8	Haripur	ICT	[44]
Health Care Workers	824	493	331	4.10%	21	13	KPK	ICT/PCR	[45]
	125	83	42	2.40	2	1	Abbottabad	ELISA	[46]
Recruited Civil	4,639	3,605	1,034	3.98	158	27	KPK	ELISA/ICT	[47]
IDPs	590	290	300	4.20	8	17	Swat	ICT/PCR	[48]

Table 2: Descriptive characteristics of studies reporting HCV prevalence in High-risk population of KPK

High-risk population	Sample size	Male	Female	HCV %	Male (+)	Female +)	Region	Methods	Reference
Hospital Patients	25,944	13,953	11,911	3.27	554	296	Bannu	ELISA/ICT	[49]
	1,443	922	521	4.00	39	19	Lakki Marwat	ICT/ELISA	[50]
	700	523	177	9.00	41	22	Mardan	ICT	[51]
	224	124	100	55.1	76	43	Dera Ismail Khan	PCR	[52]
Thalassaemia Patients	180	75	75	41.7	36	39	Peshawar	ELISA	[53]
MTBT	170	94	76	21.76	21	16	Swat	ELISA	[54]
Cataract Patients	1,130			2.57	13	16	Dera Ismail Khan	ELISA	[55]
CSCH	500	163	337	30	53	97	Peshawar	ICT/Elisa/ RTPCR	[56]
Dental Patients	1,540	561	979	2.98	9	37	Peshawar	ELISA/ICT	[57]
Gynae Patients	352		352	5.10		20	Peshawar	PCR	[58]
Hemophilia Patients	396	331	65	18	63	9	Peshawar	CMIA	[59]
High-Risk Groups	167	104	63	15.57	18	8	KPK	ICT/Nested PCR	[60]
Liver disease	1,500	810	690	29.20	210	228	Mardan	ICT/ELISA/	[61]
Patients								q-PCR	
Orthopaedical Patients	1,630	1,205	425	3.12	33	18	Abbottabad	ELISA	[62]

MTBT, Multi- transfused Beta- Thalassaemi a Major Patients; CSCH Clinically Suspected Cases of HCV

show maximum contribution in the heterogeneity, the rest of the studies are lying at the left bottom corner having nominal share on both scales. From the direct observation of the funnel plot (Figure 4 a, b, c) the heterogeneity is quite evident. Not only, but most of the plotted dots are also lying outside the funnel, some of the dots are too far on the right, which causes a significant amount of publication bias (p-value=0.01358). In Group 1 and Group 2 upward bias of magnitude 5 and 8 respectively exists, however, the standard error of the bias is substantial, which consequently makes the publication bias statistically.

GENDER WISE COMPARISON

The combined estimate of 26 studies shows that the odds of HCV infection in males as compared to females is fractionally lower for a fixed effect model but the same odds are fractionally higher for the random effect model, however, these odds are lacking in statistical significance (Figure 5). Both Q and I^2 statistics are substantially higher (p-value < 0.0001),

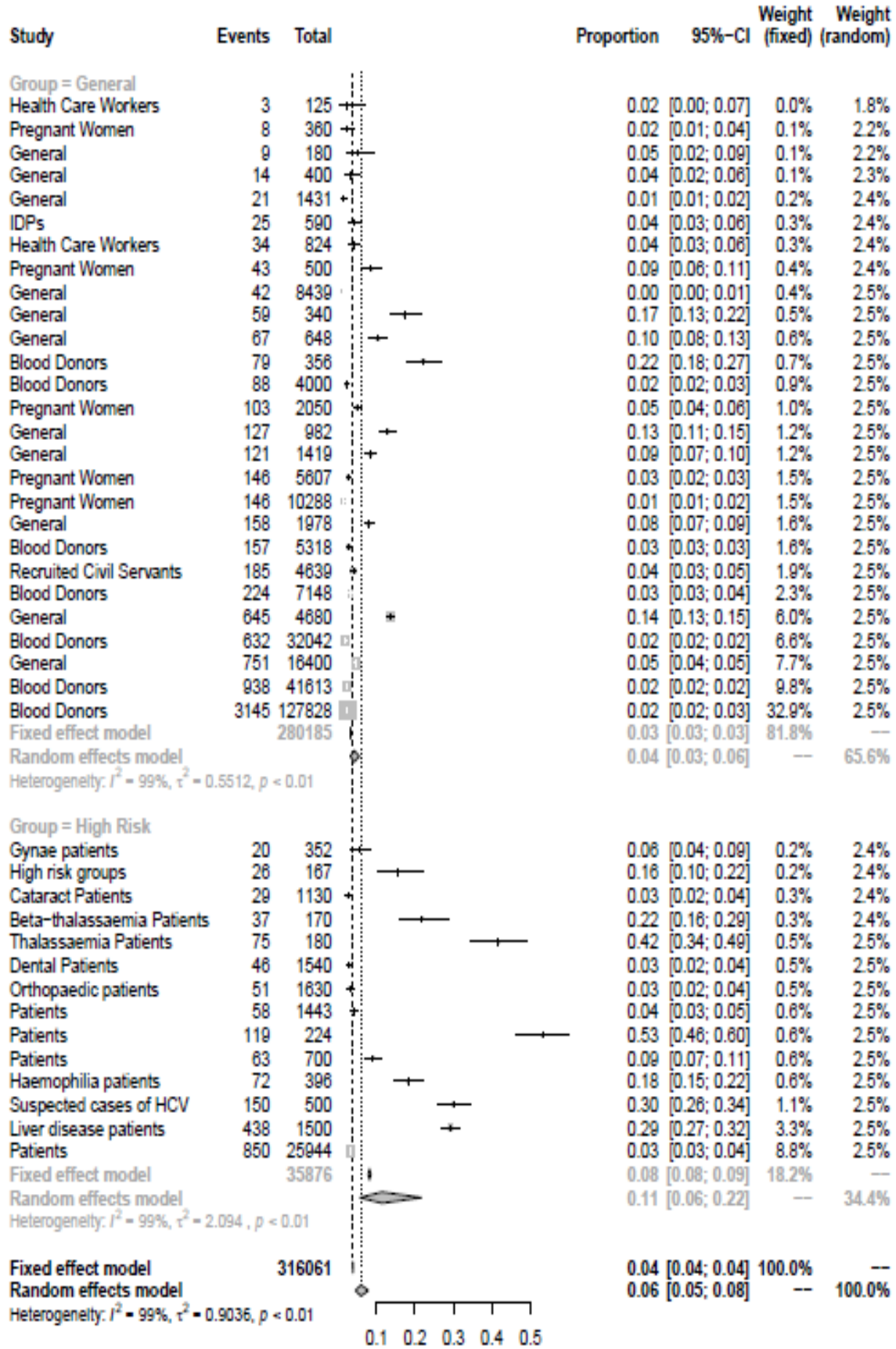

Figure 2: Forest Plot of studies reporting prevalence of HCV in KP

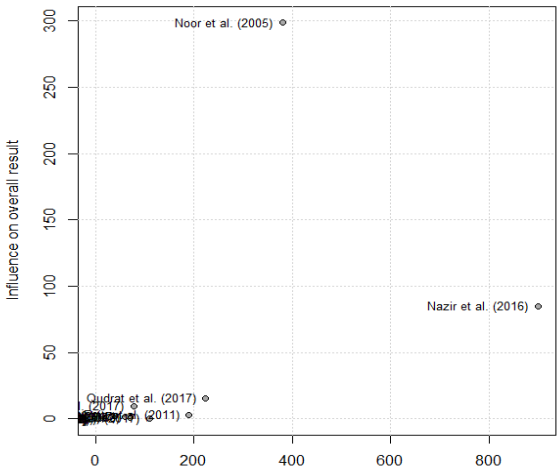

Figure 3: Baujat Plot of studies reporting the prevalence of HCV in KP

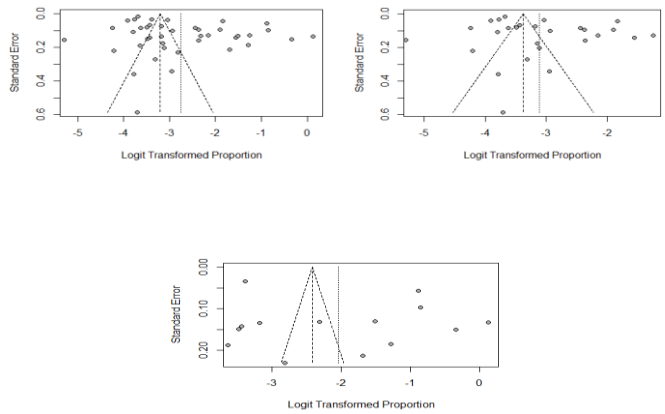

Figure 4: Funnel plot of reporting the prevalence of HCV in KP for (a) All (b) General and (c) High-Risk studies

indicating a high degree of heterogeneity in gender-wise odds given by these studies. The subgroups analysis based on General and High-Risk Population reveal that when fixed effect model is used for the general population group, the odds are significantly lower (0.78) for a male being affected by Hepatitis C as compared to female whereas in the high-risk population the odds are significantly higher (1.20 times) for male as compared to females. Further, the Q and I^2 statistics reveals significant heterogeneity in both groups of populations. As far as the difference between the two population groups is concerned, it is found from the relevant Q statistics that this is highly significant (p-value< 0.0001). Considering a large amount of heterogeneity in the two groups of populations, it will be sensible to assume a random effect model for analysis. The odds of HCV are fractionally higher in the male as compared to female in both types of populations; to assume a random effect model for analysis. The odds of HCV are fractionally higher in the male as

compared to female in both types of populations; however, they are statistically not significant. The heterogeneity, as before, is substantially higher in both groups but between groups comparison now shows the insignificant difference between the two types of populations

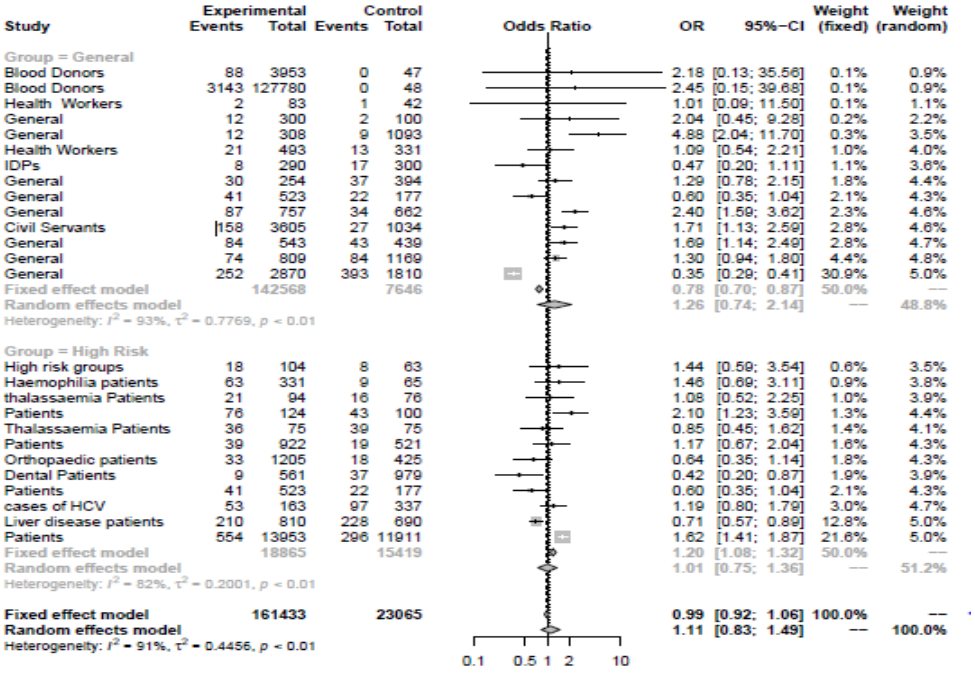

Figure 5: Forest Plot of studies reporting gender-wise prevalence of HCV in KPK

HETEROGENEITY ASSESSMENT

It is evident from the funnel plot (Figure 6a) since the total estimate represents most dots laying close to the central line while a few of them are evenly scattered on both sides of the central line, not causing serious threats to symmetry. Further, in Group 1 it is visible that most dots are laying to the right of the central line, which creates a considerable amount of upward bias, consequently the publication bias and asymmetry is statistically significant (Figure 6b). In Group 2, it is clear that dots are widely scattered, somewhat on the left side, creating negative bias, however, not attaining statistical significance (Figure 6c). Linear regression test of funnel plot asymmetry (efficient score) and publication bias indicates that there is a bias of magnitude 0.87 but it is statistically insignificant (P-value= 0.45) as the large standard error is substantial (Table 3). The studies of group 1 contain a considerable amount of bias (3.39), which produces significant (p-value = 0.046) asymmetry and publication bias among the studies. The studies of group 2 show the downward bias of magnitude 1.58; however, this amount of bias does not create significant asymmetry and publication in the funnel plot (Table 4)

CONCLUSIONS

HCV is a major global health problem for both high and low-income countries. This study could serve as a model study of the epidemiology of HCV in a province of a developing country, KP-Pakistan. In a world where advanced

Table 3: Linear regression test of funnel plot asymmetry for total studies, Group 1, and Group 2

Studies	**T**	**d,f**	**p.value**
All Studies	0.7625	24	0.4532
Group 1	2.2176	12	0.0466
Group 2	-1.3965	12	0.1928
Studies	**Bias**	**se.bias**	**Slope**
All Studies	0.8654	1.1350	-0.1468
Group 1	3.3886	1.5280	-0.8488
Group 2	-1.5848	1.1349	-0.1468

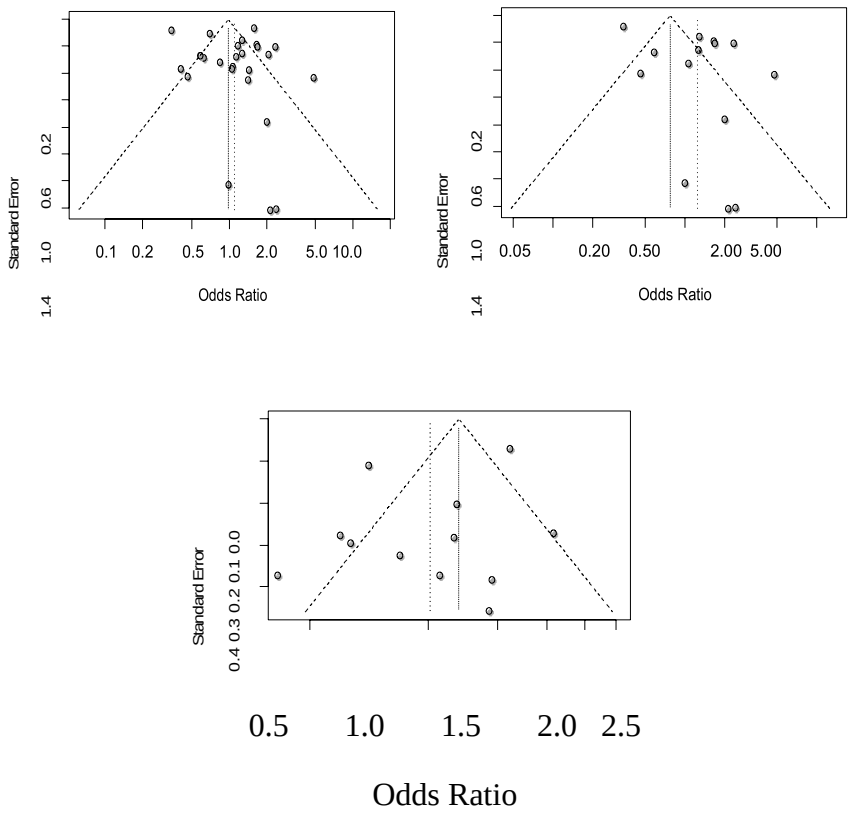

Figure 6: Funnel plot of studies reporting gender-wise prevalence of HCV in (a) All (b) General and (c) High-Risk studies

countries like Australia, Brazil, Germany, Japan, and the Netherlands have been stated to be on the path to eliminate HCV by the end of 2030, developing countries like Pakistan still struggle with an alarming prevalence rate [64]. Current study emphasizes to the prevalence rate of HCV in a war-affected province of Pakistan, by going through the previously published studies.

Our findings showed that the combined weighted estimate of HCV prevalence in KP is 4% using the fixed-effect model and 6% from the random-effect model.

Further, the general and high-risk population have 3% and 8% of prevalence of HCV respectively by fixed effect model and 4% and 11% by random effect model, while comparison between the two groups is highly significant (p-value < 0.0001). These findings are comparable with previously published reports on the prevalence of HCV in Pakistan. Umer et al. (2016) found active HCV infection in approximately 6% of the Pakistani population [25]. Similarly, Arshad et al. (2017) report a prevalence rate of 6.07% in KP and Jiwani et al. (2011) report a prevalence rate of 4.5% to 8% in Pakistan [65]. We noticed that the prevalence of HCV ranges from 1% to 22% in the general population while in high-risk populations it varies from 3% to 53%. Results of our findings are following Memon et al. 2012, which reports the prevalence of HCV in high-risk populations of Pakistan: 25% in health care workers, 10.88% in security personnel, 54.43% in prisoners, and 9.69% in PWID [66]. Similarly, these results are also following Waheed et al. (2009), which reports HCV prevalence of 4.95% +/- 0.53% in the general adult population, 1.72% +/- 0.24% in the pediatric population, 3.64% +/- 0.31% in a young population applying for recruitment, very high 57% +/- 17.7% prevalence in PWID and 48.67% +/- 1.75% in a multi-transfused population [68]. The current study based on General and High-Risk

Population reveals that when fixed effect model is used for the general population group, the odds are significantly lower (0.78) for a male being affected by Hepatitis C as compared to female where, as in the high-risk population, the odds are significantly higher (1.20 times) for the male as compared to the females. Both males and females are vulnerable to HCV and the reason why the odds are significantly higher (1.20 times) for the high-risk male population as compared to the high-risk female population, is maybe due to more exposure of males to the behavioral and social risks (e.g., unsafe injection practices, etc.). This observation is following "The report on Drug use in Pakistan 2013", which states that the male population was found to use more drugs than the female population, and approximately 50% of PWID in KP share syringes regularly [68]. Our findings have some limitations. Most of the previously published data that has been selected for the analysis has a small sample size and is not fully representative of the different populations at risk. Indeed, the lack of information on the age of different sample populations included in the estimates is a clear limitation given the geo-historical relationship between HCV transmission and prevalence across age groups. These qualitative and quantitative limitations of included studies are probably due to high variability within the specifically studied subpopulation, sampling technique, and participant recruitment.

The present study explores and updates current information on the prevalence of HCV in KP. All the basic requirements to deal with HCV are lacking in KP: lack of adequate surveillance systems, appropriate policies, register management, reliable health systems, and adequate public awareness. Pakistan still has no proper system to determine the actual incidence of HCV. The country lacks a proper surveillance system and most of the actual epidemiology data remain unrecorded and unpublished. The Federal Expanded Program on Immunization (EPI) reports that properly functioning surveillance systems are still non-existent. Major problems faced by the country is lack of effective study, disorganization, deficiency of knowledgeable staff and

an extremely poor data management. Unless strong public health surveillance systems are not organized and reliable data is not collected, it would be very hard to determine the actual incidence of HCV, and health programs could not accomplish their purpose. HCV infection can be adequately addressed only when the data generated from surveillance systems will be gathered and statistically analyzed [71].

It is important to note that that the knowledge about HCV is quite inadequate among the people of Pakistan. Previous studies have also reported inadequate awareness of causes, vaccination, transmission, consequences, and preventive methods for HCV [29,70,71]. A significant level of public awareness about HCV is very important for fighting disease especially because the people of Pakistan are generally exposed to all sorts of risk factors for transmission of HCV, i.e., the unsafe and reuse syringes, which is the biggest factor contributing to increased HCV in this region [67,72,73]. KP-Pakistan is still struggling with regard to the elimination of HCV. Pakistan needs to take some important steps. First of all, they must establish adequate surveillance systems, able to estimate the epidemiology of HCV. Once collected correctly, the data must be reported and statistically analyzed so that the politicians can draft the policies accordingly. Health sectors should be strictly asked to keep their records and report them to the concerned management. Pakistan also needs adequate health policies that can eliminate all the possible risk factors for HCV transmission. Unregulated blood transfusions still remain a dilemma in Pakistan, country's health systems need to be strengthened Public awareness about HCV is a very important factor for reducing its bioburden; it should be addressed regularly through print and electronic media.

In conclusion, once the actual incidence of HCV infection is accurately determined, the epidemic can be monitored, a high-risk population is identified and the success of interventions be measured [74–77]. HCV treatment and prevention must become a national priority to reach the WHO HCV elimination targets in Pakistan.

ABBREVIATIONS

CMIA,Chemiluminescent microparticle immunoassay; **DAA,** Direct-acting antiviral; **ELISA,** Enzyme-linked immunosorbent assay **EPI,** Expanded Program on Immunization **HBV,** Hepatitis B virus; **HCV,** Hepatitis C Virus; **HDV=** Hepatitis Delta virus; **ICT,** Immunochromatographic test **IDPs,** Internally Displaced People **IMX,** Immunoassay analyzer; **KP,** Khyber Pakhtunkhwa; **MENA,** Middle East and North Africa region; **ORF,** Open reading frame; **PRISMA,** Preferred Reporting Items for Systematic Reviews and Meta-Analyses; **PWID,** People who inject drugs; **WHO,** World Health Organization

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The author(s) declare they have no competing interests.

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Use of microbial consortium along with biosurfactants in oil sludge treatment

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ABSTRACT

Due to the excessive use of various oils, a large amount of oil sludge or waste water is produced annually which is causing various environmental and health issues. Bioremediation through the use of microbial consortium is an effective method. The use of consortium instead of single specie of bacteria has shown 89-94% improved results. However the microbes are still not able to work effectively due to bioavailability issues. This is mainly because of the low solubility of hydrophobic contaminants and microbes. This problem is overcome by use of biosurfactants which enhance the solubility and emulsification of hydrophobic substrates and microbes. These biosurfactants are produced by various organisms (mainly microbes) which are later isolated and used. Certain new and efficient insitu techniques are used for the application of microbial consortium which includes suspended growth, attached growth and hybrid growth. Waste water treated this way can be reused in the refinery procedures or can be dumped safely.

KEYWORDS: Microbial Consortium, Biosurfactants In Oil Sludge Treatment, Bioremediation Through The Use Of Microbial Consortium

INTRODUCTION:

The need for oil as a fuel and lubricant is increasing drastically with advancing technology.There are about 700 oil refineries in the world(2012 census). Water is used in refining and oil drilling processes. Some of this water comes in direct contact with crude oil producing contaminated waste water or sludge.This sludge is dumped in ponds, marshes and open pits, which possess various environmental hazards to superficial & ground water,soil and air. It also causes serious ecological problems.The pollutants in this sludge includes polycyclic aromatic hydrocarbons e.g. benzene, toluene,ethyl benzene[1], asphaltene, phenol and its derivatives[2],metal contaminants[3],some carcinogens and immunotoxicants. Exposure to these contaminants can cause variety of health problems including damage to lungs, respiratory problems, mutations, cancer,nausea, irregular heartbeats, and birth defects. It also effects plants, decreases the fertility of soil and suppresses seed germination[4].

Various physiochemicalmethods have been devisedfor the treatment of sludge e.g. oily sludge solidification method, chemical demulsification method, solid –liquid separation method etc. But,the bioremediation (through microbes) is the most cost effective,environment friendly and widely used method. In this paper use and preparation of microbial consortium in

the treatment of oily sludge is discussed along with the hindrance in microbial action and the methods to use microbial consortium.

PRETREATMENT OF OIL SLUDGE:

Hydrocarbons and other non polar compounds are good source of energy for the microbes but their bioavailability is a limiting factor because of the low solubility, non uniform spatial distribution of microorganisms and pollutants, and retardation of substrate diffusion by soil matrix[5].

To overcome these obstacles a pretreatment of oil sludge is required. The pretreatment methods include electro coagulation, electro chemical oxidation and other physiochemical methods [5][1] [6]. These methods are effective (63% COD removal and 92.8% COD removal respectively) but are inconvenient and costly[1]. However, pretreatment with bio surfactants (or bioemulsifiers) is a widely used method. Biosurfactants are the agents released by certain organisms which enhance the solubility and emulsification of hydrophobic substrates. It reduces the interfacial tension between water and hydrophobic pollutant (removing low solubility and retardation obstacles), allowing microbes to work effectively[7] [8].But, the phenomenon behind this activity of biosurfactants is not known exactly [6]. Some scientists believe that the biosurfactants produce certain agents which increase the adhesion of microbes to the hydrocarbons. Table 1[9] shows the different classifications of biosurfactants and the organisms they are isolated from.

No.	Type of biosurfactant	Name	Bacterial sp.
1.	Glycolipids	Rhamolipids	Nocardioides sp.
		Sophorolipids	Candida sp.
		Trehalose lipids	Rhodococcus sp.
2.	Lipopeptides& lipoproteins	Fengycin	Bacillus sp.
		Arthrofactin	Arthrobacter sp.
3.	Phospholipids & Fatty acids	Bile salts	Myroides sp.
		Fatty acids	Mycobacterium sp. Nocardia sp. Candida sp. Cladosporin sp.
		Phosphotidylthanlamine	Rhodococcus sp.
4.	Polymeric Biosurfactants	Alasan	Acinetobacter sp.
		Bioemulsan	Gordonia sp.
5.	Particulate Biosurfactants	Whole cells	Yarrowia sp.
		Vesicles	Serratia sp.

The addition of Biosurfactants improves the efficiency of microbial activity for example At 20 °C, rhamnolipids (11.2 mg/L) increased the removal efficiency of crude oil from 17.7% (in the absence of rhamnolipids) to 63%. At 25 °C, the removal efficiency of crude oil was over 80% with the presence of rhamnolipids compared with 22.3% in the absence of rhamnolipids.[10]

MICROBIAL AGENTS FOR OIL SLUDGE TREATMENT:

The indigenous microbes can degrade the contaminant to large extent but if the concentration of these pollutants is higher than it becomes difficult for the microbes to work efficiently and effectively. So, these microbes have to be supplemented with nutrients e.g. Nitrogen, Potassium and Sulphur[7].

Moreover, it is difficult for a single type of microorganism to degrade the wide range of hydrocarbons and non polar compounds. This was a hindrance in the process of bioremediation so scientists started using microbial consortium [2]. Some of the microbes known to degrade polycyclic aromatic hydrocarbons include Pseudomonas sp (P. aeruginosa, P. fluorescence and P. putida) [7] [2], *Acinetobacterbaumannii strains [11], micrococcus sp, cornybacteriumsp, flavobacteriumsp[12] etc. Some Agrobacterium sp, rhizobia, leguminosarum and bv. Trifolli are used to remove metallic contamination [3].*

To prepare consortium soil samples are collected from different areas (Oil rich soil). The different bacterial strains are isolated using standard serial dilution procedure. The isolated strains were further characterized on the basis of their substrate specificity and gram character. They are maintained on nutrient agar slants at 4°C and with 50% glycerol at - 20 °C for future use[12]. The isolated strains are then individually inoculated by single streaking on selective media (i.e. with oily substrate) and checked for a zone of clearing around each bacterial isolate. The strains showing the positive results are subjected to gram staining to check morphology. To prepare successful microbial consortium, bacterial cultures must be compatible with each other in order to simultaneously produce all these enzymes required for the degradation. After the successful degradation of oil substrates in lab trials by the bacterial consortia large scale trials were also set up in closed container. The consortium needs a carrier material for the safe transfer of microbes e.g. corn cob.

It is possible to assess the performance of a waste water treatment plant by measuring the BOD of the inflow and the outflow. The use of consortium has shown to cause 60% reduction in BOD level of waste water as compared to inflow [13] and in another research where Baumannii strains were used 89 to 94% removal of total petroleum hydrocarbons was observed depending upon the consortium used. [4]

METHODS OF APPLICATION OF MICROBES:

Basically the role of microbes in oil sludge treatment is to convert the complex hydrocarbons into simple compounds like water, carbondioxide and methane. After the pretreatment phase the sludge is treated with microbial consortium. Typically There are three methods for the application of microbial consortium: suspended, attached and hybrid [1].

Suspended growth: Aerobic microbes are used in this form of remediation. The microbes are present in the form of suspension i.e. they float freely in the reactor. It is of 5 types i.e. CSTR (continuous stirred tank reactor), SBR (sequence batch reaction), plug flow, complete mix and membrane bioreaction. The basic mechanism behind all these processes is the same which is demonstrated in the Figure 2 below.

This method is efficient but the waste water has to be re-purified from the consortium of microbes. So, this method is replaced by attached and hybrid methods.

Attached growth: In this method the effective microbe is immobilized on an inert surface (e.g. rocks, slag or plastic). This material is then placed in the bioreactors. When these immobilized bacteria come in contact with the sludge (hydrocarbon substrate) enzymes are released which produces a bio film. These biofilm act as sieve that filters the sludge as it passes through. (This filtration is done by converting the complex compounds into simpler ones)

Hybrid growth: It is a combination of attached growth and suspended growth method. Fixed bio filters are present in the

bioreactors accompanying suspended microorganisms and carrier material. These biofilters are made up of polyurethane. As the activated sludge (sludge with microorganisms and carrier material) passes through the urethane filters, the microbes in the sludge immobilize themselves on the surface of the filters. Urethane filters also filter sludge from micro granules.The process has been termed Activated Sludge Biofilm Waste Water Treatment System (ASBWTS)[12].

CONCLUSION:

Oil is the need of this era but the refining procedures of oil produce a large amount of sludge or waste water. This oil sludge is responsible for causing environmental pollution leading to health problems. Treating this with microbial consortium has proven to be an effective remedy. Microbial consortium used is a combination of hydrocarbon degrading microbes which work together to secrete enzymes that convert the complex hydrocarbons into simpler ones. Generally used microbes for treating oil sludge include Pseudomonas sp, Acinobactersp, Micrococcus sp, cornybacteriumsp and flavobacter sp.

These bacterial species are isolated from oil rich soil samples and then checked for efficiency and their compatibility to work with other microbes. These species are then shifted to a carrier material and applied in the treatment of sludge. However, the hydrocarbons in the sludge are not readily available for microbial activity because of solubility problems. Biosurfactants resolve this problem by increasing the solubility and adhesion rate of hydrocarbons to microbes. So, sludge has to be treated with biosurfactants before microbial consortium is applied. The insitu application techniques of microbes include suspended growth in which microbial consortium float freely in the bioreactor, attached growth in which the microbial consortium is first immobilized on an inert surface and then inserted in the bioreactor, and hybrid growth which is a combination of both suspended and attached growth methods.

So, using advanced technologies, biosurfactant and consortium we can clean our environment from hydrocarbon contaminated waste water.

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Anemia and its consequences on human body; A comprehensive overview

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ABSTRACT

Anemia is a pathological condition characterized by a reduction in the mass of red blood cells or the amount of hemoglobin. Anemia affects one-third of the world's population, with iron deficiency accounting for half of the cases. It's a major global public health problem that has an effect on maternal and child mortality, physical fitness, and referral to health-care providers. Underweight children have a greater prevalence of anemia, which can produce long-term developmental outcomes. Particular risk is presented by children 0-5 years, child-bearing mothers and pregnant women. Efforts to avoid anemia should concentrate on improving current supplementary iron and folate programs and on preventing folate and vitamin B12 anemia deficiency. In this review biological mechanism and condition of anemia development has been discussed. A further study is necessary to examine the function of additional nutrient deficits, the contribution of infectious and chronic illnesses in some populations, and the significance of hereditary hemoglobin disorders.

Key Words: Anemia, Inflammation, Hemoglobin, Nutrition, Iron deficiency.

INTRODUCTION

Hemoglobin is the red blood cell protein molecule which transports oxygen from the lung to the tissues of the body and returns carbon dioxide from the tissues to the lungs. Hemoglobin consists of four protein molecules bound together (the globulin chains). Two alpha-globulin chains and two beta-globulin chains form the normal adult hemoglobin (Hgb or Hb abbreviated). Anemia is a disease in which the concentration of hemoglobin (Hb) and red blood cells (RBC) are below normal levels, which are inadequate to satisfy the physiological demands of the person affects approximately a third of the world's population. The name comes from ancient Greek: anemia ("lack of blood"), anemia ("not"). Anemia is linked to elevated female and infant morbidity and mortality, adverse birth outcomes in adult employment and reduced cognitive and behavioral growth in infants (Chaparro & Suchdev, 2019). “Anemia is highly common in developing and developed countries and is regarded as a public health concern. It happens at all stages of life, especially in women and children who are pregnant, 1.62 billion people worldwide are anemic”. In pre-school children the highest prevalence is 47.4%, in men the lowest prevalence is 12.7%. Anemia is the second leading disease cause in the world and thus one of

the most significant public health issues in the world" (WHO, 2018). Anemia has multifactorial causes with complicated interactions among nutritional and other factors, which are a challenge to resolve effectively population determinants of anemia (Antwi-Bafour *et al*., 2016). Anemia has many causes including: "infectious diseases, for instance, malaria, hookworm and shistosomiasis. Micronutrient deficiencies including folate, vitamin B12 and vitamin A. Increased chance of having anemia is also seen in individuals with chronic diseases like kidney diseases, cancer, diabetes and associated conditions". There are different varieties of anemia classifications. Anemia is caused by a variety of red cell defects such as a production defect (AP), a maturation defect (MAM), a hemoglobin synthetic defect (AID) or a genetic defect in the maturation of hemoglobin (THA) or the synthesis of anomalous hemoglobin (HHEA) or thalassemia (Balarajan *et al.*, 2011).

Many studies suggests that fetal/neonatal Iron deficiency discusses long-term risks to brain function. Early iron deficits (ED) not only affect the brain and function, but also have an after-treatment effect. Dopamine synthesis, myelination, composition and function modifications for the long run are part of the path. The brain does not normally function, as it has an iron deficiency. Deficiency in iron Headache, vertigo, delirium, restless leg syndrome are all linked with anemia. Anemia. Anemia is today the main global risk factor for the wellbeing of adolescents and pregnant mothers. Anemia should be diagnosed and treated early in order to achieve a stable generation (Soundarya & Suganthi, 2017).

IRON DEFICIENCY ANEMIA

Iron deficiency, which affects 2 billion people globally, is the most prevalent nutshell for infants. Worldwide, the rate of iron failure is twice as high as anemia of iron deficiency. (Lanzkowsky *et al*., 2016). Microorganisms have evolved advanced mechanisms such as the siderophore system to extract iron from extremely low amounts in their atmosphere. Human beings have evolved ways to withhold iron from microorganisms as a primitive defense mechanism. In human ferrokinetics, iron-binding proteins such as transferrin, ferritine, and lactoferrin play a central role. These iron-bound proteins also contribute to a reduction in the supply of iron for microorganisms. They achieve this by reducing the use of iron (Camaschella *et al.,* 2015). Iron deficit can lead to anemia-unrelated symptoms. The most effective iron deficiency test is serum ferritin. Oral iron with vitamin C is best administered once a day (DeLoughery *et al*., 2017).

TYPES OF ANEMIA

Aplastic Anemia

Aplastic anemia (AA) is a rare bone marrow deficiency condition that is particularly lethal when seriously treated and not properly treated. Aplastic anemia is a seldom condition with a prevalence of around two to three cases annually based on specialized trials, but in Asian populations it may be three times higher. It is a young disease that usually develops during the first 30 years with an average age of about 20 (Shallis *et al.,* 2018). The most prevalence of acquired aplastic anemia is a result of the immune-mediated elimination, either by immune suppressive or haematopoietic stemcell transplant, of hematopoietic stem cells inducing pancytopenia, with empty bone marrow (Young *et al.,* 2008).

Aplastic Anemia

Aplastic anemia (AA) is a rare bone marrow deficiency condition that is

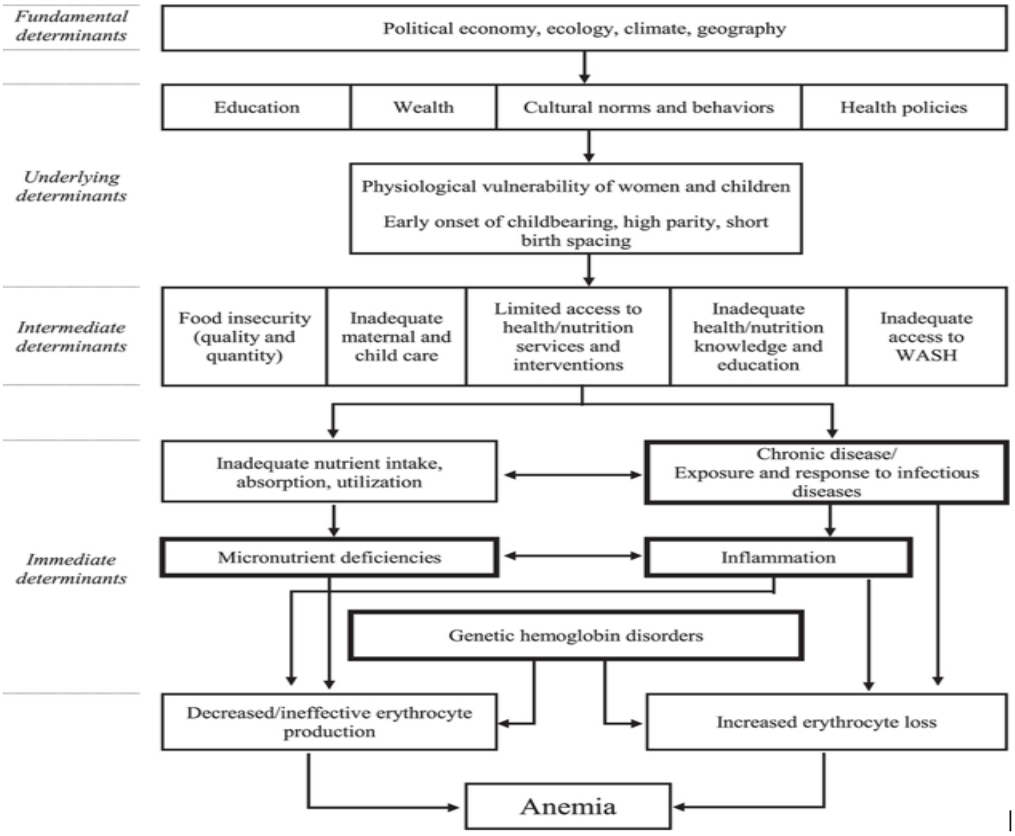

Figure 1. conceptual model of anemia etioloy

particularly lethal when seriously treated and not properly treated. Aplastic anemia is a seldom condition with a prevalence of around two to three cases annually based on specialized trials, but in Asian populations it may be three times higher. It is a young disease that usually develops during the first 30 years with an average age of about 20 (Shallis *et al.*, 2018). The most prevalence of acquired aplastic anemia is a result of the immune-mediated elimination, either by immune suppressive or haematopoietic stemcell transplant, of hematopoietic stem cells inducing pancytopenia, with empty bone marrow (Young *et al.*, 2008).

Sickle Cell Anemia

Sickle cell anemia is disorder known as sickle cell disease in which there aren't enough healthy red blood cells to carry oxygen throughout the body. In sickle cells RBC's are of sickle shape or looks like crescent moons. "These rigid can stuck in small blood vessels and can stuck or block flow of blood. Signs and symptoms start to appear around 5 months of age". They vary person to person and changes over time. Sickle cells break apart easily and then die leaving too few red blood cells. "The life span of RBC's is 120 days. But sickle cells usually die in 10 to 20 days, leaving a shortage of red blood cells (anemia)" (Adams *et al.*, 1998). It is caused by a gene mutation that instructs the body to produce the iron-rich compound that gives blood its red color and allows red blood cells to transport oxygen from the lungs to the rest of the body (hemoglobin). "Pain occurs as sickle-shaped red blood cells obstruct blood flow to the chest, belly, and joints through tiny blood vessels, develop ulcers, as well as damage to the spleen, joints, and bones, as a result of a chronic pain crisis". If the condition persists, a hospital stay may be

required. Hand and foot swelling, viruses on a regular basis, delayed growth and puberty, pale skin, confusion, headache, fatigue (Sabarense *et al.*, 2015).

Hemolytic Anemia

With an MCV of 80 to 100 fL, hemolytic anemia is classified as normocytic anemia. It is a type of low hemoglobin caused by red blood cell loss, increased hemoglobin catabolism, decreased hemoglobin levels, and increased efforts by the bone marrow to regenerate products. It may be either intrinsic or extrinsic in nature. Infections, cancer, cancers, and drug side effects are examples of extrinsic causes. Intrinsic factors: Red blood cells don't work well, and their hemoglobin is irregular. It contains sickle cell anemia and thalassemia. Often known as erythrocytosis. Hemolytic anemia will strike someone at any age (Rai *et al*., 2020). Hemolytic anemia can damage various organ systems in the body. In hemolytic cases, ischemia and thrombotic complications are also possible. Patients can feel muscle pain and fatigue when muscles are deprived of blood and oxygen, and extra iron from hemolysis may cause kidney failure or complications (Nemeth & Ganz, 2014). Fever, intolerance to physical activity, chills, a larger liver or spleen, confusion, heart palpitations, jaundice, back pain, headache, shock, arrhythmias (irregular heart rhythms), and cardiomyopathy (in which the heart expands larger than normal) are all symptoms of severe hemolytic anemia. Treatment includes blood transfusions, corticosteroids, and other medicines (Cappellini *et al*., 2020).

COMPLICATIONS DUE TO ANEMIA

Complications during Pregnancy

Anemia is normal in breastfeeding, with rates ranging from 5.4 percent to more than 80 percent in developing countries. For expansion of the maternal blood volume, placental development, and fetal growth, iron requirements rise sharply during pregnancy. A total of 1,040 to 1,240 mg of iron is expected to be needed (Beard & Durward, 2012). Anemia's severity is linked to an elevated risk of LBW and prematurity. Anemia due to iron deficiency accounts for 75% of all anemia in pregnancy. IDA is linked to an elevated risk of preterm labor, low neonatal weight, and perinatal risks in pregnant mothers. Owing to a decreased threshold for acute blood loss during gestation and an elevated risk of infections, severe IDA is linked to an increased risk of infant and maternal mortality (Beard & Durward, 2012).

Maternal Mortality and Morbidity in Anemia

Anemia, and its most common symptom, iron deficiency (ID), is a common cause of morbidity in both developed and developing countries. “Women with hemoglobin levels below 8 gm/dl have a higher risk of maternal morbidity. Additional causes, such as social status, medical treatment, and dietary status, can be linked to maternal morbidity (Breymann, 2015). When maternal hemoglobin levels dip below 5.0 g/dl, maternal mortality rates skyrocket. Women with moderate anemia are more likely to die from antepartum and postpartum hemorrhage, pregnancy-induced hypertension, and sepsis. The severity of the iron deficiency anemia affects maternal mortality as well. An increased risk of cardiovascular disease, a high risk of hemorrhagic shock, and higher rates of infection and delayed wound healing during the puerperium are among the causes” (Breymann, 2015).

CHILD GROWTH DEFECTS

Anemia in children is characterized as a hemoglobin (Hb) concentration below the World Health Organization's cutoff thresholds. “Iron deficiency anemia (IDA)

is the most common cause of anemia in children aged 6–59 months, 11.5% in children aged 5–11 years, and 12 g/dl in older children. (Ashraf *et al.,* 2017). Inadequate iron consumption during the quickly developing years of infancy and childhood is the most frequent cause of iron deficiency anemia. During infancy, growth is particularly rapid. Iron reserves available at birth will be exhausted by 6 months in a full-term baby and by 34 months in a premature infant if no iron is present in the diet or if blood loss happens" (Lanzkowsky, 2016). Anemia has been associated with growth retardation including stunting and being underweight. Complications such as growth and pubertal delay are normal. Defective IGF-I secretion is one of the mechanisms of defective development in children with IDA. (Ashraf *et al*., 2017). ID also has a detrimental effect on the mother-child relationship and the neurological growth of children, with effects lasting up to ten years despite iron replacement (Camaschella, 2015).

Complications in Adolescence

Adolescents are people aged 10 to 19 years old, according to the World Health Organization. This age group accounts for roughly 20% of the global population, and it is during this time that people transition from dependent childhood to independent adulthood (Teji *et al.,* 2016). Human babies may have chronic symptoms of early life iron deficiency that continue into adulthood, causing cognitive dysfunction in the elderly and restless leg syndrome (Cappellini *et al.,* 2020). On chronically anemic teenagers, adolescence is a susceptible time in the human life cycle for nutritional anemia. According to a survey, the most prevalent form of anemia in adolescents was megaloblastic anemia (42.5%), with iron deficiency accounting for 15% of cases. Deficiency in folate, vitamin B12 and iron are all normal in anemic teens. Vegetarianism was shown to be linked to extreme anemia. Menarche was also interrelated to an increased risk of anemia, according to researchers who found that high menstrual blood loss was linked to an increased risk of anemia. Iron, folic acid and vitamin B12 supplementation should be provided in the community (Cappellini *et al.,* 2020).

Lowered Cellular Immunity and Increased Morbidity

Iron is required for the immune system's normal growth. A healthy iron homeostasis is crucial in deciding infection tolerance and outcome. Its absence impairs the immune system's ability to respond appropriately because it is needed for immune cell proliferation and the production of complex responses to infection (Das *et al.,* 2014). Iron is a critical component for the immune system's normal growth, according to research from the last few decades. Iron is required for immune cell proliferation, especially lymphocyte proliferation, which is linked to the generation of a specific response to infection. Iron is needed for monocyte/macrophage separation, and macrophages need iron as a cofactor to carry out essential antimicrobial effector mechanisms, such as the nicotinamide adenine dinucleotide phosphate hydrogen-dependent oxidative blast (Hassan *et al.,* 2016). Anemia is the most common nutritional condition in the world, with iron deficiency being the most common cause (ID). ID is particularly dangerous to children and women of reproductive age. Hemoglobin levels below 10 gm/dl hinder cell-mediated immune responses, resulting in bacterial growth in leucocytes in young children. Cause hemodynamic Instability, reduced Immune Response which can make older adults more vulnerable to infections (Cappellini *et al.,* 2020).

Anemia and Chronic kidney disease

Renal function will not be harmed by mild to severe anemia because blood is drawn to

the kidneys from peripheral tissues. The kidneys are also one of the organs that helps the bone marrow make RBCs by increasing erythropoietin secretion. The synthesis of this hormone is reduced in people with chronic (long-term) kidney disease, which lowers RBC production (Abbaspour *et al.*, 2014). As a result, kidney function is compromised, resulting in anemia. Since erythropoietin deficiency is the most common cause of anemia in chronic renal failure. With declining hemoglobin levels, there is a cumulative rise in the risk of pre-dialysis death or the occurrence of end-stage renal disease in predialysis patients (around 2- to 3-fold for hemoglobin values <120 vs >130 g L^{-1}). (Cappellini *et al.*, 2020).

Anemia and Cardiovascular Disease

Cardio-renal anemia syndrome is characterized by anemia which is linked to a two-fold increased risk of cardiovascular hospitalization. Lack of iron Fatigue, tachycardia, cardiac murmer, and angina are also symptoms of anemia, as well as decreased physical function and quality of life (Cappellini *et al.*, 2020). Anemia was observed in 17 percent of heart failure patients in a survey, with chronic disease anemia accounting for the majority of the cases (58 percent). In this significant cohort of individuals with heart disease, those with anemia have a 10% higher 5-year mortality risk (Abid *et al*., 2019). As Hb falls below a certain threshold, compensatory processes fail, lactic acid levels increase, and cardiac failure may occur. Congestive heart disease patients are anemic on average in 40% of cases. "Hypoxia caused by anemia, regardless of the cause, causes peripheral vasodilation, a drop in blood pressure, and activation of the sympathetic and renin angiotensin aldosterone systems (RAAS) to keep blood pressure in check. Increased sympathetic activation raises heart rate and stroke volume, which, when combined with an activated RAAS, will result in renal ischemia, fluid accumulation, and increased plasma volume". Also in healthy hearts, the elevated cardiac burden caused by both of these pathways will contribute to CHF. TNFa and other cytokines are produced by compromised myocardium and can cause additional damage to the heart and kidneys. This, in fact, will worsen anemia (Silverberg *et al.,* 2001).

Anemia and Gastrointestinal Tract (GIT)

Anemia is associated with gastrointestinal disturbances. Any of them, including duodenal ulcer, gastrointestinal tract carcinoma or glossitis, and atrophy of the tongue papillae in pernicious anemia, may be symptoms of the underlying condition. In anemic patients, indigestion and irregular bowel movements have also been identified (Percy *et al.*, 2017).

Chronic Inflammatory disorders and Anemia

Inflammatory bowel diseases (IBD), include "Crohn's disease" and "ulcerative colitis", are chronic inflammatory disorders. Anemia is a frequent symptom of inflammatory bowel disease (IBD). In IBD, persistent anemia is normal. Anemia affects 5%–71% of IBD patients, according to reports. Anemia is more frequent in children than in adults with IBD (Goodhand *et al.,* 2012). According to a new meta-analysis of European studies, Crohn's disease (CD) has a prevalence of 27% and Ulcerative colitis has a prevalence of 21%. (UC). Crohn's disease (CD) often progresses from an inflammatory state to a more complex state of stenoses or fistulae. Ulcerative colitis (UC) can spread over time, raising the likelihood of a colonoscopy or cancer. Persistent or chronic anemia in patients with IBD is linked to more aggressive or debilitating illness, according to a prospective study of 410 patients. The most prominent extraintestinal manifestation of inflammatory bowel disease is iron

deficiency anemia, which can have a negative impact on one's quality of life (Cappellini *et al.*, 2020).

Anemia and Genitourinary Tract

Symptoms of the genitourinary tract are common in anemia patients, and they can be caused in part by a reduction in sexual hormone secretion. Amenorrhea, menorrhagia, and erratic menstrual cycles are among the more frequent symptoms (Percy *et al.*, 2017).

Anemia and Altered Brain Function

Late fetal/early neonatal life, toddlerhood, and puberty are three peak ages for iron deficiency in early life, all of which are associated with lower brain development during the duration of ID. According to several findings, fetal/neonatal iron deficiency is associated with long-term risks to brain development. (Cappellini *et al.*, 2020). Early iron deficiency (ID) has long-term effects on brain and behavioral function, not just during the ID phase. Long-term changes in dopamine synthesis, myelination, and hippocampal development and function are among the pathways. When the brain is iron deficient, it does not function properly. Headache, vertigo, syncope, delirium, and restless leg syndrome are all symptoms of anemia. In iron deficiency, restless leg syndrome has been characterized as an uncontrollable movement of the legs. According to a meta-analysis of five tests, a 10 g/L rise in hemoglobin was linked to a 173 (95%) increase in IQ marks (Teji *et al.*, 2016). However, whether or not impaired cognitive performance in iron-deficient children is exacerbated by other causes leads to poor cognitive functions remains to be seen (Balarajan *et al*., 2014). Iron is needed for normal energy metabolism, neurotransmitter synthesis, and myelination in neurons and glia. "Acute neurobehavioral effects of neonatal ID include altered temperament and child-mother interaction, slower neural conduction velocity, a higher prevalence of abnormal neurologic reflexes, and poorer discrimination memory, whereas long-term effects are related to dopamine and monoamine or neurotransmitter metabolism in general." (Yohannes & Ershler, 2011).

PREVENTIVE MEASURES OF ANEMIA

Other types of anemia, such as inherited anemia, cannot be prevented. However, consuming a healthy diet can help avoid anemia caused by iron deficiency, vitamin B12 deficiency, and vitamin B9 deficiency. This involves consuming a diet rich in foods rich in iron and these vitamins, as well as vitamin C-rich foods to aid absorption. Make sure you're getting enough water. Anemia can occur for a variety of reasons, but one of the most common is inadequate diet (WHO, 2017). Other micronutrients can be deficient in insufficient and unbalanced diets, contributing to micronutrient shortages and the development of anemia. To combat this, a variety of dietary-improvement-focused interventions have been applied at the community level or are specifically aimed at disadvantaged populations such as babies, small children, and pregnant women. It includes food-based methods for reducing micronutrient deprivation and increasing micronutrient consumption, such as supplementation, food fortification, and improving the diversity and consistency of food (Zimmermann *et al*., 2007).

DIETARY STRATEGIES

A nutritious, well-balanced diet can help avoid deficiencies. Strong sources of iron include liver, red meat, beans, lentils, tofu, fish, dried fruit, and dark leafy greens. Vitamin B12 and folic acid are both needed for RBC processing. These are abundant in dairy products, milk, bananas, and spinach.

Minerals, vitamin B12, and folic acid are also present in fortified breads, cereals, and pastas. Vitamin C is abundant in citrus fruits and other types of food, which is also essential (Maldonado, 2013).

SUPPLEMENTATION

Daily or occasional oral iron, nutrient, or mineral supplementation alone or in combination (especially vitamin B12, folate, vitamin A, or pro vitamin A, but also vitamin C, vitamin E, zinc, and other minerals). Supplementation during breastfeeding has been linked to the prevention of maternal anemia in people who live in areas where vitamin A deficiency is prevalent. For most diets, meeting the high physiological demand for iron during pregnancy is challenging. During breastfeeding, a woman requires about 2–2.8 mg of iron every day. "During breastfeeding, iron needs range from 450 to 1,150 mg, with a median of 790 mg. Folic acid supplements are more effective than dietary folate at raising serum levels. Adults should take 400 mcg/day, pregnant women 600 mcg/day, and breast-feeding mothers 500 mcg/day. In all cases, intake is limited to 1000 mcg/day" (Sabarense *et al.*, 2015).

FOOD FORTIFICATION WITH IRON AND VITAMIN B12

Iron fortification involves the addition of iron, usually with folic acid. The presence of iron, normally in the form of folic acid, is known as iron fortification. Thus, iron fortification of foods has emerged as a potential strategy for avoiding iron deficiency anemia during breastfeeding. Iron was fortified into a number of foods, including cereal flour (maize or wheat), salt, beverage, milk, and sugar, pasta, rice, and fish sauce, and used effectively as nutritional supplements to avoid anemia (Girard & Olude, 2012). Even though vitamin B12 does not exist naturally in plant foods, fortified foods should be used in these situations (Sabarense *et al.*, 2015). The daily recommended intake of "vitamin B12 in adults is 2.4 mcg/day, and 2.6 mcg/day and 2.8 mcg/day in pregnant and breast feeding women." (Soundarya & Suganthi, 2017).

CONCLUSION

Anemia is the biggest nutrition problem occurring these days. Iron deficiency anemia is most common type affecting children and pregnant women. Another type (genetic) Sickle cell anemia is caused due to crescent shape red blood cells. Hemolytic anemia is low hemoglobin due to the destruction of red blood cells. Common symptoms of anemia includes Dizziness, Weakness, Tiredness, pale skin pallor, irritability, anorexia, and pica. On average 80% women during pregnancy are anemic that increases the chances of death of mother or fetus, or result in early deaths or multiple post birth disorders like birth defects, impaired thermoregulation, Lowered Cellular Immunity and Increased Morbidity and Compromised development in infants and Young children. Chronic anemia cause organ damage effecting kidneys, heart, inflammation in gastrointestinal tract and impaired brain functions. Inherited anemia can't be treated. Iron levels can be maintained and regulated by proper dietary practices eating varieties of foods including fruits, vegetables, pulses and legumes high protein diet and iron rich diet and fortified food products. Micro nutrient supplementations are recommended according to need and nutritional status.

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