Student thesis

NTNU
Norwegian University of Science and Technology
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Department of Clinical and Molecular Medicine

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Prevalence of anemia and iron deficiency among women in rural Nepal

A cross-sectional cohort study

Student thesis in Profesjonsstudium i medisin
Supervisor: Unni Syversen
Co-supervisor: Kamilla Stunes
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Abstract

Objective: There are few data on the prevalence of anemia among women in rural Nepal, and the study aimed to expand the knowledge on the subject. We also wanted to examine the frequency of iron deficiency, as well as study the risk factors associated with anemia.

Methods: Data were collected in 2019-2020 from non-pregnant, married women in Bolde. The women were residing at an altitude of about 1890 meters above sea level. To adjust for altitude, we used a cut-off of 12.7 g/dL for hemoglobin instead of 12.0 g/dL to define anemia, as recommended by WHO. Ferritin, iron and TIBC were analyzed, and transferrin saturation was calculated to assess iron status.

Results: Altogether, 714 women aged 21-82 years were included. The median hemoglobin was 14.4 g/dL (10.4-18.2 g/dL). Most of the study population belong to the Adhivasi/Janajati ethnicity (82.8%), had a monthly income less than NPR 24 000 (65.7%) and did not have any formal education (84.9%). The prevalence of anemia was 5.7%, and the frequency varied from 5.4% to 6.1% among postmenopausal women and women of reproductive age respectively. Women aged 21-30 displayed the highest prevalence of anemia (13.2%), but the difference was not significant. Median iron level was 110 µg/dL (15-301 µg/dL), and the median transferrin saturation was 27.1% (3.9-79.4%). Based on ferritin levels of ≤15 ng/mL and ≤30 ng/mL, 4.1% and 18.3% had iron deficiency. Among those with anemia, 20% had iron deficiency anemia. We found that 7% of the population exhibited hemoglobin levels above the upper reference range (>16 g/dL).

Conclusion: The prevalence of anemia among women in rural Nepal was low compared to the nationwide rate, however in line with previous studies from the same area. Furthermore, we found the frequency of iron deficiency anemia to be proportionally lower compared to other studies from the region.

Abstrakt

Mål: Det er lite kunnskap om prevalens av anemi blant kvinner i rurale Nepal, og denne studien ønsket å utvide kunnskapsgrunnlaget. Vi ville også undersøke forekomsten av jernmangel.

Metode: Data ble samlet inn i 2019-2020 fra ikke-gravide, gifte kvinner i Bolde. Kvinnene bodde på 1800 meters høyde over havet. Som anbefalt av WHO, brukte vi grenseverdi på 12.7 g/dL for hemoglobin, i stedet for 12.0 g/dL for å definere anemi. For å finne jernstatus hos kvinnene brukte vi markørene ferritin, jern og TIBC, i tillegg til å kalkulere transferrinmetning.

Resultater: Totalt bestod studien av 714 kvinner fra 21 til 82 år. Median hemoglobin var 14.4 g/dL (10.4-18.2 g/dL). Den vanligste etniske gruppen var Adhivasi/Janajati (82.8%), de fleste hadde månedlig inntekt under NPR 24 000 (65.7%) og manglet formell utdanning (84.9%). Prevalensen av anemi var 5.7%, og varierte fra 5.4% til 6.1% blant henholdsvis postmenopausale kvinner og kvinner i reproduktiv alder. Kvinner fra 21-30 år hadde en høyere prevalens med 13.2%, men forskjellen var ikke signifikant. Median jernverdi var 110 µg/dL (15-301 µg/dL) og median transferrinmetning var 27.1% (3.9-79.4%). Basert på ferritin ≤15 ng/mL og ≤30 ng/mL, hadde henholdsvis 4.1% og 18.3% jernmangel. Blant kvinnene med anemi var det 20% som hadde jernmangelanemi. Vi fant hemoglobinverdier over referanseområdet (>16 g/dL) blant 7.0% av kvinnene.

Konklusjon: Vi fant en lavere forekomst av anemi sammenlignet med landsgjennomsnittet, men funnene våre var sammenlignbare med studier fra samme område. I tillegg fant vi en proporsjonalt lavere forekomst av jernmangelanemi sammenlignet med andre studier fra regionen.

Abbreviations

BMI	Body Mass Index
CI	Confidence Interval
CRP	C-reactive protein
CVD	Cardiovascular diseases
CPI	Corruption Perception Index
DBP	Diastolic blood pressure
DM	Diabetes mellitus
T2D	Type two diabetes
EPO	Erythropoietin
FCHVs	Female community health volunteers
FPG	Fasting plasma glucose
Hb	Hemoglobin
HIV	Human immunodeficiency virus
IQR	Interquartile range
NCDs	Non-communicable diseases
NDHS	Nepal Demographic and Health Survey
NPR	Nepalese rupees
OGTT	Oral glucose tolerance test
OR	Odds Ratio
REK	Regionale komiteer for medisinsk og helsefaglig forskningsetikk
SBP	Systolic blood pressure
S.D.	Standard deviation
TIBC	Total iron binding capacity
WC	Waist circumference
WHO	World Health Organization
YLD	Years lost to disability

1 Introduction

1.1 Background

1.1.1 Nepal

Nepal is a country with great diversity, in both geography and in culture. In 2020 the country had approximately 30 million inhabitants. There are over 100 different ethnic groups in Nepal due to large immigration, with large differences in economics and culture. Consequently, there are multiple languages, with Nepali as the official language. Around 80% of the population are Hindus, and the second largest religion is Buddhism. Geographically the landscape in the north consists of mainly mountains with several glaciers and the climate is cold, whereas the climate in the south is subtropical. The plain landscape in the southern part of the country is widely used for agriculture (1).

Nepal is a republic since 2008 but has big issues with corruption. According to the Corruption Perceptions Index 2022, by Transparency International, Nepal is ranked as number 110 out of 180 countries from the least to the most corrupted (2). On the Human Development Index Nepal is ranked as number 141 out of 188 countries. Approximately half of the population lacks access to electricity and running water. As one of the poorest countries in the world, a large portion of the population only eats what they grow themselves. Hence malnutrition is a big health problem. Gender inequality makes women's rights limited: they are often forced to marry, many do not partake in working life and domestic violence is common (1).

1.1.2 Women's health

Over the last decades, the global burden of diseases has shifted from communicable to non-communicable diseases (NCDs), also known as the epidemiological transition. NCDs include among others cardiovascular diseases (CVD), obesity, diabetes, cancer, dementia, respiratory diseases, musculoskeletal disorders, and depression. NCDs give rise to the majority of the mortality and disabilities amongst women and are according to WHO responsible for 65% of all female deaths. In developing countries women are especially at risk due to low socio-economic, political, and legal status. Women constitute sixty percent of the world's poor population and two-thirds of the world's illiterate adult population. Twice as many women suffer from malnutrition compared to men (3).

1.1.3 Prevalence of anemia

Anemia is a major public health problem, affecting one third of adults and almost two billion people worldwide (4). WHO estimates that 42% of children less than 5 years of age and 40% of pregnant women worldwide are anemic (5). I In low-income and middle-income countries, the etiology of anemias is typically classified into three broad categories: infectious diseases, including malaria, tuberculosis, HIV and parasitic infections; nutritional deficiencies; and genetic hemoglobin disorders (6). The most common nutritional deficiency causing anemia is iron deficiency, followed by deficiencies in folate, vitamins B12 and A (5). Iron deficiency accounts for more than 60% of all cases of anemia (4), and was associated with roughly 120,000 preventable deaths in 2010, two thirds of them among women (7). Furthermore, more than 5% of all years lost to disability (YLD) globally is due to iron deficiency. In 2012, South Asia accounted for 38% of the entire world's YLD associated with anemia (4), (8). Despite decades of economic growth in this region, there has been little progress in resolving this problem.

According to the 2016 Nepal Demographic and Health Survey (NDHS), the prevalence of anemia in children aged 6-59 months (Hb < 11.0 g/dL) was 53% (9). This included 26% with mild anemia, 26% with moderate anemia, and 1% with severe anemia. There was an increase in the total prevalence of anemia, from 46% in 2011 to 53% in 2016. The study also observed anemia to be more frequent among the rural population (56%) compared to the urban citizens (49%). Additionally, the study emphasized the significance of a mother's education in relation to children's anemia. The NDHS 2016 showed that 41% of women aged 15-49 were anemic. Furthermore, 34% of these women were mildly anemic, 7% were moderately anemic and less than 1% severely anemic. Similar to anemia in children, the prevalence also increased slightly among women from 35% in 2011 to 41% in 2016. Anemia occurred more frequently among younger women, and the highest risk was seen among pregnant and breastfeeding women (9). According to a study from 2018 the overall prevalence of anemia among 3780 adolescents (10-19 years old) was 31% (10). This study included both male and female participants, with a significantly larger percentage among female participants (38%) compared to men (24%). The study pointed out eating habits, bad sanitation, and lack of iron as important reasons for the high prevalence. Several other studies support these numbers; hence one can conclude that anemia in Nepal is a health issue to be taken seriously (11, 12). All the above-mentioned studies followed WHO recommendations concerning definition of anemia, using a 12.0 g/dL cutoff for hemoglobin at sea level, adjusting for altitude when appropriate.

1.1.4 Consequences of anemia

Anemia is an indicator of both poor nutrition and poor health. Children and pregnant women with anemia are especially vulnerable. Anemia during pregnancy may among others cause suboptimal growth of the offspring, as well as impaired learning (13). This is of concern, as anemia is prevalent among young children and women of childbearing age in low-income countries. Anemia gives rise to symptoms such as fatigue, weakness, dizziness, and drowsiness. Iron deficiency anemia has also been shown to affect cognitive and physical development in children and to reduce productivity in adults (5). Moreover, due to lack of energy, individuals with anemia may also be more prone to overweight and obesity.

1.2 Diagnostic criteria of anemia and iron deficiency

1.2.1 Definition of anemia

WHO defines anemia as hemoglobin less than 12.0 g/dL for non-pregnant women, and less than 11.0 g/dL for pregnant women (14). Given that people living at high altitudes display higher hemoglobin levels, WHO has recommended to adjust for altitude by increasing hemoglobin cut-off by 0.1 g/dL at 1000 meters above sea level, and 0.3 g/dL per 500 meters for altitude above this (15). The inhabitants of Nepal population live at altitudes varying from 0 to approximately 5100 meters above sea level. The population in our study resided at about 1890 meters above sea level (16). Accordingly, we defined anemia as hemoglobin < 12.7 g/dL as recommended by WHO. Unless otherwise stated, this correction is used throughout the manuscript.

The human body has several ways of adapting to the lower oxygen levels at high altitude. Short time adaptation consists of increased ventilation and cardiac output, enhanced oxygen extraction from capillary blood and changes in the distribution of blood flow (15). Within days of arrival to higher altitude, the plasma volume decreases, leading to an increased hemoglobin level in arterial blood. Over time, weeks to months, the body will adapt by promoting a slow increase in hemoglobin. The process entails an increase in erythropoietin (EPO) production in the kidneys, followed by a rise in erythropoiesis and hemoglobin level in the blood.

Polycythemia

Polycythemia, or erythrocytosis is defined as an increase in the absolute red blood cell (RBC) mass, and the etiology varies (17). We used hemoglobin, and not RBC, to define both anemia and polycythemia in our study. Hemoglobin is measured in a blood sample containing erythrocytes that are lysed and hemoglobin is dissolved in serum of the same sample. The hemoglobin value is the concentration of hemoglobin in this solution and is mainly affected by two factors, the number of erythrocytes and the volume of plasma in the sample. This means that the hemoglobin value measured could be falsely increased. Spurious polycythemia is due to low plasma volume and can be a chronic situation or occur transiently due to dehydration. True polycythemia (increased production of red cells) can be separated into two groups depending on serum EPO levels. Low serum EPO level, primary polycythemia, indicates polycythemia vera or primary familial and congenital polycythemia. High serum EPO levels indicates a secondary polycythemia which can occur due to high altitude and respiratory disorders such as COPD. Secondary polycythemia is also seen in cyanotic heart diseases, renal disorders, elevated carboxyhemoglobin as can be seen in smokers, people exposed to carbon dioxide in closed rooms, hemoglobinopathies and EPO-secreting tumors. Iatrogenic causes include both use of anabolic steroids and testosterone replacement therapy (17).

1.2.2 Iron deficiency

Ferritin is commonly used to diagnose iron deficiency anemia because it is the most feasible measure. However, ferritin is an acute-phase protein and will therefore increase due to inflammation, which in turn could lead to an underestimation of iron deficiency (18). To rule out iron deficiency in the case of normal or high ferritin levels, CRP and transferrin saturation should be analyzed. Regarding ferritin, a cutoff of 30 ng/mL ensures a high sensitivity as well as specificity within a normal population (19). Existing literature is, however, not consistent, and many use 15 ng/mL as a cutoff for empty iron storage (20). We will therefore use both cut-offs in our analyses.

Transferrin saturation is considered as a valuable tool to evaluate the iron status, as it takes both plasma iron and its main transport protein into account (21). Transferrin saturation is calculated using the formula iron/TIBC*100 and given in percentage (22). It is less affected by inflammation, although it tends to be slightly decreased during inflammation as well as if estrogen levels rise, due to pregnancy or use of contraceptive pills. Transferrin saturation

<16% is commonly used as cut-off for iron deficiency, while increased values of transferrin saturation, > 45%, is a sign of hemochromatosis or transfusion hemosiderosis (23). In this study we used both ferritin and transferrin saturation to assess iron deficiency. Iron deficiency is the most common cause of anemia.

2 Aims

As previously mentioned, studies have shown that anemia is a growing problem in Nepal. In this thesis we want to estimate the prevalence of anemia and iron deficiency among women in a rural community of Nepal, as the knowledge on the subject is limited. Our study may provide new knowledge and competence that may be applied to develop interventional strategies and health policies.

Aims of the study:

1) Assess the prevalence of anemia (hemoglobin) among women in rural Nepal
2) Examine the prevalence of iron deficiency
3) Study risk factors associated with anemia

3 Methods/methodology

3.1 Study method and participants

This project is part of a large cross-sectional study among women in a rural district of Nepal s entitled “Early onset and increasing burden of diabetes in Nepalese women. Risk factors, complications, and relation with vitamin A and D. A prospective cohort study in rural Nepal” (24). The women were included in this study from September 2019 till January 2020. They were recruited from the participants in a previous study conducted in 2012-2013, where the main objectives were: 1) Cervical cancer and prevalence of sexually transmitted infections, and 2) Prevalence of NCDs with focus on obesity and diabetes. The intention of the current study was originally to follow up progression of diabetes, complications and risk factors (24). However, whereas approximately 1500 women participated in the original study, only about 800 women responded to the invitation in the current study. This could partly be attributed to the fact that some of the women had died during the earthquake in 2015, and some had moved to another region.

Married and nonpregnant women were included in the study, using the same inclusion criteria as in the previous. In the original study women above 15 years of age were included, the second data collection they were obviously 6-7 years older. Both studies excluded women with physical or mental disturbances that made participation difficult (24).

The recruitment of women in this study was led by female community health volunteers (FCHVs), selected by the government to carry out preventive health initiatives. After being informed about the study´s purpose and procedures, FCHVs visited women from the first study in 2012-2013, informed them about the new study and invited them to participate (24).

3.2 Data collection

Data collection was done in Bolde and the neighboring villages. This site was chosen because of its readily access to one of the outreach centers of Dhulikhel Hospital and possibility to transport the biological samples to the central laboratory at Dhulikhel Hospital during the day. One-day screening sites that were prepared at health centers, local schools, and village halls.Data were collected using Open data kit (ODK) free software (aggregate V1.4.11, University of Washington, Seattle, WA, USA) in three stages: interviews, physical measurements and collection of blood samples. A standardized questionnaire addressing sociodemographic and lifestyle factors was used by trained health workers. Anthropometrics (weight, height, waist circumference) and blood pressure were measured before blood samples were collected. Body weight was measured in kilograms using a portable digital weighing scale (secca220, Hamburg Germany) standing barefoot. Height was measured in cm using a stadiometer attached to the wall Body mass index (BMI) was calculated as weight in kilogram per square of height in meter and categorized according to Asian cut-off values as underweight (< 18.5 kg/m^2), normal weight (18.5 -22.9 kg/m^2), overweight (23.0 -27.4 kg/m^2) and obese (> 27.5 kg/m^2) (25). In standing position, the waist circumference was measured in cm using a non-stretchable measuring tape between last palpable rib at midaxillary line and top of the iliac crest at the end of a natural expiration. Blood pressure was measured two times on the left arm in a sitting position, at the mid and end of the interview, using a digital device (omron-5 series digital blood pressure monitor). An average reading of the blood pressure was used for the analysis (26).

3.3 Blood sample collection and analyses

The blood samples were collected from a cubital vein while fasting. Whole blood was sampled and kept on ice under transportation to the Department of Biochemistry, Dhulikhel hospital (DH), Kathmandu University hospital. Hemoglobin levels were measured using colorimetric analyses by an automated analyzer coulter counter. The reference range was 12.0-16.0 g/dL for women of age 18 to 74 years. The remaining blood underwent centrifugation before being stored for 3-4 hours at the study site, first at 2 to 8 °C and later at −30 °C. Thereafter, sera were transported to DH for storage at -80 °C and further analyses. Ferritin was measured using chemiluminescent imunoassays, while TIBC and iron were measured using colorimetric analyses. CRP was analyzed with immunoassay. For CRP the reference range was below 5 mg/l. For TIBC from 265 to 497 ng/dL, for ferritin reference range was 4.0-104.2 ng/dL for women below 45 years of age and 4.9-232.3 ng/dL for women older than 45 years, and for iron the reference range was 40.0-255 µg/dL for women aged 25 years, 35-168 µg/dL for women aged 40 years and 40-120 µg/dL for women aged 60 years. This is summarized in table 1 (26).

Table 1. *Reference range and methods.*

Test	**Unit**	**Reference range**	**Method**
Hb	g/dL	Females: 12.0-16.0 (Adult aged 18-74 years)	Colorimetric analyses
CRP	mg/L	<5.0	Immunoassay
TIBC	ng/dL	Females: 265-497	Colorimetric
Ferritin	ng/dL	Females: < 45 yrs (4.0-104.2), > 45 yrs (4.9-232.3)	Chemiluminescent Imunoassays
Iron	µg/dL	Females: (25 yrs) 40.0–255.0 (40 yrs): 35.0-168.0 (60 yrs): 40.0–120.0	Colorimetric

3.4 Statistical analysis

The analyses of this study were performed using IBM SPSS Statistics version 29. Normally distributed variables are presented with mean and standard deviation, while non-normally distributed variables are presented with median with inter quartile range (IQR). Categorical variables are presented as counts and percentages. For normally distributed data we used a two-tail independent sample T-test. For non-normally distributed data we used Mann-Whitney-U-test. To examine the association between outcome variables and covariates we used binary logistic regression, and the association is presented as crude odds ratio (OR) and adjusted odds ratio (AOR) (adjusted for BMI and age) with 95% confidence interval (CI).

4 Ethical considerations

The project "Early onset and increasing burden of diabetes in Nepalese women. Risk factors, complications, and relation with vitamin A and D. A prospective cohort study in rural Nepal" was approved by REK Midt-Norge (13003), May 2019, The National Health Research Council, Nepal (2715) May 2019, and Kathmandu University School of Medical Sciences (124/19), May 2019. The participants were informed about the purpose, procedures and that they could withdraw from the study at any time without consequences. Informed consent was obtained from those who agreed to participate by either a signature or a thumb print.

5 Results

5.1 Characteristics of the study population

Altogether, 813 women were included in the study. However, not everyone completed all steps with standardized questionnaires, anthropometrics, and blood samples. These were excluded from the study, and in total 714 women aged 21 to 82 years were included in the analyses.

Most of the women were aged 31-50 years (56.9%). The women belonged to three ethnic groups, with 82.8% among the Adhivasi/Janajati/other ethnicity, 12.3% belonged to Brahmin/Chhetri and 4.9% to Dalit/lower cast. Most women did not have any formal education (84.9%) and 65.7% had monthly income less than NPR 24 000. Previous smoking

was reported by 15.6% (N=111), current smoking by 20.1% (143). Almost one third of the population (29.1%) reported drinking alcohol previously or currently, most of them with current alcohol consumption. The median BMI was 24.2 kg/m2, with 5.7% of the population being underweight (BMI<18.5), 32.6% being normal weight (BMI 18.5-22.9) and 61.7% being overweight (BMI≥23). Median hemoglobin was 14.4 g/dL (10.4-18.2), median ferritin was 69.7 ng/mL (3.9-647.2), median iron was 110 µg/dL (15-301), and median TIBC was 406 µg/dL (203-672). Transferrin saturation was calculated to be 27.1 (3.9 –79.4) and the median CRP was 2.3 mg/L (0.1-70.8).

Table 2*. Characteristics of the study population.*

Characteristics	N (%) or mean +/- S.D. or median (IQR).
Overall	714
Age (years)	47.6 ± 12
Age groups (years)	
21-30	38 (5.3%)
31-40	184 (25.8%)
41-50	222 (31.1%)
51-60	162 (22.7%)
≥ 61	108 (15.1%)
Ethnicity	
Brahmin/Chhetri	88 (12.3%)
Adhivasi/Janajati/ Others	591 (82.8%)
Dalit/lower cast	35 (4.9%)
Level of education (n=700*)	
No formal education	594 (84.9%)
Secondary or lower	81 (11.6%)
Upper secondary school and above	25 (3.6%)
Monthly household income (n=708)**	
< NPR 24 000	465 (65.7%)
> NPR 24 000	243 (34.3%)
Currently smoking (n=713*)	

Yes	143 (20.1%)
No	570 (80%)
Alcohol consumption (n=712)**	
Never	505 (70.1%)
Past/current	207 (29.1%)
BMI (kg/m2) (n=692*)	24.2 (14.3-41.7)
SBP (mmHg) (n=692*)	123 (88–233)
DBP (mmHg) (n=692*)	81 (51-124)
WC (cm) (n=692*)	78 (55-115)

Abbreviations: NPR: Nepalese rupees (24 000 NPR = approximately 180 USD), WC: waist circumference, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure.

**Number not equal to n=714 due to no answer/missing data*

***Number not equal to n=714 due to inconclusive answer*

***Table 3**. Biochemical parameters of the study population.*

Characteristics	Median (IQR)
Hb (g/dL) (n=692*)	14.4 (10.4–18.2)
Iron (µg/dL) (n=692*)	110.0 (15.0-301.0)
TIBC (µg/dL) (n=692*)	406 (203 – 672)
Ferritin (ng/mL) (n=692*)	69.7 (3.9 – 647.2)
CRP (mg/L) (n=692*)	2.3 (0.1–70.8)
Transferrin (%)	27.1 (3.9 –79.4)

Abbreviations: Hb: hemoglobin, TIBC: total iron binding capacity, CRP: C-reactive protein

**Number not equal to n=714 due to no answer/missing data*

5.2 Prevalence of anemia

The prevalence of anemia was 5.7% (N=41). Hemoglobin levels above reference range (>16 g/dL) occurred in 7.0% (N=50) of the women. Figure 1 demonstrates the distribution of hemoglobin levels among our study population.

Figure 1. *Prevalence of anemia, non-anemia, and hemoglobin above reference level in the total population.*

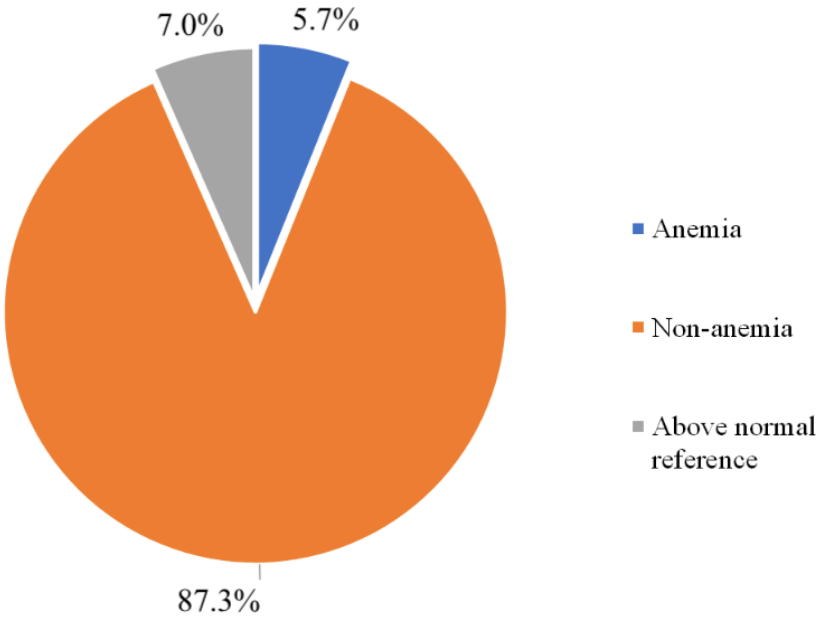


Table 4 shows the overall prevalence of anemia and the prevalence according to characteristics of the population. Anemia was most prevalent in the youngest and oldest age groups, 21-30 years and ≥ 61 years, but not significantly. There was no significant difference in prevalence of anemia with respect to ethnicity, monthly income, smoking or alcohol consumption, although the prevalence was slightly higher in the Adhivasi/Janajati ethnicity group, as well as among former smokers.

Table 4. *Prevalence of anemia by sociodemographic and lifestyle factors.*

N (%)

Characteristics	**N (%)**	**Anemia**		
		Yes	**No**	**p-value**
Overall	714 (100)	41 (5.7)	673 (94.3)	
Age groups (years)				0.098
21-30	38 (5.3)	5 (13.2)	33 (86.8)	
31-40	184 (25.8)	12 (6.5)	172 (93.5)	
41-50	222 (31.1)	9 (4.0)	213 (96.0)	
51-60	162 (22.7)	6 (3.7)	156 (96.3)	

≥ 61	108 (15.1)	9 (8.3)	99 (91.7)	
Ethnicity				0.310
Brahmin/Chhetri	88 (12.3)	3 (3.4)	85 (96.6)	
Adhivasi/Janajati	591 (82.8)	38 (6.4)	553 (93.6)	
Dalit/lower cast	35 (4.9)	0	35 (100)	
Level of education				0.659
No formal education	594 (84.9)	34 (5.7)	560 (94.3)	
Secondary or lower	81 (11.6)	5 (6.2)	76 (93.8)	
Upper secondary school	25 (3.6)	2 (8.0)	23 (92.0)	
Monthly household				0.511
< NPR 24 000	465 (65.7)	26 (5.6)	439 (94.4)	
> NPR 24 000	243 (34.3)	14 (5.8)	229 (94.2)	
Currently smoking				0.651
Yes	143 (20.1)	6 (4.2)	137 (95.8)	
No	570 (80.0)	35 (6.1)	535 (93.9)	
Alcohol consumption				0.892
Never	505 (70.9)	30 (5.9)	475 (94.1)	
Past/current	207 (29.1)	11 (5.3)	196 (94.7)	

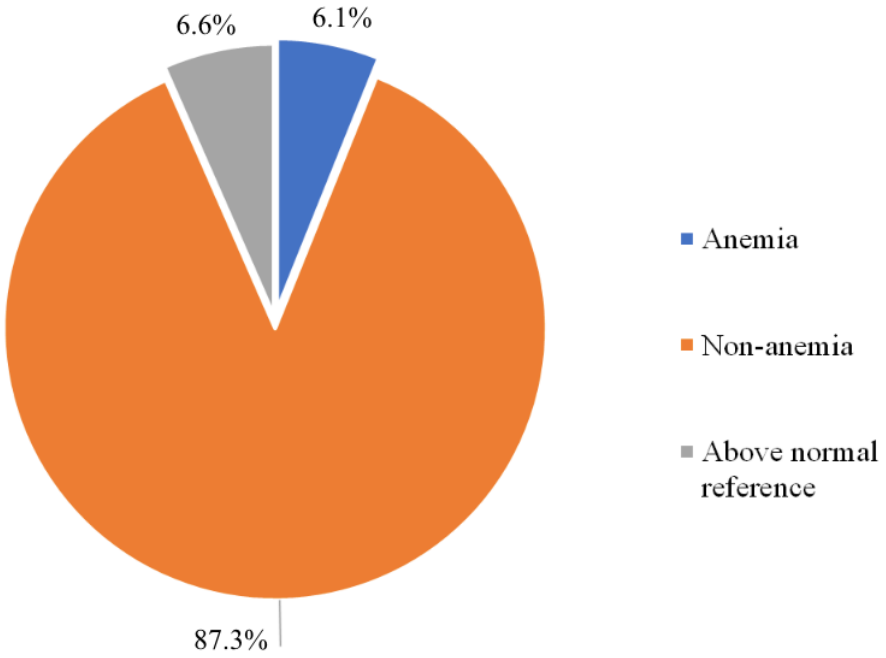
**Number not equal to n=714 due to no answer/missing data*

***Number not equal to n=714 due to inconclusive answer*

5.2.1 Menopause

Altogether 708 women answered questions about menopause. Among them, 332 (46.9%) women were postmenopausal at the time of the study, while 376 (53.1%) women were still in their reproductive age. The median age of menopause was 48 years (29-70 years). The prevalence of anemia was 6.1% among women of reproductive age compared to 5.4% among postmenopausal women. Median hemoglobin level was 14.5 (11.3–18.2) g/dL in postmenopausal women and 14.3 (6.8–17.4) g/dL in women of reproductive age (p = 0.308).

Figure 2. *Prevalence of anemia, non-anemia, and hemoglobin above reference level among women of reproductive age*



5.3 Biochemical parameters stratified by anemia yes or no

Table 5 shows specific covariates in the different groups stratified by anemia yes or no. As observed in table 5, we found significant p-values regarding iron levels, ferritin levels and TIBC. We found a significantly higher level of iron and TIBC among anemic women, while ferritin levels were significantly higher among non-anemia women. We found no significant differences concerning waist circumference, systolic blood pressure, diastolic blood pressure and BMI.

Table 5. *Biochemical parameters stratified by anemia yes or no presented as mean +/- S.D or median (range).*

Characteristics	**Anemia**		
	Yes	**No**	**p-value**
CRP (mg/L)	2.4 (0.1–32.5)	2.3 (0.1–70.8)	0.317
Iron (µg/dL)	76.4 ± 30.5	112.0 (15.0–301.0)	**<0.001**
Ferritin (ng/mL)	55.0 (3.8–322.4)	70.5 (3.9–647.2)	**0.022**
TIBC (µg/dL)	353 (272–576)	408 (203–672)	**0.002**

Abbreviations: Hb: hemoglobin, CRP: C-reactive protein, TIBC: total iron binding capacity

5.4 Iron deficiency

5.4.1 Ferritin

Iron deficiency was seen in 18.3% (N=131, total N=714) using 30 ng/mL as a cut-off for ferritin. When applying 15 ng/mL as the cut-off, 4.1% (N=29) had iron deficiency. Among the women with ferritin levels below 30 ng/mL only 3.8% (N=5) had anemia, and among the women with anemia only 29.4% (5) had iron deficiency.

We found a higher median ferritin level in women with CRP >5 compared to those with CRP<5. The median ferritin level was 92.4 (10.9 – 473.6) and 66.2 (3.8 – 647.2) respectively (p-value = 0.003). Median CRP among women with ferritin values below 15 was 1.7 (0.1–8.9), and the median value of CRP in the group of women with ferritin values above 15 was 2.3 (0.1–70.8) (p-value = 0.45).

5.4.2 Transferrin saturation

Altogether, the median value of transferrin saturation in the total population (n=713) was 27.1% (3.9-79.4). We found 9.9% (N=70) of the women to have transferrin saturation < 16%, implying iron deficiency. On the other hand, we observed 5.5% (N=39) with increased transferrin saturation > 45%, implying hemochromatosis or transfusion hemosiderosis. Among women with CRP values below and above 5 mg/L the median transferrin saturation was 28.1% (5.0-79.4) and 24.6% ± 8.8 respectively (p-value = <0.001). Among women with ferritin values below 15 ng/mL, the mean transferrin saturation value was 17.7±10.4, as opposed to 27.6 % (3.9-79.4) for women with ferritin values above 15 ng/mL (p-value = <0.001). Mean transferrin saturation was 22.2% ±10.2 for the anemic women, compared to 27.6% (3.9-79.4) for the non-anemic women (p-value = <0.001).

5.4.3 True iron deficiency anemia

In total we found 169 (23.7%) women with either ferritin levels below 30 ng/mL or transferrin saturation below 16% implying possible iron deficiency anemia. There was 32 (4.5%) women with both ferritin levels below 30 ng/mL and transferrin saturation below 16%. Among those with anemia, 20% had iron deficiency anemia. In the group of women with transferrin saturation and ferritin within reference range 25 (4.9%) had anemia.

5.5 Associated risk factors

Linear regression was performed to measure association between hemoglobin and sociodemographic characteristics, lifestyle factors, anthropometrics and SBP. To examine the association between anemia and the abovementioned covariates we used binary logistic regression, and the association is presented as odds ratio (OR) with 95% confidence interval (CI). The odds for anemia decreased in the age-group 41 to 60 displaying the low odds COR 0.279 (95% CI: 0.088, 0.883) and 0.254 (95% CI: 0.073, 0.881) compared to the 21-30 group. For the remaining varieties all the AOR 95% CI crossed 1 meaning we cannot say with confidence that the effect is either increased or decreased probability. In addition, no p-value was significant.

Table 6. *Factors associated with anemia in the study population*

Characteristics	**COR (95% CI)**	***p* value**	**AOR (95% CI)**	***p* value**
Age groups				
21-30 (n=38)	1			
31-40 (n=173)	4.60 (0.152, 1.394)	0.170		
41-50 (n=207)	0.279 (0.088, 0.883)	**0.030**		
51-60 (n=149)	0.254 (0.073, 0.881)	**0.031**		
>60 (n=97)	0.600 (0.188, 1.918)	0.389		
Menopause				
No (n=376)	1.137 (0.602, 2.145)	0.693	1.128 (0.584, 2.178)	0.720
Yes (n=332)	1		1	
Education				
No formal education (n=593)	1		1	
Secondary or lower (n=46)	0.727 (0.170, 3.117)	0.668	0.750 (0.174, 3.224)	0.699
Upper secondary school and above (n=25)	1.391 (0.316, 6.128)	0.662	1.463 (0.329, 6.506)	6.17
Income				
<24 000 (n=440)	1		1	
>=24000 (n=224)	1.032 (0.529, 2.015)	0.926	0.960 (0.484, 1.905)	0.907
Smoking				

No (n=534)	1		1	
Yes (n=130)	0.669 (0.276, 1.624)	0.375	0.663 (0.270, 1.628)	0.370
Alcohol				
Never (n=474)	1		1	
Yes (n=190)	0.889 (0.437, 1.809)	0.745	0.914 (0.448, 1.868)	0.806
SBP				
<140 mmHg (524)	1		1	
>140 mmHg (140)	0.515 (0.198, 1.337)	0.173	0.422 (0.147, 1.210)	0.109
WC				
<80 (n=370)	1		1	
>=80 (n=294)	1.376 (0.732, 2.586)	0.322	1.790 (0.790, 4.058)	0.163
BMI				
<18.5 (n=40)	0.697 (0.154, 3.157)	0.640	0.732 (0.149, 3.600)	0.701
18.5-22.9 (n=228)	1		1	
>22.9 (n=431)	0.713 (0.367, 1.386)	0.318	0.664 (0.240, 1.834)	0.429
CRP				
< 5 (n=544)	1		1	
>5 (n=120)	1.472 (0.703, 3.083)	0.306	1.500 (0.713, 3.156)	0.285

Abbreviations: COR, crude odds ratio; AOR, adjusted odds ratio for age and BMI; menopause, education, income, smoking, alcohol, SBP, WC, BMI, CRP, HbA1C.
The odds of anemia decreased with age compared with the 21-30 group.

6 Discussion

6.1 Main findings

In this large, comprehensive study among women in a rural district of Nepal, we observed a low prevalence of anemia. The women were living at an altitude of 1890 meters above sea level, hence the cut-off to define anemia was adjusted for altitude as recommended by WHO. The median hemoglobin level was 14.4 g/dL. We observed a prevalence of anemia of 5.7% in the total population, and 6.1 and 5.4% among women of reproductive age and postmenopausal women, respectively. Anemia occurred in 13.2% of the youngest women and 8.3% of the oldest women. Former smokers also displayed a higher prevalence, however, non-

significant. We found no significant differences between different ethnicities, or association with monthly income, level of education, or alcohol consumption. Altogether, 7.0% of the women had hemoglobin levels above the upper reference level of 16 g/dL. The prevalence of iron deficiency was 18.3% using ferritin < 30 ng/mL as cut-off, and 4.1% using ferritin < 15 ng/mL as cut-off, while the prevalence was 9.9% using transferrin saturation below 16% as criterion. Among women with anemia 20% had iron deficiency anemia.

6.2 Comparison with previous studies

6.2.1 Prevalence of anemia

We observed a substantially lower prevalence of anemia compared to previous studies in Nepal, which have reported prevalence ranging from 0% to 41% with considerable variability between different districts of Nepal (9, 10, 12, 27-29). In a nationwide study addressing adolescent females 10 to 19 years (N=3780), the prevalence was 38% (10). A similar prevalence of 41% was observed among women across the country aged 15-49 years (N=12 476) (9). The discrepancy in prevalence between the studies could partly be attributed to the altitude of the study sites. The two latter studies used WHO's recommended adjustment for altitude. Given that the altitude at our study site was about 1890 meters above sea level, we corrected the cut-off point for hemoglobin according to WHO. After adjustments for altitude, the prevalence of anemia was 5.7% in the total population, versus 2.4% when using conventional cut-off (Hb<12.0 g/dL). The frequency of anemia was 6.1% and 5.4% among women of reproductive age and postmenopausal women respectively. A study from Bhaktapur (2006) found the prevalence to be 12 % and 16% before and after adjusting for altitude, among women aged 13-35 without ongoing infections (28). Another study from the same area from 2014 reported a prevalence of 20% after adjusting for altitude (Hb<12.3 g/dL) among 500 lactating women (27). It is evident that the distribution of anemia is uneven across the country, with the highest prevalence in Terai regions close to the Indian border (10, 29). According to data from the 2016 Nepal Demographic and Health Survey anemia was less prevalent in most of the hilly regions and varied from 0-19.9% around our study site (29). Accordingly, our findings are in line with previous studies.

The prevalence of anemia varied from 5.4% to 6.1% between postmenopausal women and women of reproductive age respectively. We found the mean age of menopause to be 46.2 ± 7.7 years. This is comparable to existing literature, such as a study from 2021 in rural Nepal

which observed the mean age at menopause to be 47.2 ± 6.2 (30). Another study from the Kaski district in the western part of Nepal found the mean age to be 49.9 ± 5.6 (31). However, it is worth noting that this is lower than in European countries such as Norway (51.2 years) and Italy (51.2 years) (32, 33). The reason for the low prevalence of anemia in our study population could partly be because the women were healthy when selected for the study, which we also observed with regards to diabetes where the prevalence was found to be only 4.5%. The findings could also be explained by underlying diseases or dehydration, but as our results are in accordance with previous studies from the area this is less likely.

Polycythemia

We found that 7.0% of women had hemoglobin levels above the upper reference range (> 16 g/dL) compatible with polycythemia vera. There could be several factors contributing to this – such as dehydration, high altitude, respiratory diseases, cyanotic heart diseases, renal disorders, elevated carboxyhemoglobin as can be seen in smokers, people exposed to carbon dioxide in closed rooms, hemoglobinopathies and EPO-secreting tumors. In a study from Saudi-Arabia they observed that the mean hemoglobin concentration was 14.2 g/dL for women residing at about 1800 meters above sea level (34). This is similar to the altitude conditions in our study population, and we observed the median hemoglobin level to be 14,4 g/dL. Hence it is reasonable to believe that altitude is an important factor contributing to the high levels of hemoglobin in our study population.

6.2.2 Iron deficiency and anemia

Data on the prevalence of iron deficiency in Nepal are diverging. According to a study from Bhaktapur from 2014 the prevalence was 5% (ferritin ≤ 15 ng/mL) (27). This is comparable to our findings with a prevalence of 4.1% using the same cut-off. Another study from 2006 observed a prevalence of 20% (28). Both studies concluded that the prevalence of true iron deficiency anemia (defined as a condition with both anemia, low ferritin, and increased transferrin receptors) was lower than the prevalence of anemia, but still the major reason for anemia. This was not the case in our population, where only 20% of the women with anemia had iron deficiency anemia. When examining the ferritin levels among women with CRP > 5 mg/L compared to those with CRP < 5 mg/L we found the median ferritin to be higher: 92.4 mg/L (10.9 –473.6) and 66.2 mg/L (3.8 –647.2), respectively (p-value = 0.003). This could be explained by inflammation, as ferritin is an acute-phase protein, and could in turn lead to an

underestimation of iron deficiency (18). Iron deficiency was also assessed by calculating transferrin saturation. Based on this we found 9.9% of the women to have transferrin saturation below 16%, implying iron deficiency. There are few studies reporting transferrin saturation in Nepal. In a study of 200 school children aged 6-12 years from Eastern Nepal, the median transferrin saturation was 19.21% versus 27.1% as observed in our study (35). Accordingly, there are no reports on iron deficiency based on transferrin saturation in Nepal.

6.2.4 Influence of smoking and alcohol consumption on hemoglobin

Smoking is associated with elevated hemoglobin levels due to elevated carboxyhemoglobin (17). In our study population we observed 20.1% current smokers, 15.6% past smokers – 35.7% in total. The Nepal Demographic and Health Survey from 2016 observed a prevalence of 8.9% current smokers, with a significantly higher prevalence among women over 40 years of age and in the group with no education (36). Smoking was also more common in the mountain area compared to the hilly and Terai regions. Compared to this we observed a high prevalence of smokers. Smoking could be a contributing factor to the high hemoglobin levels, although we did not find a significant p-value (36). Smoking was also more common in the mountain area compared to the hilly and Terai regions. Compared to this we observed a high prevalence of smokers. Smoking could be a contributing factor to the high hemoglobin levels, although we did not find a significant p-value.

A high consumption of alcohol over time is associated with malnutrition, which is associated with various health issues, among them anemia (37). We observed 29.1% past or current alcohol consumption in our population. A study from 2013 found the national prevalence of alcohol consumption (past or present) among married women of reproductive age to be 24.7% (38). A different study from 2013 observed 11.7% users of alcohol in their lifetime, more among women older than 30 years of age, women from hilly regions and those with no formal education (39). The alcohol consumption in our study population appears to be high. We do not have information about amount and frequency, but it is reasonable to assume that alcohol consumption can contribute to malnutrition, which in turn can lead to anemia. It would be of great interest to follow up the population to observe the possible development of disease.

6.3 Limitations of the study

There are no hemoglobin levels available from our study population from the original data collection in 2012-2013. Due to this it is difficult to determine whether the values among our study population are within their individual normal ranges. Furthermore, we are lacking more blood samples that could be used to determine the cause of anemia and differentiate between different types, and to explore the underlying causes of the high hemoglobin. The lack of differential count of WBC and measurement of platelets makes it challenging to discuss the presence of myeloproliferative diseases, which could be a possible cause for the high hemoglobin values.

With respect to the study population there was a very small percentage representing the Dalit ethnicity (N=35), resulting in a 0% prevalence of anemia in this group. Although it is reasonable that the study population mainly consists of Newar ethnicity as that is also the case in the general population in the area, it would be interesting to examine a larger group of Dalit ethnicity as well to explore whether the prevalence of anemia is different.

Some of the women were reluctant to give blood samples or refused to answer essential parts of the questionnaires, resulting in them being excluded from the study. As we do not have the characteristics of this group, we do not know whether it differentiates from the rest of the study population in any way, enabling exclusion bias.

7 Conclusion

The objective of the study was to estimate the prevalence of anemia among women in a rural community of Nepal, with the purpose of providing new knowledge and competence that may be applied to develop interventional strategies and health policies. We found the prevalence of anemia among women in rural Nepal to be low compared to the nationwide rate, however in line with previous studies from the same area. Furthermore, we observed the frequency of iron deficiency to be proportionally lower compared to other studies from the region. The findings in our study underscore the considerable variation in prevalence of anemia throughout Nepal, highlighting the need for further research on the topic. There are still blood samples from the women in our study available for further analyses. Vitamin A will be analyzed and can bring valuable new insight regarding the etiology of anemia in the population.

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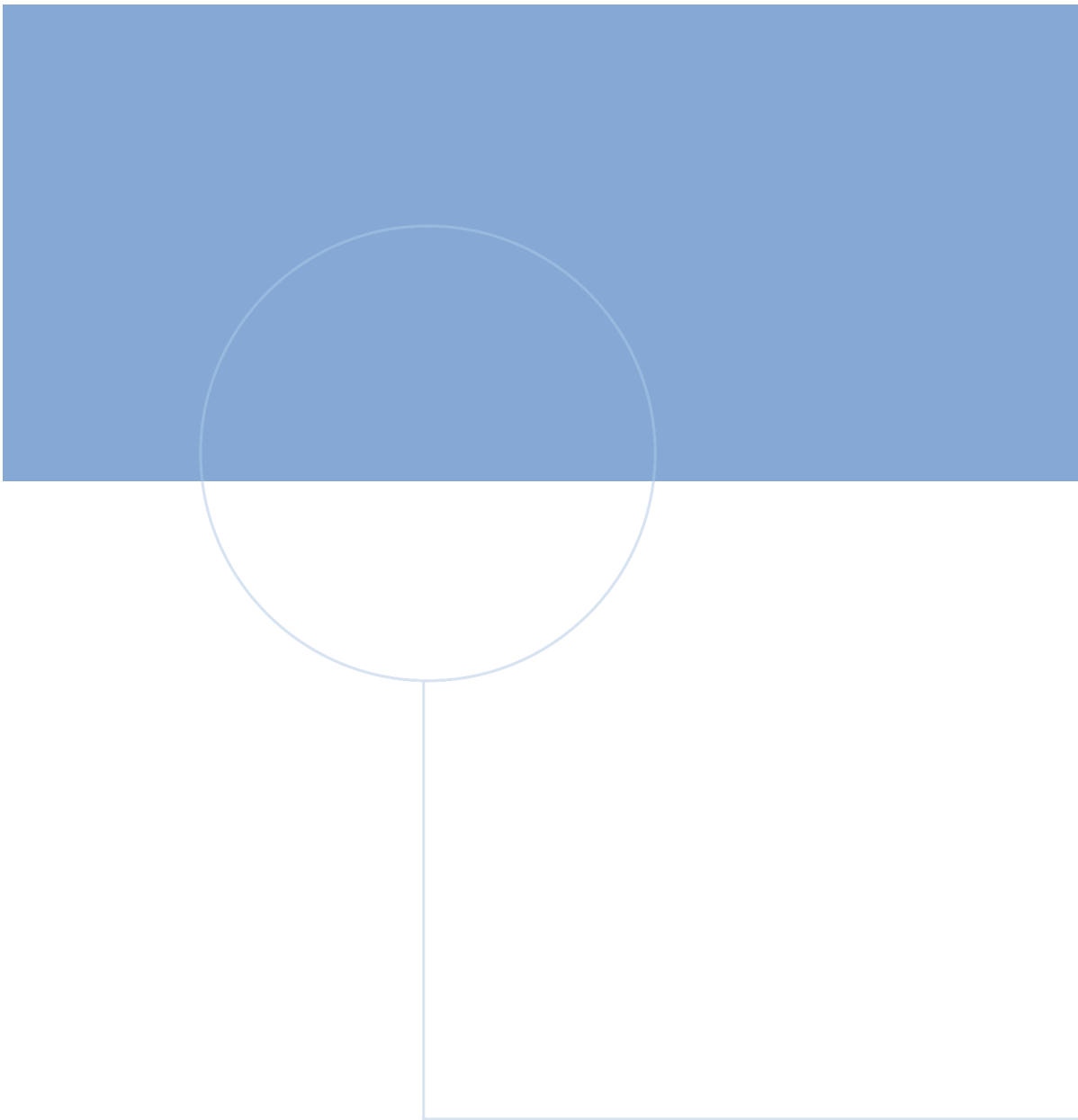

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