Spirulina Supplementation in Pregnant Women in the Dakar Region (Senegal)

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Abstract
The fight against iron deficiency anemia in pregnant women is a public health priority, especially in sub-Saharan Africa. Spirulina is an alga very rich in iron, used as a dietary supplement. This research proposes to study its effect on the hemoglobinemia of pregnant women. This was a blind randomized cohort study with a supplemented group of spirulina (1500 mg/d) and a second supplemented with iron and folic acid supplementation (IFAS). Included in this study were pregnant women attending four health centers in the Dakar region, with pregnancy at 28th amenorrhea, agreeing to participate and not having complications or twin pregnancies. Follow-up of the two groups was identical throughout the study from the 28th week of amenorrhea to the 42nd postnatal day. The study was conducted among 920 women, evenly distributed among the groups. IFAS allowed a statistically significant increase in hemoglobinemia with an average gain of 0.3 g/dl between the 28th and 32nd weeks of amenorrhea (p = 0.022) and 0.2 g/dl between the 28th week and the delivery (p = 0.043). Spirulina supplementation showed statistically higher gains than those observed with IFAS. These gains were 0.48 g/dl between the 28th and 32nd weeks of amenorrhea and 0.36 g/dl between the 28th week and the delivery, with p values less than 0.001. Spirulina, more accessible to developing countries, is a good option to strengthen the arsenal of iron deficiency anemia control, and therefore maternal mortality.

Keywords
Maternal Mortality, Spirulina, Iron, Anemia, Supplementation, Senegal

1. Introduction
According to the World Health Organization, everyday 1500 women die from complications related to pregnancy or childbirth [1]. In 2005, there were an estimated 536,000 maternal deaths worldwide.
Despite several advances in the accessibility and availability of maternal health services during this decade, maternal mortality remains high and it is estimated at 450 maternal deaths per 100,000 live births (LB) in sub-Saharan Africa [1] [2]. In Senegal, there are 392 deaths per 100,000 LB against 723 deaths per 100,000 LB in the region of Kolda where the health situation is unsatisfactory [3].

80% of maternal deaths are related to severe hemorrhages, infections, hypertension disorders during pregnancy and dystocia. The 20% would be attributable to indirect factors including anemia [1]. Prevention of this anemia requires iron supplementation since the only source of iron, which is the diet, sometimes does not sufficiently meet the needs of the pregnant woman [4] [5] [6].

Spirulina is a natural alga, rich in protein and containing a high concentration of nutrients. The term “spirulina” is considered to be a vernacular name attributed to Arthospira and Spirulina, two cyanobacteria known as “blue-green algae”. It is known for its virtues in the fight against malnutrition, anemia and the strengthening of immune defenses [7]. It is in this direction that this research studies the contribution of a supplement in spirulina in the pregnant women of the region of Dakar.

2. Framework, Tools and Methods

2.1. Framework

This study was carried out in the region of Dakar, capital of Senegal located in the Peninsula of Cape Verde. The population of the Dakar Region was estimated at 2,647,748 inhabitants in 2011. Although it covers only 0.28% of the national territory, Dakar has the highest population density of the country with 4554 inhabitants per km². The rate of natural increase is 2.5% and young people represent more than two thirds of the population of the Dakar Region. Almost all of the population is urban (97%).

The medical region of Dakar has ten health districts, which are the operational entities of the health system. This study was carried out at the health districts of Guediawaye, Mbao, Pikine and Dakar Nord, selected at random. The use of NPCs was 71.5% in 2013, or 18.5% below the national target of 90%. Of the 108,285 pregnancies expected in 2012, 68,454 deliveries were recorded in the facilities, of which 58,328 were attended by skilled personnel.

2.2. Type of Study

This was an exposed-unexposed comparative cohort study on pregnant women from the 3rd trimester of pregnancy, prenatal consultation (PNC) in the 4 health centers of the selected districts.

Were included, all women with a pregnancy of 28 weeks of completed amenorrhea (WCA). The refusal to participate, the presence of complication requiring special management of pregnancy and twinning were the criteria for non-inclusion. Women who were lost sight of or had dropped out of the study were excluded from the study.

By taking a prevalence of anemia among women in the Dakar region of 31% (corresponding to the prevalence expected in the non-exposed group), an exposure ratio of 1, a power of 90% and an expected relative risk of 0.75, the desired size of the sample was 866 women [8]. A 5% height adjustment was made to compensate for the loss of
sight, which resulted in a rounded size of 920 women to be surveyed. Thus, 115 women exposed and 115 not exposed to spirulina had to be selected and monitored at each of the four health centers. All cases excluded from the study were replaced until the desired strength was achieved.

The designation of the search tool was made after the woman’s consent had been obtained, based on a randomization list previously established at the start of the study.

2.3. Ethical Considerations

Participation in this study was voluntary after obtaining the informed consent of the woman. Anonymity was respected and the data collected were secured in order to preserve their confidentiality. All care costs for this study (consultations, biology, childbirth, supplements) were free for all women enrolled. An insurance system to deal with possible incidents and complications related to the taking of spirulina has also been taken into account. The approval of the National Ethics Committee for Health Research in Senegal was obtained before the start of the field activities, as well as the necessary administrative authorizations.

2.4. Women’s Monitoring

Women were recruited after informed consent at 28 weeks of amenorrhea. Follow-up, carried out by midwives, consisted in visits including an interrogation, a clinical examination and biological tests (hemoglobin levels, glycosuria and proteinuria). Women were evaluated at baseline (28th week of amenorrhea), followed by 32 weeks of amenorrhea, at delivery and 42 days after delivery.

Women in the Exposed group received a daily dose of 1.5 g (3 tablets of 500 mg) of spirulina. Women in the Non-exposed group received conventional iron (ferrous sulfate) 90 mg and folic acid supplementation 1 mg/day. In both groups, catches were made of inclusion on the 42nd day after childbirth.

The Spirulina tablets were green, and those of iron were pink or red. Spirulina used in this survey was provided free of charge by the health education association after acquisition of administrative authorizations for use in the research, but also in the context of the supplementation of children and adults as a food supplement.

2.5. Data Collection Tool

A previously tested and corrected questionnaire was administered to the women included in the study. This questionnaire included several aspects such as personal characteristics, women’s gynecological and obstetric history, anthropometric measurements (weight, height), clinical and biological data (temperature, hemoglobinemia, glycosuria, albuminuria) and any problems encountered.

The records were filled out during consultations and childbirth by the midwives responsible for following pregnant women at the health centers concerned.

2.6. Data Capture and Analysis

The data thus collected had been entered in duplicate on Epi info version 3.5.3 and analyzed with software R according to a per protocol procedure.
The primary endpoint was the evolution of hemoglobinemia in women. Secondary endpoints were the clinical and biological tolerance of spirulina, pregnancy outcome, hemoglobinemia, and neonatal weight. A univariate analysis made it possible to describe the variables and to have their proportions for the qualitative variables or their averages and standard deviations for the quantitative variables.

A comparison of the characteristics of the two groups was made at the inclusion to determine the level of comparability of the two groups, and then on the subsequent contacts. The Chi² and Fisher tests were used for proportional comparisons and the Student test for mean comparisons (in paired or not paired) by setting the risk of alpha error at 5%.

Data analysis in the post-natal period only concerned women who gave birth to a newborn child.

3. Results

The study was conducted on a total of 1201 women recruited over a period of 16 months. Of these, 920 received full follow-up until the 42nd post-natal day, or 76.6%. A difference in protocol was observed in 281 women because they were lost to follow-up (227% or 80.8%), missed appointments (44% or 15.7%) or abandoned for family reasons (10% or 3.6%). There was no statistically significant difference in the reasons for exclusion between the two groups studied (p = 0.48).

Statistical analysis showed that the two groups studied had superimposable characteristics at the time of inclusion (Table 1). The mean hemoglobin levels of women in the two groups were statistically superimposable at the time of inclusion in the study with a rate of 10.7 g/dl for women in the Exposed group versus 10.5 for the second group and a p-value of 0.21. Hemoglobinemia showed an upward trend in each group throughout the study and in a statistically significant manner (Table 2).

The comparison of the evolution of hemoglobinemia between the two groups showed a statistically significant difference. Indeed, the increase in hemoglobinemia in

<table>
<thead>
<tr>
<th>Variables</th>
<th>Settings</th>
<th>Exposed Spirulina Group</th>
<th>&quot;Non-Exposed&quot; Spirulina Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Average</td>
<td>26.6</td>
<td>26.9</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>6.22</td>
<td>6.13</td>
<td></td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>Average</td>
<td>1.75</td>
<td>2.01</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>1.87</td>
<td>2.02</td>
<td></td>
</tr>
<tr>
<td>Weight (in Kg)</td>
<td>Average</td>
<td>66.3</td>
<td>66.3</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>13.1</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>Size (in cm)</td>
<td>Average</td>
<td>1.65</td>
<td>1.65</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>6.3</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Hb (en g/dl)</td>
<td>Average</td>
<td>10.7</td>
<td>10.5</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>1.36</td>
<td>1.26</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Evolution of hemoglobinemia during the various follow-up phases (N = 920).

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Follow-up phases</th>
<th>Average increase</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirulina Non Exposed</td>
<td>Between 28th WCA and 32nd WCA</td>
<td>+0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Between 28th WCA and childbirth</td>
<td>+0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Between birth and 42nd day post natal</td>
<td>+0.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spirulina Exposed</td>
<td>Between 28th WCA and 32nd WCA</td>
<td>+0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Between 28th WCA and childbirth</td>
<td>+0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Between birth and 42nd day post natal</td>
<td>+1.37</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*t test paired with a difference of 0.

Table 2. Evolution of hemoglobinemia during the various follow-up phases (N = 920).

The group exposed to spirulina was statistically higher than that of the exposed group from the first month of exposure with respective gains of 0.48 g/dl and 0.30 g/dl and a p-value of 0.022. The same observation is made when comparing the evolution of hemoglobinemias between the two groups between inclusion and delivery. The more consistent increase in hemoglobinemia in the group exposed to spirulina also remained after childbirth (Table 3).

The evolution of pregnancy between the two groups was statistically superimposable (Table 4). Indeed, the mode of delivery, the occurrence of a hemorrhage of the delivery, the viability of the newborn and the presence of a malformation in the latter. In women no case of albuminuria was objectivized from inclusion to the 42nd postnatal day. Only one case of glycosuria was found in the group exposed to spirulina (Table 4).

The average weight of newborns at birth was 3018 g for women in the group exposed to spirulina and 2999 g for the group under iron and folic acid supplementation (IFAS). This difference was not statistically significant (p = 0.54). On the 42nd postnatal day this weight was 4445 g for the group under spirulina and 4323 g for the group under IFAS. The mean weight gain between birth and the 42th postnatal day was 1432 g in infants under spirulina group and 1318g for those under IFAS group with a statistically significant difference (p = 0.01).

The average hemoglobinaemia of infants at day 42 postnatal was 13.8 g/dl for those in the group exposed to spirulina, and 12.6 g/dl for those in the group whose mother was under IFAS. This difference was statistically significant (p < 0.001).

4. Discussions

This study sought to determine the contribution of spirulina supplementation to hemoglobin in pregnant women. The main limitations of this study are the absence of groups receiving neither spirulina nor iron. This was not possible because of the ethical constraints that this design could have posed to the over-exposure to the risk of anemia that this could entail. The same constraint also led us to start the spirulina supplementation at the 28th week, so that the women included had already received iron and folic acid supplementation before being enrolled in this study. The other limit is represented by the absence of a follow-up of the catches of the products at home. Indeed, adherence to treatment was only indirectly determined on the basis of the women’s statement, which exposes them to a prevarication bias. However, the study was able to reach the desired number of subjects, and the latter could be followed under the
Table 3. Comparison of the evolution of hemoglobinaemia between the two groups during the various follow-up phases (N = 920).

<table>
<thead>
<tr>
<th>Follow-up phases</th>
<th>Evolution in spirulina exposed</th>
<th>Evolution in non-exposed spirulina</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between 28th WCA and 32nd WCA</td>
<td>+0.48</td>
<td>+0.30</td>
<td>0.022</td>
</tr>
<tr>
<td>Between 28th WCA and childbirth</td>
<td>+0.36</td>
<td>+0.20</td>
<td>0.043</td>
</tr>
<tr>
<td>Between birth and 42nd day post natal</td>
<td>+1.37</td>
<td>+0.92</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4. Comparison of the characteristics of the evolution of pregnancy between the two groups (N = 920).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Terms and Conditions</th>
<th>Spirulina Exposed Group</th>
<th>Spirulina Non Exposed Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childbirth mode</td>
<td>Childbirth</td>
<td>440</td>
<td>438</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Caesarean delivery</td>
<td>20</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Haemorrhage of the deliverance</td>
<td>Yes</td>
<td>8 (1.7%)</td>
<td>8 (1.7%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>452 (98.3%)</td>
<td>452 (98.3%)</td>
<td></td>
</tr>
<tr>
<td>Stillborn</td>
<td>Yes</td>
<td>6 (1.3%)</td>
<td>3 (0.7%)</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>454 (98.7%)</td>
<td>457 (99.3%)</td>
<td></td>
</tr>
<tr>
<td>Malformation in the Newborn</td>
<td>Yes</td>
<td>1 (0.2%)</td>
<td>2 (0.4%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>459 (99.8%)</td>
<td>458 (99.6%)</td>
<td></td>
</tr>
<tr>
<td>Albuminuria</td>
<td>Yes</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>460 (100%)</td>
<td>460 (100%)</td>
<td></td>
</tr>
<tr>
<td>Glycosuria</td>
<td>Yes</td>
<td>1 (0.2%)</td>
<td>0 (0.0%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>459 (99.8%)</td>
<td>460 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

same conditions until the 42nd postnatal day. Randomization also allowed two groups of pregnant women with superimposable statistical characteristics to study the supply of spirulina supplementation versus iron and folic acid supplementation with good reliability.

The efficiency of iron supplementation in improving hemoglobinemia in pregnant women has been strongly shared in the literature [9] [10] [11]. Indeed, the World Health Organization [9] recommends iron supplementation through iron-rich medicines and foods for women in countries with limited to moderate resources. Peña-Rosas [10] pointed out in a randomized, systematic review that iron supplementation in pregnant women during pregnancy would result in a hemoglobin gain of 8.8 g/l compared to non-supplemented women. The results of our study made it possible to objectify this contribution of iron and folic acid supplementation on the hemoglobinemia of the pregnant woman. This hemoglobinemia showed an increase of 0.3 g/dl between the 28th and 32nd week of amenorrhoea and 0.2 g/dl between the 32nd week of amenorrhoea and childbirth. A more significant increase was observed between childbirth and the 42nd postnatal day. This increase could be attributed to the disappearance of the phenomenon of haemodilution observed during pregnancy [12].

This study demonstrated improvement in hemoglobinemia through spirulina supplementation. Indeed, the hemoglobinemia of women in the group exposed to spirulina
showed a statistically significant increase in inclusion at the 32nd week of amenorrhoea, but also of this 32nd week at delivery and of delivery in the 42nd Day post-natal.

This increase could be explained by the richness of spirulina in iron. It could also be explained by the effects of phycocyanin contained in spirulina. Phycocyanin is a respiratory pigment of a polypeptide nature specific to spirulina. This complex molecule acts on the bone marrow by stimulating the evolution and differentiation of stem cells from red and white bloodlines. This action was demonstrated by the work of Zhang C. [13], who compared the action of phycocyanin to that of the hormonal systems (kidneys and bone marrow) regulating erythropoiesis. This action of phycocyanin could explain the greater increase in hemoglobinemia observed in women on spirulina compared to women under iron supplementation.

In addition to having good efficacy in improving hemoglobinemia in pregnant women, spirulina was well tolerated. Indeed no case of albuminuria was objectified from inclusion to the 42nd day of follow-up. Only one case of glycosuria was diagnosed in the group of women on spirulina. This shows the good biological tolerance of spirulina. With regard to the outcome of pregnancy, the proportion of stillbirths, caesarean sections, delivery haemorrhages and malformation in newborns was statistically superimposed between the two groups studied.

In the newborn and infant, spirulina has been shown to be effective in improving weight and hemoglobinemia. Indeed, measuring the weight of newborn babies at birth had not made it possible to objectify a statistically significant difference between the two groups. However, weight gain between birth and 42 days post-natal was higher in the group of women exposed to spirulina (p = 0.01). This is in accordance with data from the literature in older children [14] [15].

To this weight gain was added hemoglobinemia of infants at the 42nd postnatal day higher in the group exposed to spirulina. The mean hemoglobin level of infants was 13.7 g/dl in the spirulina group versus 12.6 g/dl in the IFAS group with a statistically significant difference (p < 0.001).

5. Conclusion

Spirulina supplementation in pregnant women in the last trimester is more effective than iron and folic acid supplementation in improving hemoglobinemia and hence preventing and treating anemia. This higher efficacy is accompanied by a good tolerance of spirulina and a significant gain in the weight and hemoglobinemia of infants if supplementation is continued until the 42nd postnatal day. Being a natural product accessible to the vast majority of the population and having already proved its action against hunger, it is an element that could strengthen the arsenal of maternal and newborn health interventions in developing countries.

Acknowledgements

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Conflict

No conflict of interest to declare.

References

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